Biological control of plant pathogenic fungi and the regulation of mycotoxins by soil fauna communities in a conservation tillage system as ecosystem services for soil health

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The
earth
is not only
the common heritage
of all humankind but
also the ultimate source of life.
By over-exploiting its resources we
are undermining the very basis of our own life.
All around, signs abound of the destruction caused by
human activity and of the degradation of nature.
Therefore, the protection and conservation of
the earth is not a question of morality or
ethics but a question of our survival.
How we respond to this
challenge will affect
not only this
generation
but also
many
generations
to
come.

- Dalai Lama -

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Preliminary Remark

This PhD-thesis was carried out in the Working Group "Structural and Functional Soil Zoology" of Prof. Dr. Stefan Schrader, Thünen-Institute of Biodiversity in Braunschweig. Funding of this project was provided by the German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU). This thesis is a collection of already published papers and unpublished results. Most of the results were presented at scientific congresses. Journal contributions are specified below.

Author contributions:

Dipl.-Biogeogr. Friederike Meyer-Wolfarth developed the idea and designed all studies of the PhD-thesis with scientific advice of Prof. Dr. Stefan Schrader. Mycotoxin concentrations and contents of *Fusarium* biomass were analysed by Friederike Meyer-Wolfarth at the laboratories of the Julius Kühn-Institute in Braunschweig with advice of Dr. Elisabeth Oldenburg and Dr. Joachim Weinert. The quantitative analysis of the mycotoxin deoxynivalenol for the paper of Wolfarth et al. (2015) published in Pedobiologia (see below) was carried out by Sina Wedekind M.Sc.. The lab study was carried out at the Thünen-Institute of Biodiversity, the field studies were established in agricultural fields of and with suggestions of Dr. Joachim Brunotte. All experimental studies were conducted by Friederike Meyer-Wolfarth. Data analysis, collecting and processing were carried out by Friederike Meyer-Wolfarth. Friederike Meyer-Wolfarth wrote all manuscripts with contributions of all co-authors as specified above.

Full Papers (peer-reviewed)

The papers 1, 2 and 3 represent the core of the present thesis.

1. **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 and Soil* doi:10.1007/s11104-015-2772-2

2. **Wolfarth F**., 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 doi:10.1016/j.pedobi.2015.01.003

3. **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 Biology and Biochemistry* 57, 903-910 doi:10.1016/j.soilbio.2012.11.001

4. Schrader S., **Wolfarth F.**, Oldenburg E., (2013). Biological control of soil-borne phytopathogenic fungi and their mycotoxins by soil fauna – A review. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, serie Agriculture* 70 (2), 291-298

5. **Wolfarth F.**, Schrader S., Oldenburg E., (2013). Bodenfauna als Ökologischer Dienstleister - Collembolen und Nematoden fördern den Abbau von Deoxynivalenol in Fusarium-infiziertem Weizenstroh. *Gesunde Pflanzen* 65(4), 169-176 doi:10.1007/s10343-013-0309-2

6. **Wolfarth F.**, Schrader S., Oldenburg E., Weinert J., Brunotte J., (2011). Earthworms promote the degradation of *Fusarium* biomass and deoxinivalenol content in wheat straw under field conditions. *Soil Biology and Biochemistry* 43, 1858-1865 doi:10.1016/j.soilbio.2011.05.002

7. **Wolfarth F.**, Schrader S., Oldenburg E., Weinert J., (2011). Contribution of the endogeic earthworm species *Aporrectodea caliginosa* to the degradation of deoxynivalenol and *Fusarium* biomass in wheat straw. *Mycotoxin Research* 27, 215-220 doi:10.1007/s12550-011-0098-3

Non peer-reviewed

8. **Wolfarth F**., Schrader S., Oldenburg E., Brunotte J., (2015). Dekontamination von Mykotoxinen in der Strohauflage: Ökologische Dienstleistung pilzfressender Bodentiere. *Journal für Kulturpflanzen* 67, 236

9. Schrader S., **Wolfarth F**., Oldenburg E., (2015). Aktive Bodentiere fressen Pilze und deren Mykotoxine. *dlz agrarmagazin* Oktober 2015, 40

10. Schrader S., **Wolfarth F.**, Oldenburg E., Brunotte J., (2014). Förderung der Bodengesundheit: Bodentiere dezimieren Schadpilze und ihre Toxine. *Forschungsreport, Ernährung-Landwirtschaft-Verbraucherschutz* 1-2014, 4-7

11. Wolfarth F., Schrader S., Oldenburg E., (2013). Bodentiere fördern den Abbau von Deoxynivalenol (DON). *Landwirtschaft ohne Pflug* 9/10-2013, 32-36

12. Wolfarth F., Schrader S., Oldenburg E., (2012) Abbau von Deoxynivalenol in Weizenstroh durch Nematoden und Collembolen in Abhängigkeit von der Bodentextur. *Journal für Kulturpflanzen* 64, 174-175

13. Schrader S., **Wolfarth F.**, Oldenburg E., (2011). Regenwürmer als natürliche Gegenspieler. *Landwirtschaft ohne Pflug* 5-2011, 11-15

14. Wolfarth F., Schrader S., Oldenburg E., Weinert J., Brunotte J., (2011). Abbau von *Fusarium*-Biomasse in Weizenrückständen durch Regenwürmer – ein Beitrag zur biologischen Kontrolle eines phytopathogenen Schaderregers. *Journal für Kulturpflanzen* 63, 189

Contributions to symposia and congresses

International

1. **Wolfarth F.,** Schrader S., Oldenburg E., Brunotte J. (2014). Decontamination of pollutants by soil faunal communities as an ecosystem service for soil health. – **Poster** auf der First Global Soil Biodiversity Conference, Dijon, Frankreich, Dezember 2014.

2. **Wolfarth F**, Schrader S., Oldenburg E., Brunotte J., Weinert J. (2014). Functional linkage between soil fauna activity and mycotoxin degradation in straw cover. – **Vortrag** auf dem 44th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Hildesheim, September 2014.

3. **Wolfarth F.,** Schrader S., Oldenburg E. (2014). Mycotoxin degradation in wheat straw by soil faunal communities under field conditions – An ecosystem service for soil health. – **Vortrag** auf dem 36th Mycotoxin-Workshop, Göttingen, Juni 2014.

4. **Wolfarth F.,** Schrader S., Oldenburg E. (2012). Degradation of deoxynivalenol by nematodes and collembolans in wheat straw depending on soil texture. – **Vortrag** auf dem 34th Mycotoxin-Workshop, Braunschweig, Mai 2012.

5. **Wolfarth F**., Schrader S., Oldenburg E. (2012). Mycotoxin degradation by nematodes and collembolans in wheat straw depending on soil texture. – **Vortrag** auf der Eurosoil, Bari, Italy, Juli 2012.

6. **Wolfarth F**., Schrader S., Oldenburg E., Weigel H-J. (2012). Interaction between soil micro- and mesofauna regarding mycotoxin degradation in wheat straw as a function of soil texture. – **Poster** auf dem 12th Congress of the European Society for Agronomy, Helsinki, Finland, August 2012.

7. **Wolfarth F**, Schrader S., Oldenburg E., Weinert J., Brunotte J. (2011). Biological control of the phytopathogenic fungus *Fusarium culmorum* and its mycotoxin deoxynivalenol by earthworms. – **Vortrag** auf dem 33rd Mycotoxin-Workshop, Freising, Mai 2011.

8. **Wolfarth F**, Schrader S., Oldenburg E., Weinert J., Brunotte J. (2011). Functional linkage between earthworm activity and *Fusarium* infection in wheat straw under conservation tillage. – **Poster** auf dem Workshop der GfÖ Soil Ecology Group, Microbial faunal Microbial-interactions shaping soil processes, Berlin, Oktober 2011.

National

9. **Wolfarth F**., Schrader S., Oldenburg E., Brunotte J. (2015). Dekontamination von Mykotoxinen in der Strohauflage: Ökologische Dienstleistung pilzfressender Bodentiere. – **Vortrag** auf der 28. Tagung der DPG-Projektgruppe „Krankheiten im Getreide“, JKI, Braunschweig, Februar 2015.

10. **Wolfarth F.**, Wedekind S., Schrader S., Oldenburg E., Brunotte J., Weinert J. (2013). Interaktion zwischen Collembolen und Nematoden als Beitrag zur biologischen Kontrolle pilzlicher Pflanzenpathogene und deren Mykotoxine. – **Vortrag** auf der Jahrestagung der Deutschen Bodenkundlichen Gesellschaft, Rostock, September 2013.

11. **Wolfarth F.**, Schrader S., Oldenburg E., Weinert J. (2012). Abbau von *Fusarium* Biomasse und Deoxynivalenol (DON) in Weizenstroh durch Nematoden und Collembolen in Abhängigkeit von der Bodentextur. – **Vortrag** auf der 58. Deutschen Pflanzenschutztagung, Braunschweig, September 2012.

12. **Wolfarth F.**, Schrader S., Oldenburg E. (2012). Abbau von Deoxynivalenol in Weizenstroh durch Nematoden und Collembolen in Abhängigkeit von der Bodentextur. – **Vortrag** auf der 25. Tagung der DPG-Projektgruppe „Krankheiten im Getreide“, JKI, Braunschweig, Januar 2012.

13. **Wolfarth F.**, Schrader S., Oldenburg E., Brunotte J., Weinert J. (2011). Abbau von pilzlichen Pflanzenpathogenen und deren Mykotoxinen in Weizenrückständen durch Regenwürmer im Freiland. – **Vortrag** auf der Jahrestagung der Deutschen Bodenkundlichen Gesellschaft, Berlin, September 2011.

14. **Wolfarth F.**, Schrader S., Oldenburg E., Brunotte J., Weinert J. (2011). Abbau von Fusarium-Biomasse in Weizenrückständen durch Regenwürmer – ein Beitrag zur biologischen Kontrolle eines phytopathogenen Schaderregers. – **Vortrag** auf der 24. Tagung der DPG-Projektgruppe „Krankheiten im Getreide“, JKI, Braunschweig, Januar 2011.

Abstract

Besides well-known positive aspects of conservation tillage combined with mulching, a drawback may be the survival of phytopathogenic fungi like *Fusarium* species on plant residues. This may endanger the health of the following crop by increasing the infection risk for specific plant diseases. In infected plant organs, these pathogens are able to produce mycotoxins like deoxynivalenol (DON). Mycotoxins like DON persist during storage, are heat resistant and of major concern for human and animal health after consumption of contaminated food and feed, respectively.

Among fungivorous soil organisms, there are representatives of the soil fauna which are obviously antagonistic to a *Fusarium* infection and the contamination with mycotoxins. Earthworms, collembolans and nematodes provide a wide range of ecosystem services including the stimulation of decomposition processes which may result in the regulation of plant pathogens and the degradation of environmental contaminants. However, it is still an open question whether and to what extent key stone species of different functional groups of soil fauna (earthworms: *Lumbricus terrestris*, collembolans: *Folsomia candida* and nematodes: *Aphelenchoides saprophilus*) and their interaction significantly contributes to pathogen repression and the degradation of mycotoxins in arable fields.

Several investigations under laboratory conditions and in the field were conducted to test the following hypotheses: (1) *Fusarium*-infected and DON-contaminated wheat straw provides a more attractive food substrate than non-infected control straw (2) the introduced soil fauna reduce the biomass of *F. culmorum* and the content of DON in infected wheat straw under laboratory and field conditions (3) the species interaction of the introduced soil fauna enhances the degradation of *Fusarium* biomass and DON concentration in wheat straw; (4) the degradation efficiency of soil fauna is affected by soil texture.

Minicontainer-studies with collembolans and nematodes were conducted under laboratory and field conditions in 2011. Furthermore, mesocosm-based field studies with earthworms, collembolans and nematodes were conducted in a cropping system under reduced tillage conditions in 2011 and 2013. The soil fauna was introduced in different numbers and combinations and exposed to either *Fusarium*-infected (and DON-contaminated) or non-infected wheat straw. At the end of the experiments, biomass of *Fusarium culmorum* and concentrations of DON were detected in samples of soil and wheat straw using ELISA (Enzyme-linked immunosorbent assay)-method. Furthermore, collembolans and nematodes were counted and biomass of earthworms was determined.

The results of the present thesis pointed out that the degradation performance of the introduced soil fauna must be considered as an important contribution to the biological control of plant diseases and environmental pollutants. Under laboratory conditions interacting collembolans and nematodes play an important role in plant pathogen repression and mycotoxin degradation. Also soil texture matters in the provision of these ecosystem services by collembolans and nematodes as in particular DON was reduced more efficiently in sandy and silt loam treatments. Under on-farm conditions the interaction between the introduced soil fauna is assessed to be minor. In the absence of earthworms, collembolans and nematodes showed single species effects on *Fusarium* degradation and DON regulation. In presence of the anecic earthworm species *L. terrestris,* the introduced collembolans and nematodes did not influence its degradation capacity. As in particular *L. terrestris* revealed to be the driver of the degradation process, it could be concluded that earthworms contribute to a sustainable control of fungal pathogens like *Fusarium* and its mycotoxins in wheat straw, thus reducing the risk of plant diseases and environmental pollution as ecosystem services.

Kurzfassung

Ein Nachteil konservierender Bodenbearbeitung ist das erhöhte Risiko eines Schaderregerbefalls durch pilzliche Pflanzenpathogene. Die für konservierende Bodenbearbeitung charakteristische Mulch Schicht bietet ein ideales Nähmedium für Saprophyten, unter denen allerdings auch pflanzenpathogene Bodenpilze der Gattung *Fusarium* gefördert werden. Vor diesem Hintergrund entsteht eine potenziell erhöhte Infektionsgefahr für die Folgefrucht. Ein Befall mit Fusarien führt neben Ertragseinbußen zu Qualitätsbeeinträchtigungen des Ernteguts durch Mykotoxine, zu deren Bildung Fusariumarten in der Lage sind. Eines der in Getreide am häufigsten nachgewiesenen Mykotoxine ist das zu den Trichothecenen zählende Deoxynivalenol (DON), von dem beim Verzehr kontaminierter Lebens- und Futtermittel ein erhebliches Gesundheitsrisiko für Mensch und Tier ausgeht.

Bodentiere bieten eine Vielfalt an ökologischen Funktionen und Dienstleistungen. Dazu zählen beispielsweise die Stimulierung von Zersetzungsprozessen und der Abbau von Pflanzenrückständen, was eine Regulierung von Pflanzenpathogenen und einen Abbau von Schadstoffen zur Folge haben kann. Ungeklärt blieb bislang allerdings, inwiefern bestimmte Schlüsselorganismen (Regenwürmer: *Lumbricus terrestris*; Collembolen: *Folsomia candida* und Nematoden: *Aphelenchoides saprophilus*) und deren Interaktion einen signifikanten Beitrag zur Lösung agrarrelevanter Umweltprobleme leisten, indem sie in der Lage sind, einem erhöhten Fusarienbefall und einer Mykotoxin Kontamination entgegenzuwirken. Aus diesem Grund wurden verschiedene Untersuchungen im Labor und im Freiland durchgeführt, anhand derer folgende Hypothesen sollten geprüft werden: (1) *Fusarium*-infiziertes und DON-kontaminiertes Pflanzenmaterial stellt für die eingesetzten Bodentiere ein attraktiveres Nahrungssubstrat dar, als nicht künstlich infiziertes Kontrollstroh (2) Die eingesetzten Bodentiere fördern den Abbau von *Fusarium*-Biomasse und DON-Konzentration im Stroh (3) Die Interaktion zwischen den Vertretern der Makrofauna, Mesofauna und Mikrofauna verstärkt die Reduzierung der *Fusarium*-Biomasse und der DON-Konzentration (4) Die Bodentextur hat Auswirkungen auf die Abbauleistung der Tiere

Minicontainerstudien (mit Collembolen und Nematoden) wurden im Jahr 2011 unter Laborbedingungen und im Freiland durchgeführt. Außerdem wurden in den Jahren 2011 und 2013 Mesokosmen (mit Regenwürmern, Collembolen und Nematoden) auf Ackerflächen etabliert, die mit Verfahren konservierender Bodenbearbeitung bewirtschaftet wurden. Die Organismen wurden in verschiedener Anzahl und Kombination künstlich mit *Fusarium*-

infiziertem und DON-kontaminiertem Weizenstroh ausgesetzt. In einem jeweils zweiten Ansatz wurde den Organismen Weizenstroh angeboten, welches nicht künstlich infiziert war. Nach jeweiligem Versuchsende wurden in Stroh und Boden die *Fusarium*-Biomasse in Form von *Fusarium*-Protein-Äquivalenten (FPE) und die DON-Konzentrationen mittels der ELISA (Enzyme-linked immunosorbent assay)-Methodik quantitativ bestimmt. Außerdem wurde die Biomasse der Regenwürmer sowie die Individuenzahlen von Collembolen und Nematoden erfasst.

Die eingesetzten Bodentiere fördern den Abbau von *Fusarium*-Biomasse und DON-Konzentration in Ernterückständen. Insbesondere Regenwürmer leisten einen wichtigen Beitrag zur biologischen Schädlingsbekämpfung. Die Interaktion zwischen Collembolen und Nematoden erwies sich unter Laborbedingungen als entscheidend für die Reduzierung der DON-Konzentration in Weizenstroh. Außerdem konnte unter standardisierten Laborbedingungen ein signifikanter Einfluss der Bodentextur auf die Abnahme von *Fusarium*-Biomasse und DON-Konzentration nachgewiesen werden.

Im Freiland spielte die Interaktion zwischen Collembolen und Nematoden im Hinblick auf den Abbau von *Fusarium*-Biomasse und DON-Konzentration keine Rolle. Entscheidend für die Reduzierung hier war der alleinige Einfluss von *F. candida* oder *A. saprophilus*.

Auch in Anwesenheit von Regenwürmern stellte sich der Einfluss von Collembolen und Nematoden auf den Abbau von *Fusarium*-Biomasse und DON-Konzentration als vernachlässigbar heraus. Die Interaktion führte nicht zu einem verstärkten Rückgang der Konzentrationen von *Fusarium*-Biomasse und DON. Hier war der Einfluss von *L. terrestris* in seiner Funktion als Primärzersetzer von entscheidender Bedeutung.

Chapter 1

1 General Introduction

1.1 Soil health and the importance of conservation tillage

Soils are fundamental to life on earth (FAO, 2015). Thus the degradation of soils and the associated decline in soil productivity are among the most serious agricultural and environmental problems (Chen et al, 2002). Through human activities soils are degraded at a rapid pace. This degradation includes erosion, desertification, nitrogen deposition, salinization, chemical contamination and depletion of fertile land for food production (Wall et al., 2012). Karlen et al. (2013) hypothesised that intensive tillage has been the primary anthropogenic factor degrading soil health and soil quality. Soil health is defined as the capacity of soil to function as a vital dynamic system to sustain biological productivity, promote environmental quality, and maintain plant and animal health (Doran and Zeiss, 2000). In other words: the quality and health of soil is determining agricultural productivity.

As an alternative management measure, conservation agriculture has been shown to be a promising soil management system able to minimize negative impacts of farming operations with several beneficial consequences on soil structure, hydrology and biodiversity (Holland, 2004; Soane et al., 2012; Tamburini et al., 2016). Furthermore, conservation agriculture has been increasingly promoted to contribute to sustainable production intensification. According to Kassam et al. (2009) conservation agriculture is a system of agronomic practices that include three key components:

(1) Maintaining permanent organic soil cover by retaining crop residues and crop rotation including cover crops and intercrops
(2) Minimizing soil disturbance by reduced tillage. Furthermore eliminating tillage once the soil has been brought to a good condition.
(3) Diversifying crop rotations, sequences and associations, adapted to local environmental conditions including appropriate nitrogen fixing legumes thus promoting above- and belowground biodiversity and help avoid establishing pest populations.

Originally, conservation tillage was introduced in the US to regulate wind and water erosion in the 1930s (Baveye et al., 2011; Palm et al., 2014). Since then, the adoption of conservation agriculture has been rapid, particularly in North America, South America and Australia. During the last 15 years an ongoing expansion of the area under conservation tillage shows the increasing interest of this technology among farmers (Derpsch et al., 2010). The aim of conservation tillage is to assure production of plants and water recurrently and sustainably by favouring improvements in conditions of soils (Kassam et al., 2009). In contrast with tillage agriculture, conservation agriculture can reverse the loss of organic matter, improve and maintain soil fertility and thus preserve the bioavailability of nutrients and water in times of drought. Enhancing agro-ecological diversity and favouring biological nitrogen fixation is resulting in both, increased and better stabilised yields accompanied by lowered costs of production. By introducing conservation tillage, some objectives of the UN Conventions to Combat Desertification (UNCCD, 1994), loss of biodiversity and climate change can be achieved (Kassam et al., 2009). Consequently, a faster adoption of this sustainable production system should be encouraged in order to reverse the process of soil degradation into a process of rehabilitating or building up its health, fertility and productive capacity (Derpsch et al., 2010).

1.2 Soil organisms and their ecological role in agroecosystems

1.2.1 Earthworms

Earthworms are found in almost all land areas of the world (Lee, 1985). In terrestrial ecosystems, earthworms generate the most abundant animal biomass (Lavelle and Spain, 2001). Most earthworms are inhabitants of soils, including litter layers and aboveground habitats like animal dung, rubbish accumulations or rotting logs. Some even live under the bark of trees and in organic material accumulated at the bases of some epiphytes (Lee, 1985). According to Bouché (1977) earthworms can be categorised in three ecological groups: *epigeic*, *anecic* and *endogeic*. Epigeic earthworm species feed on accumulations of organic matter and burrow horizontally. Anecic species live in permanent or semipermanent vertical burrows that may extent deep into the soil. They are detritivorous and forage for food at the soil surface and feed on organic residues which they pull into their burrows. Because of their feeding habits epigeic and anecic earthworm species generally are considered as primary

decomposers. Endogeic earthworm species form widely branched networks of burrows which are often packed with cast or soil from overlaying horizons. They are usually geophagous and feed on a mixture of soil and organic matter (Lee, 1985; Shipitalo and Le Bayon, 2004).

In agroecosystems earthworms are probably the most important soil-inhabiting invertebrates. By changing the physical, chemical and biological properties, earthworms act as typical soil ecosystem engineers which affect soil fertility essentially and influence soil formation (Edwards et al., 1995; Lavelle et al., 1997). The activity of earthworms generally increases the availability of nutrients, accelerates mineralization of organic matter and improves soil structure. On the one hand those effects of earthworms are direct because of their feeding and burrowing activities, on the other hand earthworms affect their environment indirectly as a result of their numerous interactions with soil microorganisms and dynamic soil processes (Whalen and Sampedro, 2010).

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; van Capelle et al., 2012). It is well documented that earthworm populations and community structure were affected directly by the tillage system used (Edwards and Lofty, 1982; Ernst and Emmerling, 2009; Langmaack, 1999). Especially deep burrowing earthworm species like *Lumbricus terrestris* benefit from reduced tillage (Edwards, 1983; Edwards et al., 1995; Ernst and Emmerling, 2009; Joschko and Rogasik, 2002; Whalen and Sampedro, 2010).

1.2.2 Collembolans

Collembolans (springtails) are microarthropods which constitute an important component of soil mesofauna in almost all terrestrial ecosystems (Rusek, 1998). Together with mites, they account for 95% of total soil microarthropod numbers (Neher and Barbercheck, 1999). According to ecomorphological life-forms, Gisin (1943) distinguished three groups of collembolans which occupying different soil subhorizons: *Euedaphic* species live in deeper soil layers and are permanent soil-dwellers, *hemiedaphic* species inhabit upper soil layers and leaf litter and *epedaphic* (atmobiotic) species occurring at the soil surface or on vegetation. Collembolans depend on high humidity and live in air filled soil pores and in litter layers. In general collembolans are non-specific feeders (Filser, 2002), whereas the majority of collembolan species feed on fungal hyphae or decaying plant material but a few feed also on plants or are predators (Parkinson, 1983; Petersen and Luxton, 1982). Furthermore, collembolans are also food sources for many predators such as carabid beetles and their larvae

(Rusek, 1998). Thus, collembolans represent an essential element of the soil food web (Hopkin, 1997) and an important mesofauna group in arable soils (van Capelle et al., 2012). Despite of their small body size and their low biomass there is general consensus, that collembolans can play key roles in natural and agricultural ecosystems (Edwards, 2000). They affect primary production directly by root feeding and indirectly through their contribution to decomposition and nutrient mineralization (Neher and Barbercheck, 1999). However, the indirect catalytic effects of collembolans on the decomposition processes, like recirculation of nutrients, dispersal of spores and selective feeding are regarded to be more important than the direct contribution to energy flow (Petersen, 2002).

Concerning their abundance and population density under reduced tillage systems, the reports about the development of collembolan communities are inconsistent (Holland, 2004; van Capelle et al., 2012). In general, the population biomass and numbers of collembolans is lower in cultivated soils than in non-cultivated soils (Petersen, 2002), whereas soil cultivated with conservation tillage my lie somewhere in between the two extremes (Holland, 2004). Where soil structure is degraded a reduced collembolan density could be expected since this group is restricted by the pore space. Nevertheless, the vertical distribution of microarthropods depends on soil tillage and compaction and a higher density of macropores, which occurs under reduced tillage, is facilitating the distribution of microarthropods (Holland, 2004).

1.2.3 Nematodes

Nematodes are small roundworms and the most abundant multicellular animals on earth. They are aquatic organisms, consequently soil nematodes depend on thin water films to live and move through the soil system within existing pathways of soil pores of 25-100 µm diameter. According to the nature of food resource nematodes can be separated into different functional groups or feeding groups: *herbivores*, *carnivores* (as parasites or predators), *fungivores, bacterivores* or *omnivores* (Yeates et al., 1993; Yeates et al, 2009). Because of their central position in the soil food web and linkage to ecological processes they can be used as a tool for understanding biological mechanisms in soil and serve as indicators of environmental disturbance (Neher, 2010; Yeates et al., 2009). The structure of nematode communities has a high information content because of their food specificity, the high number of species and the high abundance in habitats where decomposition takes place (Bongers and Bongers, 1998). Most of the soil nematode species are involved in beneficial ecosystem processes (Neher, 2001). In general microbial grazing of nematodes results in greater metabolic activity and

alters microbial communities, thus regulating rates of decomposition and nutrient mineralization. Particularly bacterivores and fungivorous nematodes play important roles in influencing the turnover of soil microbial biomass and enhancing the availability of plant nutrients. Furthermore, their participation in the carbon and nitrogen cycle is of major importance (Ferris et al., 2004; Seastedt, 1988; Yeates and Bongers, 1999; Yeates et al., 2009). In arable soils the abundance, activity and community structure of nematodes are highly correlated with soil biological, chemical and physical microsite properties, which are depending on tillage interventions (van Capelle et al., 2012). Thus different tillage systems might result in different nematode communities. Furthermore, the different feeding types of nematodes are differently affected by a reduction of tillage, whereas bacterivorous and fungivorous species are reported to be more abundant when tillage intensity is reduced and consequently benefit from conservation tillage practices (Holland, 2004; van Capelle et al., 2012).

1.3 Ecosystem services and disservices

1.3.1 Soil as an important provider of ecosystem services

Soils are not only a natural resource that must be secured for future generations (Pulleman et al., 2012). In order to supply the needs of a growing world population (food, feed, fibre, clean air, clean water) the extreme importance of soils as natural capital stocks providing a huge range of ecosystem services must be approved (Dominati et al., 2010). Soils of natural and managed ecosystem are a critical and a dynamic regulatory system that generates a multitude of soil functions. These functions support the delivery of ecosystem services (Adhikari and Hartemink, 2016; FAO and ITPS, 2015). Soil ecosystem services depend on soil properties and their interaction, and are mostly influenced by its use and management. Landslides, erosion, decline in soil carbon and biodiversity lead to soil degradation which is a serious global challenge for food security and ecosystem sustainability (Adhikari and Hartemink, 2016). According to the MEA (Millennium Ecosystem Assessment, 2005), ecosystem services are the benefits people obtain from ecosystems. These ecosystem services are divided into 4 categories:

(1) *Supporting services* include primary biomass production, nutrient cycling, water cycling, nitrogen fixation, soil formation, and soil biological activity.

(2) *Provisioning services* are products obtained from ecosystems including genetic resources, food, feed, and fibre, and fresh water. Furthermore, soils provide habitats for different species which is essential because soil biota is the driver of many ecosystem services.

(3) *Regulating services* directly contributing to human welfare as they enable humans to live in a stable and resilient environment. These services include flood control, filtering of nutrients and contaminants, recycling wastes, limitation of greenhouse gas emission. By promoting beneficial species soils are involved in biological control of pests and diseases; furthermore, by degrading dead organic matter soil organisms decompose chemical compounds that can be harmful to humans.

(4) *Cultural services* include inspiration for art and spirituality, recreation and aesthetic values, as well as opportunities for ecotourism and education.

Tubé et al. (2010) divided soil organisms into three functional groups called *chemical engineers*, *biological regulators* and *ecosystem engineers*. According to Tubé et al. (2010), these functional groups contribute to provisioning ecosystem services and are described as followes:

The majority of soil organisms are microorganisms, such as bacteria, fungi and protozoans, which are attributed to the *chemical engineers* of the soil, responsible for the decomposition of plant organic matter into nutrients readily available for plants, animals and humans.

The group of *biological regulators* include a variety of small soil invertebrates including nematodes, pot worms and microarthropods such as springtails and mites. Biological regulators act as regulators of microbial activities, mainly through grazing but also through parasitic or mutualistic interactions with other microbes or invertebrates which directly regulate the abundance and the activity of chemical engineers through top-down effects. Biological regulators are also recognised as integrators of the soil food web. Furthermore, predation often suppresses microbial populations. Hence, this functional group is also essential in the development of semi-natural ecosystems, sustainable agriculture, by indirectly influencing plant abundance, invasive species outbreaks, and diseases and pests outbreaks in crop systems.

Soil organisms that modify environmental conditions for other organisms through their mechanical activities are called *ecosystem engineers*, which have the ability to build resistant organo-mineral structures and pores by moving through the soil and mixing the soil, in process known as bioturbation. Earthworms, termites, ants and roots have been identified as the most important soil engineers.

1.3.2 Ecosystem services and disservices in the context of biodiversity and agroecosystems

Besides a variety of ecosystem services provided by ecosystems and the inhabiting soil biota, there are also ecosystem disservices that have harmful effects on human well-being. These ecosystem disservices include pests, litter and impairment of infrastructure, biological hazards like diseases, animal attack, allergic and poisonous organisms and geophysical hazards such as floods, heat waves and storms (Lyytimäki and Sipilä, 2009). In general, ecological disservices have often been described as negative effects of ecological changes or as disturbed services caused by the loss of biodiversity (von Döhren and Haase, 2015). However, it should be noted that many disservices are caused by anthropogenic disturbance to natural systems (Dale et al., 2001) and societies have to pay and account for the costs of these disservices either through prevention or remedial measures such as rebuilding after natural disasters, treating diseases or applying pesticides (Shapiro and Báldi, 2014).

Agricultural ecosystems are primarily managed to optimize the provisioning ecosystem services of food, fibre, and fuel and they depend upon a wide variety of supporting and regulating services, such as soil fertility, nutrient cycling, soil retention and pollination (MEA, 2005), that determine the underlying capacity of agricultural ecosystems (Zhang et al., 2007). However, intense agriculture practices causes many ecosystem disservices that reduce productivity or increase production costs (e.g., herbivory and competition for water). Ecosystem disservices to agriculture include crop pest, like herbivores, fungivores, seed-eaters, and pathogens which decrease productivity and can result even in complete crop loss (Zhang et al., 2007). Furthermore, intense management practices result in a reduction of biodiversity compared to natural ecosystems. The application of synthetic fertilizers, pesticides and frequent cultivation affect soil organisms and often altering community composition of soil fauna (Neher and Barbercheck, 1999). This is a serious problem in terms of the provision of ecosystem services, since species composition matters as much or more than species richness when it comes to ecosystem services. The provision of these ecosystem services and disservices depend on how agricultural ecosystems are managed at the site scale

and on the diversity, composition, and functioning of the surrounding landscape (Tilman, 1999). As many processes and ecosystem services are mostly biological, their continued existence depends on the maintenance of biological diversity (Altieri, 1999). Biodiversity in natural communities is a key factor in ecosystem structure and functions that provide ecosystem services (Neher and Barbercheck, 1999). Ecosystem functioning, and hence ecosystem services are strongly influenced by the ecological characteristics of the most abundant species, not by the number of species. The relative importance of a species to ecosystem functioning is determined by its traits and its relative abundance (MEA, 2005). The value of the world´s ecosystem services was estimated by Constanza et al. (1997) to have the value of twice the gross national product of the world. Later studies showed this assessment to be probably underestimated (Breure et al., 2012).

Consequently, the loss of natural services due to biological simplification results in economic and ecological costs, which can be significant (Altieri, 1999). By the year 2050 the economic consequences of the annual loss of biodiversity on land and the corresponding loss of ecosystem services is assessed by Braat and ten Brink (2008) to a sum which is equivalent to 14,000 billion Euro.

1.3.3 Fungal plant pathogens and mycotoxin contamination as agricultural disservices

Alongside the beneficial effects of conservation tillage mentioned above, 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). The pathogens survive as a saprophyte in infected tissue (like crop residues) and produce ascospores and/or macroconidia which are dispersed by wind, rain and insects (Goswami and Kistler, 2004). Besides various types of diseases including vascular wilts, head and seed blights, stem rots, root and crown rots and canker disease caused by *Fusarium* species, the fungal plant disease *Fusarium* head blight is one of the most important diseases that affect agriculture and horticulture in all parts of the world (Leslie and Summerell, 2013). By causing significant yield losses in maize and cereals such as wheat, oat and barley (Parry et al., 1995; Pereyra and Dill-Macky; 2008, Vogelgsang et al., 2011) *Fusarium* head blight can have devastating economic and sociological impacts on farmers and communities (Leslie and Summerell, 2013). In wheat fields of temperate regions, *Fusarium graminearum, F. culmorum* and *F. avenaceum* are found predominately (Leplat et al., 2013; Wagacha and Muthomi, 2007). In infected plant organs, these pathogens are able to produce mycotoxins (Parry et al., 1995). The best known

Fusarium mycotoxins are the trichothecenes, the fumonisins, the zearalenone and the gibberellic acids (Leslie and Summerell, 2013). The trichothecene class of mycotoxins includes the deoxynivalenol (DON) (
Fig. **1.3.1**) which is the most frequently produced mycotoxin by *F. graminearum* and *F. culmorum* and therefore often detected in cereals (Curtui et al., 2005; Pestka, 2007). Although it is not the most toxic one, DON is considered to be the most economically important mycotoxin (Audenaert et al. 2013).

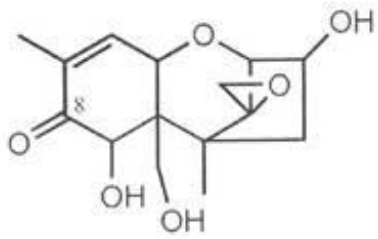

Fig. 1.3.1: Chemical structure of the *Fusarium* toxin deoxynivalenol (DON) according to Leslie and Summerell, (2013).

Mycotoxins like DON persist during storage, are heat resistant and of major concern for human and animal health after consumption of contaminated food and feed, respectively (JEFCA, 2001). An acute health risk of DON is given after consumption of large amounts of DON within a short time frame resulting in vomiting and feed refusal for animals and gastroenteritis with vomiting for humans. A chronic exposure to DON causes growth retardation, alter immune function and interfere with reproduction and development (Bianchini et al., 2015). 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). In the interest of ensuring public health protection, the European Commission set thresholds for certain mycotoxins including DON (Table 5.1.1) in foodstuffs (EC, 2006; 2007).

Table 1.3.1: Maximum levels for DON in µg kg^{-1} in selected foodstuffs as ruled by the European Commission (EC, 2006)

Foodstuff	DON [µg kg^{-1}]
Unprocessed durum wheat and oats	1750
Unprocessed cereals other than durum wheat, oats and maize	1250
Pasta	750
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500
Processed cereal-based foods for infants and young children	200

Besides the emanating health threat of DON, there are increasing concerns about the importance of DON and other mycotoxins as potential environmental contaminants (Bucheli et al. 2008; Gautam and Dill-Macky 2012; Hartmann et al. 2008b). Leaching of mycotoxins from host tissue like infected plants or residual material has been considered as environmental threat since mycotoxins including DON were detected in soil, drainage water and soil water, which percolates to the ground water table (Hartmann et al. 2008a; Kolpin et al. 2014).

Another factor affecting mycotoxin contamination that must be considered is the changing of the global climate (Paterson and Lima, 2010; 2011). Increased CO_2 levels, higher global temperature, altered precipitation regimes and increases in the frequency of extreme weather events will influence the interaction between crops and pathogens in a multiple way (Juroszek and von Tiedemann, 2011). In general, more favourable conditions for mycotoxigenic pathogen survival and colonisation are expected due to climate change in the future (Luck et al., 2011).

Against this background, it is still an open question, whether and to what extent members of the soil fauna contribute to the degradation of mycotoxins.

1.4 Biological control of fungal plant pathogens

Soil-borne diseases, caused by fungi (and nematodes), are major yield-limiting factors and they are difficult to control (EIP-AGRI Focus Group, 2015). It is estimated that over 40% of

the pre-harvest yield of the eight most important crop species is lost due to disease and pest damage and the global food production losses to plant disease have been estimated at 10% (Cheatham et al., 2009). Biological control of soil-borne plant pathogens has become a promising alternative, because it may provide an alternative measure of disease control instead of application of chemical pesticides (Choudhari and Johri, 2009). In general, there are considered to be three main ways for a biocontrol agent to control a plant pathogen (Bardin et al., 2015):

(1) by acting directly on the plant pathogen, through antibiosis, competition for nutrient or other resources, parasitism or predation
(2) by interfering with the mechanisms of pathogenesis of the plant pathogen,
(3) by modifying the interaction of the plant pathogen with its plant host for instance through the induction of local or systemic acquired resistance.

Plant growth-promoting rhizobacteria are applied to control plant pathogens. These bacterial genera include *Acinetobacter, Agrobacterium, Arthrobacter, Azospirillum, Bacillus, Bradyrhizobium, Frankia, Pseudomonas, Rhizobium, Serratia, Thiobacillus*, and many others (Kloepper et al., 2004). The underlying mechanisms of beneficial rhizobacteria include production of enzymes such as chitinases, peroxidases and proteases, and many types of antibiotics (Pieterse et al., 2014).

The ability of fungivorous nematodes as members of the soil microfauna, to control pathogenic fungi has been demonstrated by many investigators (Freckman and Caswell, 1985). By penetrating fungal cells and ingesting the cell content, fungivorous nematodes destroy the fungal cells (Yeates et al., 1993). Nematodes species, such as *Aphelenchus avenae* and to some extent also species in the genera *Aphelenchoides* and *Ditylenchus* have been tested under laboratory and field conditions to control a range of plant pathogenic fungi in crops (Friberg et al., 2005), such as *F. culmorum* (Roessner and Urland, 1983), *F. moniliforme* and *Pythium butleri* (Gupta, 1986), *Rhizoctonia solani* and *F. oxysporum* (Okada, 2006).

Among the soil mesofauna, collembolans are also considered to play an important role in biological control of fungal plant pathogens. Food selection experiments revealed that collembolans prefer feeding on plant pathogenic fungi rather than on saprophytic fungi (Friberg et al., 2005). Furthermore, several collembolan species are reported to successfully suppress fungal pathogens like *R. solani* (Lartey et al., 1994; Shirashi et al. 2003),

Gaeumannomyces graminis and *F. culmorum* (Sabatini et al., 2000) by grazing on fungal hyphae (Friberg et al., 2005).

Another important group of the soil fauna regarding biocontrol of fungal plant pathogens are earthworms. Stephens and Davoren (1997) demonstrated the potential of endogeic earthworms (*A. trapezoides* and *A. rosea*) to reduce harmful effects of *R. solani* on specific pasture plants and Wolfarth et al. (2011b) showed that the biomass of *F. culmorum* in straw residues decreased in the presence of *A. caliginosa* (endogeic). Further studies revealed that anecic earthworm species *L. terrestris* are obviously antagonistic to *Fusarium* infection in wheat straw (Oldenburg et al., 2008; Schrader et al., 2009; Wolfarth et al., 2011a). Just recently, *L. terrestris* was suggested to be an effective biocontrol agent for eyespot disease of winter wheat (Bertrand et al., 2015).

However, exploring the interaction between key stone species of different functional groups of soil fauna concerning pathogen repression has been neglected up to the present.

1.5 Study objectives and research design

This thesis generally aims to reveal the degradation performance of selected members of the soil fauna on *Fusarium culmorum* and its mycotoxin deoxynivalenol (DON) in infected and contaminated wheat straw remaining on the soil surface (common practice in conservation tillage) as crucial ecosystem services for soil health. On this account key species of the soil macrofauna (*Lumbricus terrestris*, earthworms), mesofauna (*Folsomia candida*, collembolans) and microfauna (*Aphelenchoides saprophilus*, nematodes) were introduced into meso- and microcosms.

Within the macrofauna, earthworms are one of the most important taxa (as elucidated in 1.2.1). The anecic species *L. terrestris* is a peregrine species with Palaearctic origin and worldwide distribution (Lehmitz et al., 2014). It was chosen, because it is widely distributed in Germany (Rutgers et al., 2016) and occurring mostly in arable soils and grassland with low soil organic matter content (Jänsch et al., 2013; Lehmitz et al., 2014). Furthermore, *L. terrestris* showed a clear preference for *Fusarium* species when offered different fungal pathogens as food source (Bonkowski et al., 2000).

Belonging to the mesofauna, collembolans represent an important component in soil ecosystems (see chapter 1.2.2). The collembolan species *F. candida* was chosen for the

present thesis, because it is a well-known model arthropod which is widespread and occurs in arable soils (Fountain and Hopkin, 2005). As most collembolans feed on fungal hyphae, *F. candida* is no exception (Fountain and Hopkin, 2005) and show even preferences for distinct fungal pathogen species like *Fusarium culmorum* (Larsen et al., 2008).

As members of the microfauna, nematodes are important regulators of the soil food web (see chapter 1.2.3). Arable soils usually housing several species of fungivorous nematodes (Freckman and Caswell, 1985). Furthermore, many studies were conducted investigating the ability of fungivorous nematodes to control pathogenic fungi (Hasna et al., 2008; Roessner and Urland, 1983). As the genus *Aphelenchoides* comprising predominantly fungal feeders, the fungivorous species *A. saprophilus* was chosen for the present thesis.

To determine the *Fusarium* biomass and the DON concentration at the beginning and the end of each experiment, Enzyme-linked immunosorbent assays (ELISAs) tests were applied. This method has become one of the most useful tools for rapid monitoring of mycotoxins. The great advantages of ELISAs are speed, ease of operation, sensitivity, and high sample throughput (Krska and Molonelli, 2007). A detailed description of the procedure is given by Oldenburg et al. (2008) and Schrader et al. (2009).

As there is still a gap of knowledge about the significance of species-interaction between 3 different members (earthworms, collembolans and nematodes) of the soil food web concerning biological control of plant pathogens and mycotoxin regulation, this thesis contribute to the understanding of the linkage between *L. terrestris*, *F. candida* and *A. saprophilus* and their potential role in providing ecosystem services like plant pathogen control and mycotoxin degradation in an agroecosystem.

The main hypothesis of the thesis:

The introduced soil fauna contribute in an interactive manner to the biological control of *Fusarium culmorum* and the regulation of its mycotoxin deoxynivalenol (DON) in residual wheat straw on the soil surface.

Chapter 3 outlines a study under laboratory conditions based on the minicontainer-system (Eisenbeis et al. 1995; 1999) and assessed the degradation of both, *Fusarium* biomass and

DON concentration in finely grounded wheat straw by collembolans (*F. candida*) and nematodes (*A. saprophilus*). Furthermore, soil fauna and wheat straw were exposed to 3 different types of soil texture within the minicontainers. This study focused on the hypotheses: (1) nematodes and collembolans reduce the biomass of the soil-borne phytopathogenic fungus *F. culmorum* and the content of its mycotoxin deoxynivalenol (DON) in infected wheat straw; (2) the species interaction of *A. saprophilus* and *F. candida* enhances the degradation of *Fusarium* biomass and DON concentration in wheat straw; (3) the degradation efficiency of nematodes and collembolans is affected by soil texture.

Chapter 4 summarizes two field experiments which were established in a cropping system under reduced tillage conditions: A minicontainer-study, where collembolans and nematodes were exposed to DON-contaminated wheat straw. In a second investigation mesocosm-based experiments were conducted in two different years to determine the interaction-effect of 3 soil fauna species (*L. terrestris, F. candida* and *A. saprophilus*) on the reduction of DON concentration in residual wheat straw. The hypothesis was: (1) the introduced soil fauna and their interaction (collembolans and nematodes; earthworms, collembolans and nematodes) contribute significantly to the regulation of the mycotoxin deoxynivalenol (DON) in contaminated wheat straw under reduced tillage conditions.

Chapter 5 focuses on unpublished results of *Fusarium* biomass of the two field studies described in chapter 4. A minicontainer-study, where collembolans and nematodes were exposed to *Fusarium*-infected wheat straw and a second study based on mesocosms where the interaction of 3 soil fauna species (*L. terrestris, F. candida* and *A. saprophilus*) was investigated concerning their degradation capacity on *Fusarium culmorum* in artificially infected wheat straw on the soil surface. The hypotheses were: (1) the anecic earthworm species *L. terrestris* contribute to the control of the plant pathogenic fungus *Fusarium culmorum* in infected wheat straw in a reduced tillage cropping system; (2) *F. candida* and *A. saprophilus* reduce the biomass of the soil-borne plant pathogenic fungus *Fusarium culmorum* in infected wheat straw under field conditions; (3) the degradation efficiency of *L. terrestris* is affected in the presence of collembolans and nematodes.

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Chapter 2

2 Synthesis and conclusion

The results of the present study revealed that the introduced soil fauna (earthworms, collembolans and nematodes) contribute to the degradation of *Fusarium culmorum* and its mycotoxin DON in wheat straw. The contents of FPE and DON were significantly reduced in presence of the soil fauna. Therefore, the overall hypothesis (chapter 1.5) of the present thesis can be confirmed. To what extent the reduction took place in an interactive manner of *L. terrestris*, *F. candida* and *A. saprophilus* and whether the degradation was influenced by other factors such as soil texture is discussed below.

2.1 Degradation capacity of collembolans and nematodes

It is well documented that fungivorous collembolans and nematodes reduce plant pathogenic fungi (Hasna et al., 2007; 2008; Lagerlöf, 2011; Lartey, 2006). The plant pathogenic fungi *F. culmorum*, for example, turned out to be an adequate food source for collembolans, particularly for *F. candida* (Larsen et al., 2008; Sabatini and Innocenti, 2000). In the case of nematodes, the fungivorous species *Aphelenchus avenae* has been proved to decrease severity of several fungal-induced diseases including corky root disease of tomato (Hasna et al., 2008) or *Fusarium* wilt in cotton (Karuri et al., 2014). However, results of the investigation presented in chapter 3 revealed no significant fauna effects on the degradation of *Fusarium* biomass in infected wheat straw under controlled laboratory conditions. The content of fungal biomass decreased to a similar degree in all treatments including the non-faunal control treatment (Fig. 3.1.1 and Fig. 3.1.2). Although the genus *Aphelenchoides* was tested to control fungal plant diseases (Friberg et al., 2005), it turned out to be unable to reduce the fungal pathogen *Pyrenochaeta lycopersici* in experiments of Hasna et al. (2008). The authors supposed that, *Aphelenchoides* spp. can change their food preferences temporarily when alternatives are available, possibly because to avoid toxic compounds in the food source (Hasna et al., 2008; Ruess et al., 2000). This, however, is suggested to be rather unlikely for the present study because the minicontainer field study of chapter 5 ended up with different findings: At the end of the experiment (after 4 weeks), faunal effects on *Fusarium* degradation of *F. candida* and *A. saprophilus* were detected, where collembolans and nematodes were introduced in single culture (Fig. 5.1.1). During the field experiment (chapter 5), the minicontainers were placed in close contact with the surrounding soil, which enabled an

exchange with air, water, and the soil-inhabiting microfauna- and flora close to a real agroecosystem. This might have been resulted in more favourable conditions in the field experiment for the introduced species *F. candida* and *A. saprophilus*, respectively. It also might create a more favourable microclimate for suppressive microfauna- and flora. Furthermore, *A. saprophilus* seemed to benefit from the *Fusarium*-infected substrate. Results of the lab-study (chapter 3) as well as of the field experiment (chapter 5) showed a trend towards higher individual numbers of nematodes in treatments containing *Fusarium*-infected and DON-contaminated wheat straw. In contrast, the population density of *F. candida* was not affected by the fungal infection but rather by the time span of the experiment. Under controlled laboratory conditions, the introduced soil fauna had no effect on the degradation of *Fusarium culmorum* in infected wheat straw, whereas in the field experiment, very close to real conditions, *F. candida* and *A. saprophilus* significantly promote the reduction of *Fusarium* biomass. Interaction effects of the introduced collembolans and nematodes could not be detected in the case of *Fusarium* degradation. The underlying mechanism of suppression cannot clearly be defined, but it likely involves the activity of antagonistic soil microorganisms (see Lucas, 2011). It is assumed, that mainly microbial activity (in the case of the lab-study) and interaction between inoculated soil fauna and microorganisms (in the case of the field-study) caused most of the *Fusarium* degradation.

With regard to mycotoxin degradation, the present thesis revealed significant fauna effects on the reduction of DON. The findings of chapter 3 strongly suggest, that the degradation of DON is not only related to the effect of one single member of the soil food web, as the concentration of DON was lowest in treatments containing both, collembolans and nematodes (Fig. 3.1.2). The interaction of *F. candida* and *A. saprophilus* obviously enhanced the degradation of DON in contaminated wheat straw under laboratory conditions (see chapter 3). In contrast, the results of the study presented in chapter 4.1 revealed single fauna effects of *F. candida* and *A. saprophilus*: The concentrations of DON were reduced most efficiently in treatments where *F. candida* or *A. saprophilus* were introduced in single culture (Fig. 4.1.2). As mentioned above, there is a considerable amount of reports concerning repression of fungal plant pathogens by soil fauna. But evidences for the degradation of mycotoxins are scarce up to now. In the case of collembolans, grazing of *F. candida* on mycelia of the fungus *F. graminearum* was recently reported to decrease the production of DON, 15-acetyl-DON and 3-actyl-DON (Xu et al., 2015). Since many secondary metabolites of fungi (such as mycotoxins) are potentially toxic to antagonistic arthropods (Rohlfs and Churchill, 2011), it is

concluded by Xu et al. (2015) that collembolans might have evolved mechanisms for detoxification of fungal toxins as self-protection. These mechanisms may rely on enzymatic activities or on detoxification processes provided by symbiotic microbes inhabiting the digestion track of collembolans (Karlovsky, 1999). Some of these microorganisms proved to be responsible for fungal growth inhibition or even showed antifungal effects (Xu et al., 2015). It is suggested that this mechanism of detoxification was involved in the degradation processes which took place in the investigations of the present thesis.

2.2 Degradation capacity of earthworms

The lower concentrations of *Fusarium* biomass (Fig. 5.1.4 and Fig. 5.1.5) (see chapter 5) and DON (Fig. 4.2.2) (see chapter 4.2) in the presence of earthworms may result from several mechanisms.

Firstly, soil fungi are regarded as a primary food source for earthworms (Brown, 1995), because they have the ability to accumulate high amounts of nitrogen and phosphorous, thus providing an adequate source of nutrients (Ruess and Lussenhop, 2005). Furthermore, there is clear evidence that earthworms do not feed at random (Bonkowski et al., 2000). Food selection studies with *L. terrestris* revealed distinct preferences for *Fusarium* spp. (Bonkowski et al., 2000; Cooke, 1983; Moody et al., 1995). Confirming these reports, *L. terrestris* preferred the *Fusarium*-infected and DON-contaminated wheat straw (see chapter 5) as the incorporation was faster in mesocosms where the infected straw was offered (Fig. 5.1.2 and 5.1.3). Similar results have been reported by Oldenburg et al. (2008) and Wolfarth et al. (2011).

Secondly, earthworms play a major role in acceleration of decomposition of plant material (Brown et al., 2000). Thus, it is supposed that *L. terrestris* reduced *Fusarium* biomass and DON concentration in wheat straw directly by litter decomposition and by incorporating infected plant material into the soil. Bertrand et al. (2015) also suggested the feeding activity of anecic earthworms to be responsible for pathogen repression because infected crop residues were buried into the soil and prevent fungal spread and plant infection. In temperate regions, earthworms indeed ingest large amounts of material (2-15% of organic matter inputs) (Whalen and Parmelee, 2000).

Thirdly, earthworms expend much energy in their modification of the soil (Petersen and Luxton, 1982). This modifying of soil structure by earthworms may limit water stagnation (Blouin et al., 2007), which favour fungal dissemination. Earthworms are also known as

typical ecosystem engineers (Lavelle et al., 1997). By active burrowing and mixing of soil and organic matter, earthworms have major impacts on soil conditions and other soil biota (Wurst et al., 2012). Kuzyakov and Blagodatskaya (2015) emphasise that the most ecologically relevant biochemical processes in soil are microbial mediated. Hence, the suppression of plant pathogens and mycotoxins was probably also caused indirectly by metabolic interactions between earthworms and soil microorganisms. By its casting activity and litter collection, anecic earthworms create structures at the soil surface known as "earthworm middens" (Brown et al., 2000; Subler and Kirsch, 1998), which provide improved conditions of microclimate and habitat structure (Brown et al., 2000; Schrader and Seibel, 2001). The earthworm middens are hotspots of higher microbial activity and diversity as well as increased abundance of soil mesofauna and nutrient availability (Subler and Kirsch, 1998). Ecologically relevant biochemical processes mainly occur in the small volume of such soil hotspots, which are characterised by much faster process rates and much more intensive interactions compared to the surrounding soil (Kuzyakov and Blagodatskaya, 2015). Earthworm middens also contain the binding agent calcium humate (Edwards, 1998) that reduces dehydration of earthworm casts and favours the proliferation of beneficial organisms, such as *Pseudomonas* spp. (Schmidt et al., 1997). Furthermore, Elmer (2009) found that earthworms increase population densities of fluorescent pseudomonads and filamentous actinomycetes which have been implicated in disease suppression (Mazurier et al. 2009; Lemanceau and Alabouvette 1993).

Besides direct feeding of *L. terrestris* on infected crop residues, the providing of improved conditions for beneficial microorganisms by creating hotspots of enhanced microbial activity and diversity seems to be a determining factor which promotes the reduction of *Fusarium* biomass and DON concentration.

2.3 Degradation capacity of soil fauna as a function of soil texture

Not only the introduced soil fauna revealed statistical effects in reducing DON concentration, but also the given soil texture provided an environment which significantly influenced the degradation of the mycotoxin in contaminated wheat straw, as the results clearly showed. As shown in chapter 3, soil texture is obviously an important factor for controlling the conditions of soil fauna in degradation processes. According to Dignam et al. (2016), disease suppressive properties of the soils are a function of the activity of soil microorganisms, and directly related to the amount of microbial activity at a time critical to the pathogen (Janvier et al.,

2007). Suppressive soils possess a community of microorganisms and microfauna that act together to reduce or repress pathogenic organisms (EIP-AGRI Focus Group, 2015; Wahlen and Sampedro, 2010). In these soils a large, active microbial community and associated soil fauna compete for energy, nutrients and other substances required for the pathogen. Soil microorganisms may occupy favourable habitats and thus inhibit pathogens (Wahlen and Sampedro, 2010). Soils with high fertility and high levels of organic matter are regarded to have a great potential of natural biocontrol of pathogens (Janvier et al., 2007). The more microbes are active, the greater is the chance that some of them will be antagonistic to pathogens (Altieri, 1999). Consequently, enhancing functional biodiversity in agroecosystems is important ecological strategy for sustainable crop production (Altieri, 1999).

2.4 Degradation of *Fusarium* biomass and DON concentration in soil

In the lab investigation of chapter 3, *Fusarium* biomass in soil was determined in concentrations between 10 and 20 µg kg^{-1}. These concentrations, however, are very low and can be neglected. The field investigations of chapter 5 revealed no *Fusarium* biomass in soil.

Regarding DON concentrations in soil, only in investigations with minicontainers (see chapter 3 and 4.1) DON could be detected. At the beginning of both experiments, lab-study (chapter 3) and field-study (chapter 4.1), respectively, concentrations of DON in soil were below the quantification limit (<37 µg kg^{-1}). But after 2 weeks, in both examinations, DON was found in the soil of all treatments, containing contaminated wheat straw (Fig. 3.1.3 and Fig. 4.1.2). The lab-study of chapter 3 revealed significant effects of soil texture as the DON content in clay loam was significantly higher than the DON concentrations of sandy loam and silt loam (Fig. 3.1.3 and Fig. 3.1.4). Whether DON was leached from the contaminated straw into the surrounding soil was not analysed but could be hypothesised for both studies (chapter 3 and 4.1) of the present thesis. An adsorption of DON on soil particles is thought to be unlikely as efficient binding of DON to natural and modified clay minerals or humic substances has not been detected so far (Döll and Dänicke, 2004; Sabater-Vilar et al. 2007). Furthermore, Schrader et al. (2009) reported recovery rates of DON dosed to the soil of their microcosms to be slightly exceeded 100%, thus excluding binding effects of DON on soil particles. After 4 weeks the mycotoxin was reduced significantly in all minicontainers of both investigations. In contrast to the investigation of chapter 3 where the factor “time” seemed to be crucial for the reduction of DON in soil (Table 3.1.1), results of the field investigation of chapter 4.1 indicate

a decontamination effect of both introduced soil fauna species (*F. candida, A. saprophilus*) (Fig. 4.1.2). In general, a biological degradation of DON in nature is suggested by Völkl et al. (2004), because no accumulation could be observed in agricultural soils. And no accumulation has been found in the present thesis as the DON concentrations were reduced after the experimental time span (chapter 3 and 4.1) or were below the quantification limit (37 µg kg^{-1}) (chapter 4.2). Again, the underlying mechanism was not addressed in this thesis and can only be hypothesised. Since fungal toxins are abundant in nature, microorganisms that catabolise these metabolites can reasonably be expected and detoxification mechanisms for fungal toxins are likely to be found in nature (Karlovsky, 1999). As already been suggested for the reduction of DON in wheat straw, the introduced soil fauna possibly promoted the activity of suppressive microorganisms.

Moreover, DON-degrading activity in soil extracts was reviewed by Karlovsky (2011). The soil bacterium E3-39, which was assigned to the Agrobacterium-Rhizobium group, completely removed DON from the medium after converting it to the intermediate 3-oxo-deoxynivalenol (Shima et al. 1997). A strain of the bacterium *Nocardioides* sp. (Ikunaga et al. 2011) completely removed DON from the medium, producing 3-epi-DON as an intermediate. This strain is currently the only available organism that is able to completely catabolise DON. Furthermore, fungi have often been reported to transform DON and other trichothecenes. Mycotoxin-producing fungi are often able to degrade and possibly use them as a source of energy under starvation (Karlovsky, 1999). For instance, DON was modified by *Fusarium nivale* (Karlovsky, 1999) and the conversion of DON by *Aspergillus tubingensis* was reported by He et al. (2009).

2.5 Interaction effect of the introduced soil fauna and the provision of ecosystem services

Soil inhabitants provide an enormous potential for natural suppression of infection, disease incidence and severity. There is good evidence in literature that biological control of pollutants is not based on single effects but on reciprocal effects of different soil organisms which are already inhabitants in soils (Sabatini and Innocenti, 2001). The key lies in the understanding of the soil as an ecosystem and interactions among the potential antagonists and the pathogens (Lartey, 2006). Although results of the present thesis revealed significant effects of *L. terrestris, F. candida and A. saprophilus* on the degradation of *Fusarium culmorum* (chapter 3 and 5) and its mycotoxin DON (chapter 3, 4.1 and 4.2), the results

regarding the interaction among the introduced soil fauna species showed a rather inconsistent cluster. Under laboratory conditions (see chapter 3) the interaction between *F. candida* and *A. saprophilus* was the determining factor regarding the mycotoxin degradation, whereas under field conditions the collembolan-nematode-interaction appeared not to be decisive for the reduction of *Fusarium* biomass (chapter 5) and DON (chapter 4.1), respectively. Moreover, in presence of the anecic earthworm species *L. terrestris*, the contribution of the soil meso- and microfauna (*F. candida, A. saprophilus*) considering fungal degradation (chapter 5) and mycotoxin reduction (chapter 4.2) in infected and contaminated crop residues seemed to be minor. The investigation presented in chapter 4.2 and chapter 5 pointed out clearly that *L. terrestris*, as an anecic detritivorous earthworm species has been the driver of the degradation process in reducing *Fusarium* biomass (chapter 5) and DON (chapter 4.2) in a cropping system with reduced tillage conditions. Only in 2011, differences between the faunal treatments on the degradation of *Fusarium* biomass (Fig. 5.1.4) after 4 weeks were calculated. Leading to the careful assumption that *F. candida* might have influenced the degradation performance of *L. terrestris* more positive than *A. saprophilus*. But this result should be considered with caution, as after 8 weeks the difference disappeared. Furthermore, it could not be verified in 2013 (Fig. 5.1.5). However, Wurst et al. (2012) emphasised, that across different size classes the composition of community and the traits of key species appear to be significant contributors of soil processes and function. But there is still a long way to go to understand the innumerable interactions between different components of the soil micro flora, and the specific factors regulating soil populations, including pathogens (Lucas, 2011).

As soils are hosting an enormous biodiversity, soils are recognised to be the major suppliers of critical ecosystem services (Breure et al. 2012). Soil health is defined as the capacity of soil to function as a vital dynamic system (Doran and Zeiss 2000). In this dynamic system, soil organisms like earthworms and, to a lesser extent, collembolans and nematodes provide supporting services like nutrient cycling, water cycling, nitrogen fixation, soil formation, and soil biological activity (MEA, 2005). Fig. **2.5.1** demonstrates how nematodes, collembolans and earthworms are incorporated in the circle of ecosystem services and disservices. While the disservice of fungal infection of cultivated crops is occurring aboveground, soil fauna provide their ecosystem services (supporting services and regulating services) belowground (Fig. 2.5.1). By the provision of regulating services including the repression of plant pathogens and the degradation of contaminants (Fig. 2.5.1), nematode, collembolans and earthworms play an important role in the regulation of fungal plant pathogens like *Fusarium* species and their mycotoxins like DON, thus reducing the risk for plant diseases and

environmental contaminants. Furthermore, nematodes, collembolans and earthworms significantly contribute to the preservation of soil health.

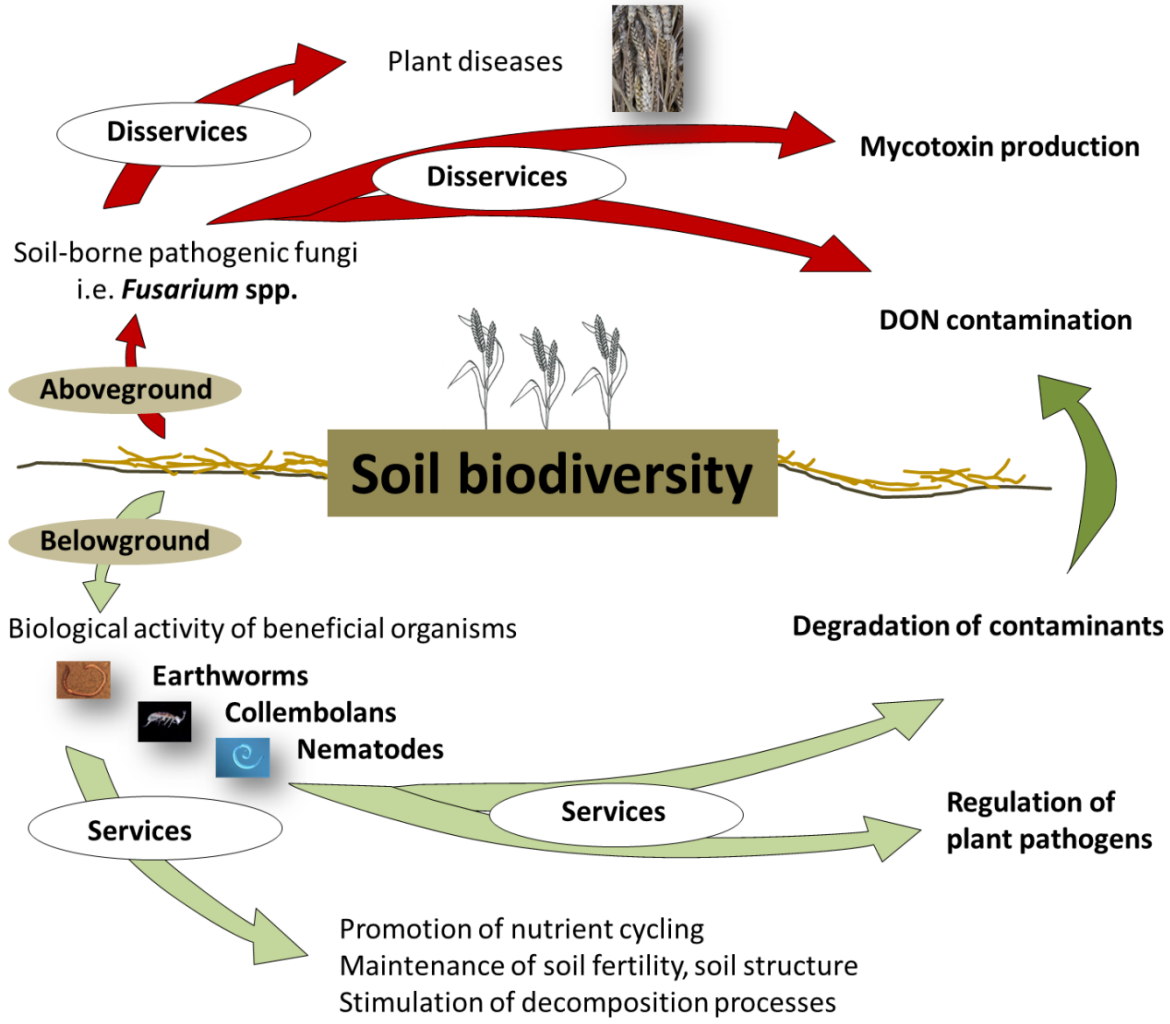

Fig. 2.5.1: Selected ecosystem services (regulating services) and disservices (infection of plants by fungal pathogens and toxin production) provided by soil organisms.

2.6 Conclusion and perspectives

The degradation performance of the introduced soil fauna of the present thesis must be considered as an important contribution to the biological control of plant pathogens (here: Fusarium culmorum) and environmental pollutants (here: deoxynivalenol).

2.6.1 Pathogen control and toxin reduction in agroecosystems

The biocontrol capacity of fungivorous earthworms, collembolans and nematodes is well-documented. But in the case of collembolans and nematodes, they have so far not been

extensively used in practical agriculture or horticulture in the form of applications or of systematic creation of favourable conditions for beneficial organisms. This is surprising, since for example, the used nematodes are easy to propagate in large numbers and can be stored in a dormant stage. They could be distributed to growers in this form and applied in the field, especially in the establishment phase of high-value crops (Ishibashi et al., 2000; Ishibashi, 2005). However, a key goal should be to define and encourage agricultural practices that increase abundance and diversity of soil organisms by enhancing habitat conditions or resource availability (Thiele-Bruhn et al. 2012). Diversified landscapes hold most potential for the conservation of biodiversity and sustaining the pest control function (Bianchi et al., 2006; Chaplin-Kramer and Kremen, 2012). Establishing a fine mosaic of crop and non-crop areas such as hedgerows, tree lines and small semi-natural habitat patches is recommended by Tamburini et al. (2016) to improve biological control. Furthermore, the authors emphasise the importance of considering both local management as well as landscape composition when planning strategies for maximizing biological control services in agro-ecosystems.

High species diversity combined with high species abundance within functional classes facilitates exploitation of resources, and greater contribution of organisms to ecosystem services (Ferris and Tuomisto (2015). Consequently, diversity of species within functional classes is an essential element of the biological component of soil health (Ferris and Tuomisto, 2015). Biodiversification can result in pest regulation, through restoration of natural control of pests and also produces optimal nutrient cycling and soil conservation by activating soil biota, which is all leading to sustainable yields, energy conservation and less dependence on external inputs (Altieri, 1999). Sustainable land use practices like conservation agriculture is linked to the conservation of soil biological diversity which is a precondition for ecosystem stability against external disturbances and the provision of ecosystem services and functions (Thiele-Bruhn et al. 2012).

Earthworms, for instance, represent an excellent potential partner for humans in managing ecosystem services (Blouin et al., 2013). Since earthworms live within agroecosystems, farming practices exert a top-down effect on earthworm populations. Earthworm species richness, the size and structure of earthworm populations (number of individuals, ratio of juveniles/adults), and their biomass depend on crop management (Bertrand et al., 2015b). Edwards and Lofty (1982) for example, observed an increase of earthworm populations up to 30% after 8 years of conservation tillage. And there are many other reports of raising earthworm populations after converting from conventional to conservation tillage (Edwards,

1983, Edwards et al., 1995, Langmaack, 1999, Joschko and Rogasik, 2002). To design crop management systems that consider the value of earthworms explicitly, it is necessary to assess the earthworm-mediated ecological services that occur in cropped fields and to evaluate the effects of agricultural practices on earthworm diversity, abundance, and activity (Bertrand et al., 2015b). Against this background, earthworm augmentation has been considered, but it revealed to be too expensive (2500-7000 $ per ha) as calculated by Elmer and Ferrandino, 2009). A better alternative should be to create improved conditions in the field that earthworm numbers can increase naturally. Bertrand et al. (2015b) conclude it is not an easy task to increase size of earthworm population in the field. But various strategies can be adopted (Bertrand et al., 2015a) depending on the objectives of the farmers. Soil management, however, plays the key role in the design of sustainable cropping systems (Roger-Estrade et al., 2010). Organic residue management, prevention of compaction, crop rotation and the timing of cultivation, must be considered together, with an assessment of their impact on pests and their natural enemies and on ecosystem engineers (Roger-Estrade et al., 2010).

Figure 2.6.1 is demonstrating how the control of fungal pathogens like *Fusarium* species and their mycotoxins is managed by farmers in interaction with the soil-inhabiting organisms in agroecosystems. Combating measures of farmers and the degrading activities of soil fauna may result in synergistic effects to control pathogenic fungi as well as their mycotoxins and improve (or at least maintain) quality and quantity of crop products and crop residues. Thus, farmers and soil fauna are benefiting from each other because their activities promote soil health (Fig. 2.6.1).

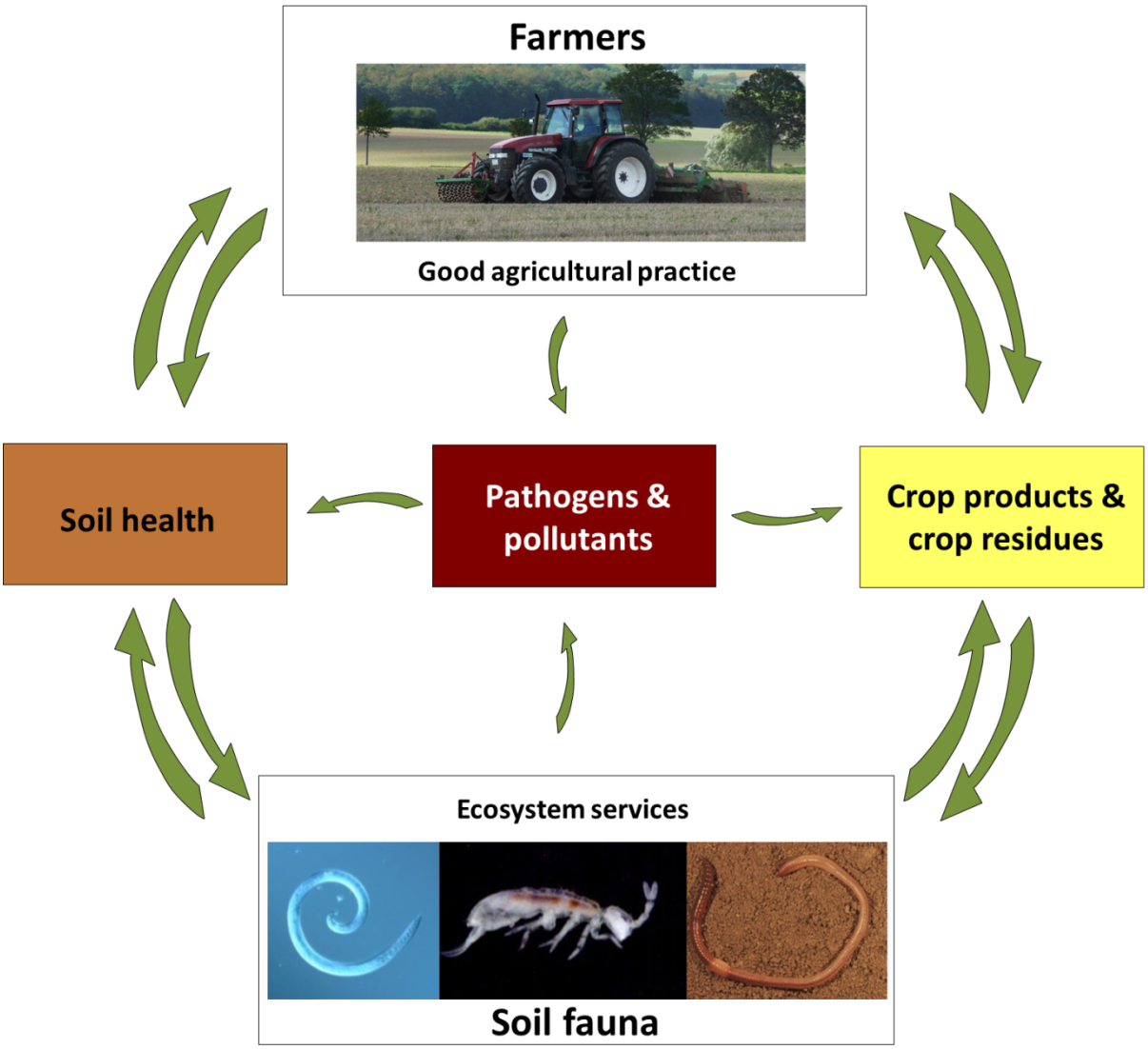

Fig. 2.6.1: Synergistic effects of good agricultural practices by famers and the provision of ecosystem services by soil fauna in controlling plant pathogens and pollutants in agroecosystems.

Through the combination of good agricultural practice by farmers (Busch et al., 2015) and the provision of ecosystem services by soil fauna an effective control of pathogens and pollutants can be achieved (Fig. 2.6.1). Furthermore, the natural mechanism of self-regulation in the soil system is promoted by farmer´s residue management. Good agricultural practices include measures such as technical residue management, sustainable crop rotation, less susceptible cultivars and sustainable fungicide application (Busch et al., 2015).

For a long time, broad spectrum chemicals have been widely used to control soil-borne pathogens (Janvier et al., 2007) and most farmers still manage their crops with high level of chemical inputs (Nave et al., 2013). But most of the products are not specific and destroy the

whole microflora, pathogenic or not (Janvier et al., 2007). Furthermore, chemical control has resulted in the occurrence of resistance to fungicides of major plant pathogenic fungal populations (Bardin et al., 2015). Moreover, despite the steady increase in chemical pesticide use over the last 50 years, estimated crop losses to pests have also significantly increased (Oerke 2006).Thus, there is rising pressure to reduce chemical inputs and the carbon footprint of intensive agriculture (Lucas, 2011). In particular, the options for control *Fusarium*-related diseases are limited and difficult to implement. Chemical control measures, for example have been turned out as unsuccessful for *Fusarium* diseases and very few fungicides are available and economically effective (Leslie and Summerell, 2013).

Therefore, alternative methods to chemical control of fungal plant diseases are necessary to find. For example measures of biological control and its benefits for human health and the environment have resulted in increased attention in controlling crop diseases, (Hasna et al., 2008) and there should be a continued progress in the discovery and utilization of biological agents especially in protected cultivation of horticultural crops, and smaller-scale, low-input cropping systems (Lucas, 2011).

Besides the complex of problems with *Fusarium*-related diseases and mycotoxin contamination of crops and crop products occurring nowadays, another factor affecting fungal colonisation and mycotoxin contamination that must be considered is the changing of the global climate in near future (Paterson and Lima, 2010; 2011). Increased CO_2 levels, higher global temperature, altered precipitation regimes and increases in the frequency of extreme weather events will influence the interaction between crops and pathogens in a multiple way (Juroszek and von Tiedemann, 2011). In general, more favourable conditions for mycotoxigenic pathogen survival and colonization are expected (Luck et al., 2011). The future climate, with predicted increasing temperature and higher precipitation rates in winter may change the dynamics of residue composition and microbial activity in soil. Mild winter conditions can increase the pathogen colonisation in particular of *F. culmorum* on crop residues (Lukas et al. 2014). Considering these aspects increases concerns about assuring agriculture production at a sufficient level. Changes in precipitation and warming will affect the soil food web directly and will also influence soil organisms indirectly by altering plant growth, structure and physiology (Pritchard, 2011). The influence of climate changes on natural or managed soil processes, including disease pressure, is likely to be inversely related to species diversity present within successive trophic levels. Effects of environmental changes will also vary depending upon whether a given soil food web is regulated mainly top down or

bottom up (Tylianakis et al., 2008). Furthermore, the impact of human activities and the role of the changing climate and land use on soils coupled with changing consumer demands over time changes soil physico-chemical processes and lead to a different set of ecosystem functions and services (Adhikari and Hartemink, 2016).

2.6.2 Perspectives on investigation of biological control in agroecosystems

The present thesis revealed clearly the potential of members of the soil macrofauna (*L. terrestris*), the soil mesofauna (*F. candida*) and the soil microfauna *(A. saprophilus*) in reducing *Fusarium* biomass and DON concentration of infected and contaminated wheat straw. Nevertheless, the underlying mechanisms still remain unexplored. Furthermore, the microorganisms (antagonistic bacteria, fungi, etc.) which are involved in the degradation process were not identified. Further studies should focus on the detoxification mechanisms and discover the involved microorganisms, as good knowledge of the dynamics of the cropland soil fauna community structure and the interactions among them is essential to design a successful biological control strategy (Lartey, 2006). In addition, investigations under practical plant production conditions should also be considered studying the effects of the natural inhabiting fungivorous soil fauna in an agricultural field after a *Fusarium*-infection of the cultivated crop.

Based on the EU initiative to produce 20% of primary energy supply by renewable resources by the year 2020, maize (*Zea mays*) is the leading energy crop and thus increasingly cultivated throughout Europe (FNR, 2014). In the last 15 years the percentage of arable land used to grow maize has increased by almost 50% in Germany (Federal Statistical Office 2010), which accounts for approximately 10% of the whole arable area and is expected to increase up to approximately 30% in the future (Gutzler et al. 2015). This trend is leading towards mono-cultivation of silage maize (Kruska and Emmerling, 2008) as well as less diverse and shorter crop rotations (Karlen et al. 1994) which increases diseases risks induced by soil and straw borne pathogens like *Fusarium* species. Against this background, studies on plant pathogen repression and mycotoxin regulation on maize stubbles or maize residues by earthworms and other fungivorous soil fauna should be taken into account in the near future.

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Chapter 3

3 Soil matters – How ecosystem services of soil organisms are affected by soil texture

3.1 Nematode-collembolan-interaction promotes the degradation of *Fusarium* biomass and deoxynivalenol according to soil texture

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Abstract

Despite well-known positive aspects of conservation tillage combined with mulching on arable fields, one drawback may be the survival of phytopathogenic fungi on plant residues. Therefore, plant pathogen repression is an important ecosystem service to prevent cultivated plants from fungal diseases and mycotoxin contamination. A microcosm-study was conducted under constant laboratory conditions to assess the impact of soil microfauna (*Aphelenchoides saprophilus,* Nematoda) and soil mesofauna (*Folsomia candida,* Collembola) on soil-borne phytopathogenic fungi (*Fusarium culmorum*) and its mycotoxin deoxynivalenol (DON). Our hypotheses were: (1) nematodes and collembolans reduce the biomass of *F. culmorum* and the content of DON in infected wheat straw; (2) the species interaction of *A. saprophilus* and *F. candida* enhances the degradation of *Fusarium* biomass and DON concentration in wheat straw; (3) the degradation efficiency of nematodes and collembolans is affected by soil texture. Therefore, microcosms were filled with soil of different texture and finely chopped wheat straw (*Fusarium*-infected *vs.* non-infected). The microcosms were inoculated with the two species in different combinations (single and mixed species, non-faunal control). After 2 and 4 weeks of incubation, the individual densities in all soil faunal treatments increased with highest individual numbers in the non-infected treatments in case of collembolans and in the infected treatments in case of nematodes. The *Fusarium* biomass (*Fusarium* protein equivalents = FPE) of all infected treatments decreased by at least one order of magnitude after 2 weeks. At the end of 4 weeks *Fusarium*-biomass was reduced by 93% in sandy and silt loam and 89% in clay loam mostly in mixed species treatments. Also DON concentrations were reduced significantly compared to the initial concentration in all treatments after 4 weeks. The highest reduction was found in mixed species treatments, where DON was degraded by 92%, 95% and 39% for sandy loam, silt loam and clay loam, respectively. We concluded that particularly interacting collembolans and nematodes play an important role in plant pathogen repression and mycotoxin degradation. In any case, soil texture matters in the provision of these ecosystem services by collembolans and nematodes.

Keywords: Plant pathogen repression; Mycotoxin degradation; Ecosystem services; Functional soil biodiversity; Conservation tillage; Wheat straw; minicontainer

Introduction

Conservation tillage has been introduced worldwide to assure sustainable agricultural production (Holland, 2004; Kassam et al., 2009). In combination with organic amendment techniques like mulching, conservation tillage promotes soil biodiversity (Hobbs, 2007; van Capelle et al., 2012) and provides benefits to ecosystem services like decomposition of organic matter (Derpsch et al., 2010; Kassam et al., 2009). However, disservices are likely to increase through the survival of mycotoxin producing plant pathogenic fungi like *Fusarium* species on plant debris (Champeil et al., 2004). They can possibly endanger the health of the following crop by increasing the infection risk for specific plant diseases like Fusarium head blight (Pereyra et al., 2004; Pereyra and Dill-Macky, 2008). This is a widespread fungal disease in small grain cereals of global importance (Champeil et al., 2004; Mudge et al., 2006). Fusarium head blight is frequently caused by *Fusarium graminearum, Fusarium culmorum* and *Fusarium avenaceum* (Parry et al., 1995). *F. culmorum* is often predominant in wheat fields of temperate regions (Wagacha and Muthomi, 2007). The trichothecene mycotoxin deoxynivalenol (DON) is one of the most common toxins produced by *F. culmorum* and is thus frequently detected in cereals (Curtui et al., 2005; Pestka, 2007). *Fusarium* infection and 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 livestock and humans (Bennett and Klich, 2003; Rotter et al., 1996). An effective stimulation of decomposition is essential to reduce the infection risk in arable land (Stemann and Lütke-Entrup, 2005). Fungi and bacteria are responsible for most of the organic matter breakdown. Both microorganism taxa are greatly influenced by protozoans, nematodes, annelids and arthropods through their feeding activities (Seastedt, 1984). More recent studies revealed earthworms to be important actors in arable soil by degrading *Fusarium* biomass and DON from infected wheat straw (Oldenburg et al., 2008; Schrader et al., 2009; Wolfarth et al., 2011a,b). Collembolans showed a selective behaviour in their food choice and a significant preference for some plant pathogenic fungi in laboratory studies (Klironomos et al., 1992; Lartey et al., 1994). Sabatini and Innocenti (2000) reported highest numbers and best fecundity for the collembolan species *Mesaphorura krausbaueri* when fed on *F. culmorum*. Larsen et al. (2008) confirmed these findings by demonstrating that *F. culmorum* is a palatable food source for the collembolan species *Folsomia candida* and *Folsomia fimetaria*. Due to their notable contribution to decomposition processes in agroecosystems and their influence on fungal succession and the altering of soil saprotrophic fungal communities through grazing, collembolans are considered to play an important role in biological control of fungal

plant pathogens (Klironomos et al., 1992; Klironomos and Kendrick, 1995; Lartey et al., 1994). Nematodes are also important regulators of residue decomposition (Ruess and Ferris, 2004). Especially bacterial and fungal feeders are key components in decomposition processes and nutrient cycling, thus indirectly affecting primary production (Chen and Ferris, 2000; Ruess and Ferris, 2004). Many studies were conducted investigating the ability of fungivorous nematodes to control soil-borne plant pathogenic fungi. Roessner and Urland (1983) for example, reported the reduction of *F. culmorum* in wheat by the fungivorous nematode phelenchoides hamatus. Other investigations on pathogen repression by the fungal feeding nematode *Aphelenchus avenae* demonstrated significant results for plant pathogens like *Fusarium moniliforme* and *Pythium butleri (*Gupta, 1986), *Pyrenochaeta lycopersici* (Hasna et al., 2008), *Rhizoctonia solani* and *Fusarium oxysporum* (Okada, 2006).

Regarding biological control of plant pathogens, the interaction between soil micro- and meso-fauna has received only little attention so far, although their members are strongly linked in belowground food webs. Furthermore, the possible role of nematodes and collembolans in mycotoxin degradation has been neglected up to now. Therefore, we conducted a microcosm-study under laboratory conditions to assess the interaction between the fungivorous nematode *Aphelenchoides saprophilus* and the collembolan species *F. candida* with respect to plant pathogen repression and mycotoxin degradation. This investigation contributes to the understanding of their functional role as key components in soil food webs of agroecosystems.

Our hypotheses were: (1) nematodes and collembolans reduce the biomass of the soil-borne phytopathogenic fungus *F. culmorum* and the content of its mycotoxin deoxynivalenol (DON) in infected wheat straw; (2) the species interaction of *A. saprophilus* and *F. candida* enhances the degradation of Fusarium biomass and DON concentration in wheat straw; (3) the degradation efficiency of nematodes and collembolans is affected by soil texture.

Materials and methods

Soil

Soil of different texture used for the experiment was taken from the Ap horizon of different agricultural fields near Hildesheim in Lower Saxony, Northern Germany (9°56’E 52°00’N, 196 m a.s.l). The soils were characterised as follows: sandy loam (15% clay, 51% silt, 34% sand) with a pH of 7.1; silt loam (12% clay, 85% silt, 3% sand) with a pH of 7.3; clay loam (21% clay, 68% silt, 11% sand) with a pH of 7.4. The soils were stored at 4°C until further treatment. The soils were defaunated by freezing at 20°C for 48 h seven days before filling the

minicontainers, and thereafter thawed at room temperature for 48 h. This freezing thawing cycle was repeated twice (Poll et al., 2007), which significantly reduces the number of soil microarthropods and annelids (Huhta et al., 1989). In addition to the defaunating procedure by freezing, the soils were autoclaved in small portions at 134°C for 30 min to eliminate nematodes, nematode eggs and larvae as recommended by Trevors (1996). The soils were macroscopically cleared of organic plant residues like straw or roots and sieved (mesh size 2 mm). According to van Vliet et al. (2004) 100 g autoclaved soil was moistened with 5 ml filtered soil extract, derived from each soil prior to defaunating and autoclaving to reinoculate microbial populations. No. 42 Whatman filter paper (2.5 mm particle retention) was used for soil extract production to ensure against the introduction of undesired nematodes during microbial reinoculation. After moistening, the water content was 15.7% (w/w), 20.5% (w/w) and 20.9% (w/w) for sandy loam, silt loam and clay loam, respectively.

Straw

Winter wheat (*Triticum aestivum* cv. Ritmo) was cultivated at an experimental site of the Julius Kühn- Institute, Braunschweig (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 x 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 318.56 ± 41.36 mg kg^{-1} when the plant material was collected. Finely chopped straw of approximately 1.5 mm length was used for the experiments. 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 2.54 ± 0.42 mg kg^{-1}.

Soil fauna

The fungal feeding nematode *A. saprophilus* was obtained from mass cultures with the mycorrhizal fungus Laccaria laccata (Scop.: Fr.) Cooke. Before introducing the nematodes to *L. laccata* for cultivation, the fungus was grown on Pachlewska agar at 17°C in darkness. A detailed description of this method is given in Ruess et al. (2002). Nematodes were extracted from agar for 24 h at room temperature by the Baermann funnel method. The collembolan species *F. candida* used for the experiment originated from laboratory mass cultures (see Fountain and Hopkin, 2005). *F. candida* was reared on a mixture of moist plaster of Paris and

charcoal (9:1) and fed with brewer's yeast and carrots twice a week. Only young adults of the same age and size were introduced to the experimental system after starving for 24 h.

Experimental design

Eisenbeis minicontainers (MCs) (Eisenbeis et al., 1995; Eisenbeis et al., 1999) were used as experimental units. Each end of the cylindrically shaped MCs (16 mm long, 11 mm in diameter) was covered with nylon-gauze. A mesh size of 15 µm was chosen to enable an exchange of air and water with the surrounding soil but prevent soil fauna from escaping or immigrating. The MCs were filled with 150 mg of soil and 200 mg of finely chopped straw (see chapter *soil* and *straw*). Those amounts of substrate were recommended by Eisenbeis et al. (1995). The soil fauna was introduced into the MCs after filling. The experimental system was divided into 2 sets: one set was applied with Fusarium-infected straw and another set with non-infected straw. Those two sets were subdivided once again by receiving 3 different soil substrates (sandy loam, silt loam, clay loam). The C org content of the soil was 0.8% for sandy loam, 1.2% for silt loam and 2.2% for clay loam. Furthermore, there were 4 different faunal treatments: single collembolan treatment (15 individuals per MC); single nematode treatment (50 individuals per MC); mixed treatment with collembolans (10 individuals) and nematodes (25 individuals) and a treatment without fauna as control. Reduced numbers of collembolans and nematodes were chosen for the mixed species treatment to avoid biased results on degradation through an overpopulation of organisms compared to the single species treatments. The prepared MCs were placed horizontally in plastic jars containing the same soil like the respective MCs. Each plastic jar (5 cm high, 10 cm in diameter) received 4 MCs (below soil surface) to ensure an adequate amount of sampling material for faunal extractions (see chapter *Sampling and sample processing*) and analyses of FPE and DON (see chapter *Determination of Fusarium protein equivalents* and *Determination of deoxynivalenol*). The experiment was carried out under constant laboratory conditions over a period of 2 and 4 weeks, respectively. A total of 240 plastic jars were set up for the 2 straw treatments, 3 soil texture treatments, 4 faunal treatments and 2 time treatments considering 5 replicates per treatment. The plastic jars were distributed randomly in a climate chamber at 17°C ± (1°C) in permanent darkness. After 2 weeks, soils and MCs in the plastic jars were additionally moisturized to maintain moisture. In fact, the initial mean soil water content of 15.7% (w/w), 20.5% (w/w) and 20.9% (w/w) for sandy loam, silt loam and clay loam, respectively, decreased slightly to 13.5%, 18.6% and 18.8%, respectively, during the 4 weeks. Small quantities of soil and straw (infected and non-infected straw) were retained at the beginning

and stored at –20°C until further processing to detect the initial FPE and DON concentrations ((see chapter *Determination of Fusarium protein equivalents* and *Determination of deoxynivalenol*).

Sampling and sample processing

At the end of the experiment, soil fauna was extracted from the MCs. The collembolans were extracted for 10 days using a MacFadyen high-gradient extractor (MacFadyen, 1961). The extraction started at 20 °C with a daily increase of 5° up to 40°C from day 5 to the end. The collembolans were first collected in monoethyleneglycol, then transferred into 96% ethanol and counted. Nematodes were extracted for 3 days at room temperature by the Baermann funnel method. The remaining substrate of the MCs was removed immediately. During sampling, great care was taken to mechanically separate adhesive soil from straw. Soil and straw samples were then stored at –20°C in readiness for analytical preparation. 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 were manually homogenized with a mortar to obtain a fine powder (< 0.5 mm).

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 100 mg of wheat straw and soil were taken for the assay. The limits of quantification were 10µg FPE kg^{-1} for all samples.

Determination of deoxynivalenol

The deoxynivalenol (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 100 mg for wheat straw and soil. The limits of quantification were 37µg kg^{-1} for soil and non-infected straw and 74µg DON kg^{-1} for infected wheat straw.

Statistics

The Kolmogorov-Smirnov test checked and confirmed data for normality. Variance homogeneity was confirmed for DON concentration in wheat straw. For DON concentration in soil, FPE content (in straw and soil) and population density of soil fauna variance homogeneity was violated. In these cases, the Greenhouse Geisser correction of F-values was considered. Then, a repeated measures analysis of variance (RM-ANOVA) was carried out to compare treatment effects of soil fauna (*F. candida, A. saprophilus*, Mix), *Fusarium* infection (infected, non-infected), soil texture (sandy loam, silt loam, clay loam) and date (start as t_0 and end of the experiment as t_1 and t_2). A posteriori test (post hoc test) was implemented to determine differences among the means. According to Day and Quinn (1989), we chose a Tukey´s HSD (honestly significant difference) test for multiple comparisons within the factors "species" and "soil". All data are presented as arithmetic means + standard errors (SE). All statistical analyses were done using the software package SPSS for Windows version 18.

Results

Individual density of soil fauna

Collembolans

Regarding the individual density of collembolans, significant effects by RM-ANOVA were recorded for the factors "fauna", "*Fusarium* infection" and "date" as well as for the interaction of the factors "date x fauna", "*Fusarium* infection x fauna", "Fusarium infection x date" and "date x *Fusarium* infection x fauna". No significant effect could be measured for the factor "soil" (Table 3.1.1). The initial individual number of collembolans introduced per MC was 15 in single species treatments and 10 in mixed treatments. After 2 weeks of inoculation the mean number of collembolans increased in almost every treatment (Table 3.1.2). After 4 weeks the individual density of all collembolan treatments continued to increase (Table 3.1.2). The highest collembolan numbers of nearly 60 individuals of *F. candida* (mixed species treatments) and around 100 individuals (single species treatments) were found in the non-infected control treatments. This was a multiple of the initial number at the beginning of the experiment.

Table 3.1.1: Individual density of collembolans and nematodes, *Fusarium* protein equivalent (FPE) and deoxynivalenol (DON) content in wheat straw and soil. *F*-values of RM-ANOVA on the effects of soil fauna (collembolans: *F. candida*, nematodes: *A. saprophilus*, mixed species), *Fusarium* infection (*Fusarium*-infected wheat straw, non-infected), date (start, 2 weeks and 4 weeks) and soil texture (sandy loam, silt loam, clay loam).

	Individual density		FPE	DON	
	Collembola	Nematoda	Straw	Straw	Soil
Fauna (F)	56.291 ***	0.398	0.033	15.631 ***	0.740
Fusarium infection (I)	94.530 ***	8.442 *	428.330 ***	2026.996 ***	27.875 ***
Date (D)	181.895 ***	54.182 ***	266.515 ***	100.468 ***	16.434 **
Soil (S)	0.707	2.663	0.035	19.234 ***	13.833 **
S x F	0.241	1.531	0.055	3.267 *	0.796
D x S	1.640	1.481	0.031	12.853 ***	8.269 *
D x F	13.846 ***	0.545	0.073	6.707 ***	0.876
I x F	14.477 ***	4.215 *	0.040	16.449 ***	0.739
I x S	1.376	0.224	0.061	18.113 ***	13.101 **
I x D	97.242 ***	5.724	240.074 ***	100.705 ***	15.246 **
I x S x F	1.749	4.056 *	0.053	3.323 *	0.817
D x S x F	0.184	0.835	0.135	2.863 **	0.631
D x I x F	20.940 ***	1.277	0.141	6.872 ***	0.857
D x I x S	1.750	0.318	0.051	12.365 ***	7.845 *
D x I x S x F	1.066	3.043	0.055	2.854 **	0.586

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

Nematodes

Regarding the individual density of nematodes, RM-ANOVA revealed significant effects for the factors "*Fusarium* infection" and "date". The interaction of the factors "*Fusarium* infection x fauna" and "*Fusarium* infection x soil x fauna" also showed significant effects. No statistical effect could be measured for the factor "soil" (Table 3.1.1). The initial individual number of nematodes introduced per MC was 50 in single species treatments and 25 in mixed treatments. During the experimental time span the individual density of *A. saprophilus* increased significantly throughout all treatments for a multiple of the initial number (Table 3.1.2). After 2 weeks the highest increase (ca. 1100 individuals in total) was found for the mixed species treatment with clay loam and *Fusarium*-infected straw (Table 3.1.2). After 4 weeks the nematode numbers resulted in around one to two orders of magnitude more than the initial densities of *A. saprophilus* (Table 3.1.2). The increase in nematode numbers was generally higher in the infected treatments as compared to the non-infected treatments. Most nematodes of ca. 4000 individuals were found in the mixed species treatment with clay loam soil.

Table 3.1.2: The mean individual number of collembolans and nematodes (single species and mixed species treatments) per minicontainer with *Fusarium*-infected or non-infected wheat straw after 2 and 4 weeks of inoculation separated for different soil textures (sandy loam, silt loam, clay loam) compared to the initial number (start).

	Collembolans				Nematodes			
	single		mixed		single		mixed	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
infected								
Start	15	15	10	15	50	50	25	25
sandy loam	25	32	11	28	167	2081	362	2607
silt loam	19	23	9	31	260	2597	940	1099
clay loam	25	23	13	14	151	1478	1102	3939
non-infected								
sandy loam	26	96	14	48	300	1209	145	552
silt loam	21	123	12	56	381	935	508	1006
clay loam	16	97	15	57	287	1923	501	1311

FPE concentrations in wheat straw

In the case of wheat straw, significant effects on the FPE concentration were recorded for the factors "*Fusarium* infection" and "date" and their interaction (Table 3.1.1).

The initial Fusarium biomass in the infected straw was 3365.30 ± 302.87µg kg^{-1}. After 2 weeks of incubation the FPE concentration of all treatments decreased by at least one order of magnitude (Fig. 3.1.1). The lowest concentrations were determined in the control treatments without soil fauna. After 4 weeks the *Fusarium* biomass showed an increase of at least twice the FPE content 2 weeks earlier (Fig. 3.1.2). The greatest increase was found in the control treatments. The FPE concentrations of the wheat straw were significantly lower compared to 26 the control in all soil fauna treatments in sandy loam and silt loam. After 4 weeks compared to t_0, the FPE content was degraded by 87% (sandy loam), 88% (silt loam) and 91% (clay loam) in control treatments without animals. In single species treatments, the *Fusarium* biomass was reduced by 92%. In the mixed species MCs, the FPE content was reduced by 93% in sandy loam and silt loam and 89% in clay loam.

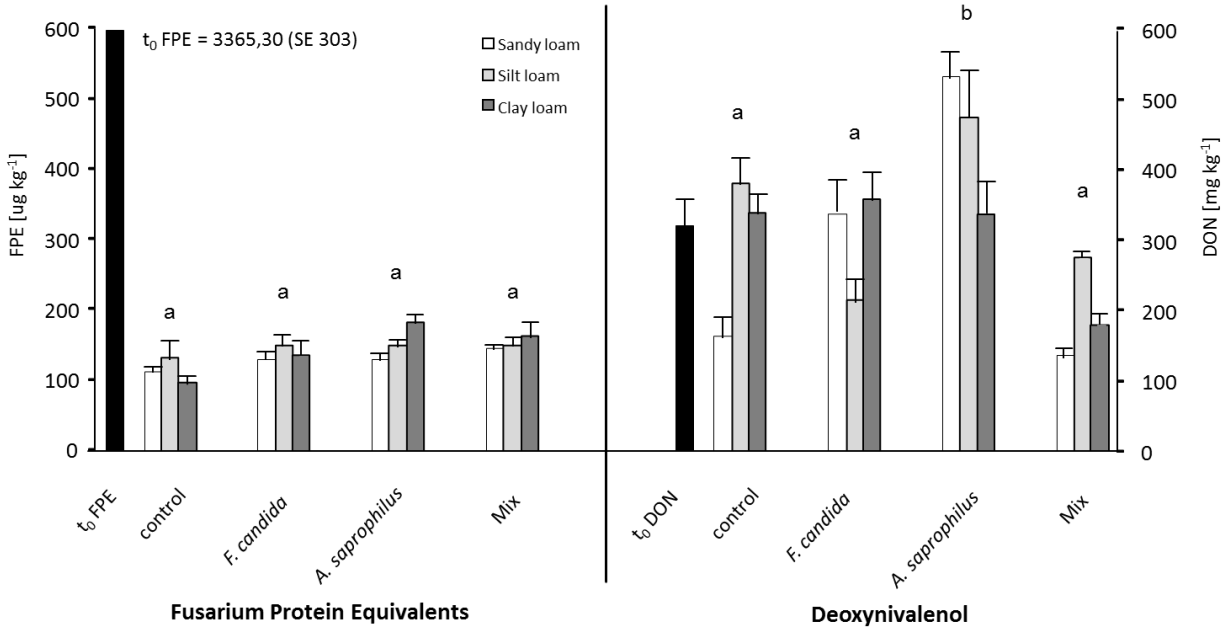

Fig. 3.1.1: Mean (+SE) concentrations of FPE (*Fusarium* Protein Equivalents) (µg kg^{-1}) and DON (deoxynivalenol) (mg kg^{-1}) in *Fusarium*-infected winter wheat straw in minicontainers inoculated with soil fauna species *F. candida* (Collembola), *A. saprophilus* (Nematoda), both species (Mix) or without soil fauna (control) at the beginning (t0) and after 2 weeks of inoculation. Bars are separated for different soil texture. Different letters indicate statistically significant differences in means (Tukey's HSD test; $P < 0.05$). The starting concentration served as reference value but was not included in Tukey's test.

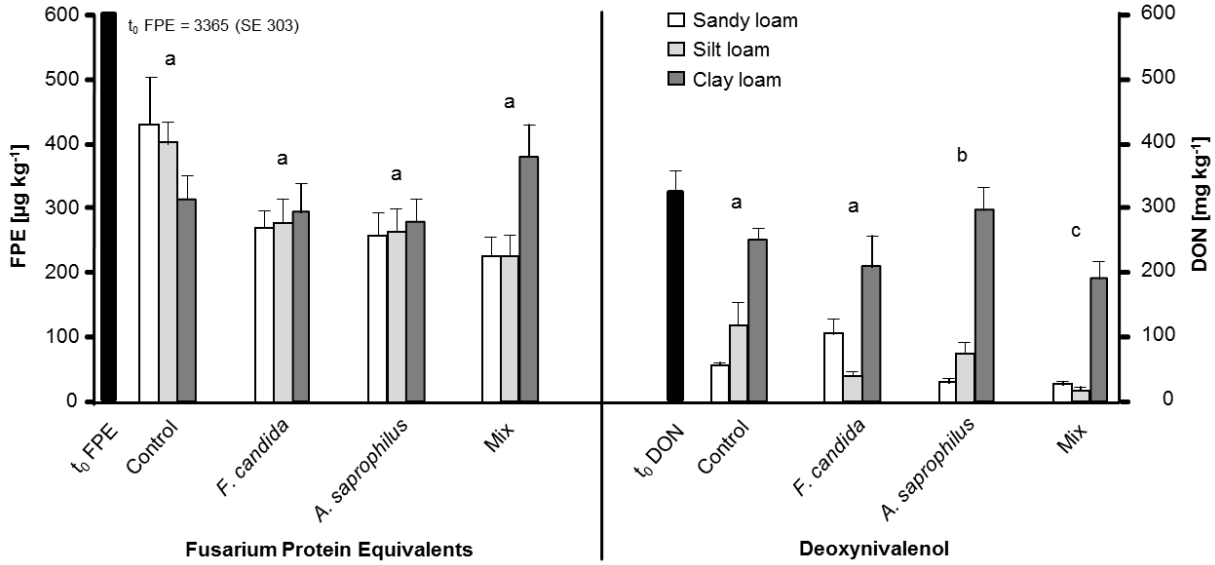

Fig. 3.1.2: Mean (+SE) concentrations of FPE (*Fusarium* Protein Equivalents) (µg kg^{-1}) and DON (deoxynivalenol) (mg kg^{-1}) in *Fusarium*-infected winter wheat straw in minicontainers inoculated with soil fauna species *F. candida* (Collembola), *A. saprophilus* (Nematoda), both species (Mix) or without soil fauna (control) at the beginning (t0) and after 4 weeks of inoculation. Bars are separated for different soil texture. Different letters indicate statistically significant differences in means (Tukey's HSD test; $P < 0.05$). The starting concentration served as reference value but was not included in Tukey's test.

The *Fusarium* biomass initially present in non-infected straw of 26.16 ± 2.06µg kg^{-1} was reduced in all treatments. After 2 and 4 weeks, FPE concentrations were below the quantification limit.

FPE concentrations in soil

At the start of the experiment the initial FPE concentration in soil could only be determined in clay loam (19.4 ± 1.96µg kg^{-1}). In sandy loam and silt loam the initial FPE concentration was below the quantification limit. But 2 weeks after soil fauna inoculation, FPE concentrations were detectable in the soil of almost all treatments with infected wheat straw (Fig. 3). After 4 weeks, *Fusarium* biomass was determined only for sandy and silt loam treatments without soil fauna (20.86 ± 11.08µg kg^{-1}; 17.84 ± 7.60µg kg^{-1}). In MCs receiving non-infected wheat straw, FPE content was generally below the quantification limit after 2 and 4 weeks.

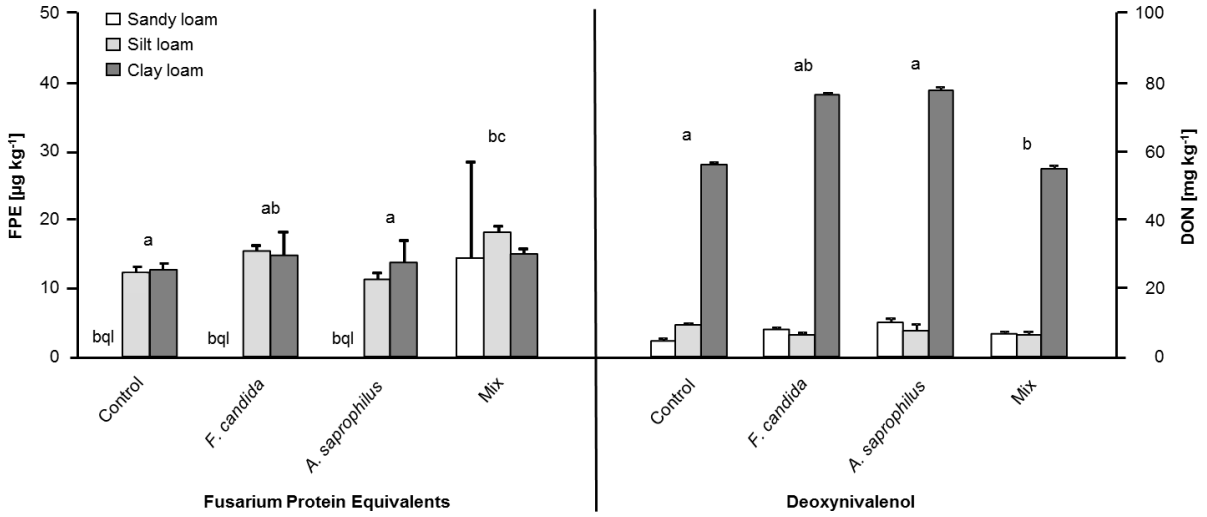

Fig. 3.1.3: Mean (+SE) concentrations of FPE (*Fusarium* Protein Equivalents) (µg kg^{-1}) and DON (deoxynivalenol) (mg kg^{-1}) in soil from the *Fusarium*-infected treatment in minicontainers inoculated with soil fauna species *F. candida* (Collembola), *A. saprophilus* (Nematoda), both species (Mix) or without soil fauna (control) after 2 weeks of inoculation. Bars are separated for different soil textures. Different letters indicate statistically significant differences in means (Tukey's HSD test; $P < 0.05$). bql = below quantification limit.

DON concentrations in wheat straw

According to RM-ANOVA the impact of the analysed factors on the DON concentration of wheat straw is presented in Table 3.1.1. Significant effects were recorded for all factors as well as all their interactions. In the infected wheat straw, an initial DON concentration of 318.56 ± 41.33mg kg^{-1} was determined. After 2 weeks the DON concentration decreased in all mixed species treatments, in the F. candida treatment with silt loam and the control with sandy loam (Fig. 3.1.1).

After 4 weeks the DON concentration was reduced significantly compared to the initial content of DON. The highest reduction was found in the mixed species MCs where the DON concentration was reduced by more than 90% in sandy and silt loam and 40% in clay loam which was significant compared to all other treatments (Fig. 3.1.2). In non-faunal MCs, the DON concentration generally decreased less compared to the single and mixed species treatments. Regarding the factor "soil", the post hoc test confirmed that clay loam treatments differed significantly from sandy loam and silt loam treatments. No significant differences were found between silt loam and sandy loam.

An increase was measured throughout nearly all treatments with regard to the DON concentrations of the non-infected straw after 2 weeks. Starting from an initial DON concentration of 2.54 ± 0.42 mg kg^{-1}, the DON content increased up to twice the initial content at the maximum. After 4 weeks the DON content of the non-infected straw decreased, except in the control treatments with clay loam or sandy loam.

DON concentrations in soil

RM-ANOVA on soil DON concentration revealed no significant influence of the factor "fauna", whereas the factors "*Fusarium* infection", "date" and "soil" as well as their interactions of "date x soil", "*Fusarium* infection x soil", "*Fusarium* infection x date" and "*Fusarium* infection x date x soil" were significant (Table 3.1.1: Individual density of collembolans and nematodes, Fusarium protein equivalent (FPE) and deoxynivalenol (DON) content in wheat straw and soil. *F*-values of RM-ANOVA on the effects of soil fauna (collembolans: *F. candida*, nematodes: *A. saprophilus*, mixed species), *Fusarium* infection (*Fusarium*-infected wheat straw, non-infected), date (start, 2 weeks and 4 weeks) and soil texture (sandy loam, silt loam, clay loam).). At the start of the experiment DON concentrations in soil of all different textures were below the quantification limit (< 37µg kg^{-1}). But after 2 weeks experimental time, DON concentrations between 4 and 11 mg kg^{-1} were detected in sandy and silt loam soil of all treatments with infected wheat straw (Fig. 3.1.3) which was significantly different from the DON content increase in clay loam up to 55 to 78mg kg^{-1}.

After 4 weeks DON concentrations were still determined in the soil of the infected treatments (Fig. 3.1.4) but all concentrations were significantly reduced compared to the DON contents after 2 weeks. The lowest DON concentrations were found in the mixed species treatments, where a decrease of DON concentration of about 90% was determined. The mixed species treatment differed significantly from the *A. saprophilus* treatment and the non-faunal control. Regarding the different soil texture treatments, the lowest DON concentrations were determined in sandy and silt loam. In both cases, DON contents were significantly lower compared to those in clay loam (Fig. 3.1.4). The greatest difference between control and soil faunal treatments was measured in clay loam (control: 24.0 mg DON kg^{-1}; mixed species: 4.5 mg DON kg^{-1}).

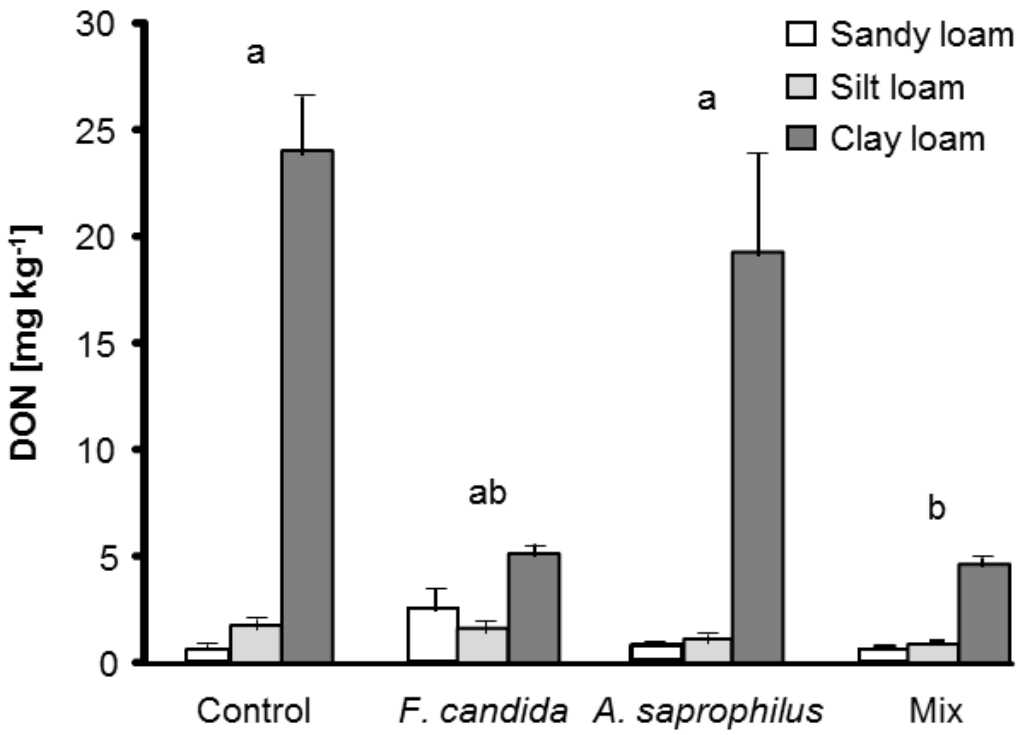

Fig. 3.1.4: Mean (+SE) concentrations of DON (deoxynivalenol) (mg kg^{-1}) in soil from the *Fusarium*-infected treatment in minicontainers inoculated with soil fauna species *F. candida* (Collembola), *A. saprophilus* (Nematoda), both species (Mix) or without soil fauna (control) after 4 weeks. Bars are separated for different soil textures. Different letters indicate statistically significant differences in means (Tukey's HSD test; $P < 0.05$).

DON concentrations below 1 mg kg^{-1} were measured in soil of treatments receiving non-infected wheat straw after 2 weeks. Similar to the development of the DON concentration in soil with infected wheat straw, the DON contents of the non-infected treatments decreased significantly after 4 weeks compared to the DON contents after 2 weeks. DON concentrations were below the quantification limit in the non-faunal control, *A. saprophilus* and mixed species treatments in sandy loam, as well as in the non-faunal control and *F. candida* treatments in silt loam.

Discussion

The biomass of the soil-borne pathogenic fungi *Fusarium culmorum* in infected wheat straw was reduced notably in all treatments. Even though is no doubt that *F. culmorum* is an adequate food source for collembolans, and particularly for *F. candida* (Larsen et al., 2008; Sabatini and Innocenti, 2000), the present study showed no statistical differences between the degradation capability of the different faunal treatments *(F. candida, A. saprophilus,* mix and without animals), neither after 2 nor after 4 weeks. Also the results of *A. saprophilus* feeding on *F. culmorum* are not in line with other authors (Hasna et al., 2007, 2008; Lagerloef et al., 2011), who showed that introduced fungivorous nematodes significantly reduce pathogenic fungi. The hypothesis, that the introduced soil fauna is responsible for the degradation of *Fusarium* biomass, could therefore not be confirmed with certainty. Possibly the time span of first 2 weeks was too long to detect a significant influence of collembolans and nematodes on FPE reduction. We assume that mainly microbial activity or interaction between inoculated soil fauna and microorganisms caused most of the degradation occurring during the first 2 weeks of the experiment (mean reduction: *F. candida,* 96%; *A. saprophilus* and mix, 95%; without animals; 97%). Fragmentation of organic residues and selective grazing of soil fauna on soil microorganisms control soil respiration and composition of microbial communities (Seastedt, 1984; Anderson, 1988; Lussenhop, 1992) which might have indirectly enhanced the degradation of *Fusarium* biomass.

Since *F. candida* is known to feed not only on fungi but also to prey on nematodes (Lee and Widden, 1995) the collembolans possibly influenced the population of *A. saprophilus*. On the other hand, collembolans may also stimulate bacterial and fungal activity by excreting substrates with high nutrient contents, thus indirectly promoting the growth of free-living nematodes (Kaneda and Kaneko, 2008), which might be assumed in case of the infected mixed treatments. Lootsma and Scholte (1997a, b) reported combined effects of fungivorous

micro- and mesofauna depending on moisture and temperature conditions as well as on individual densities. In their investigations of repression of Rhizoctonia stem canker by the nematode *Aphelenchus avenae* and the springtail *Folsomia fimentaria*, they showed a given interaction effect when *F. fimentaria* was combined with the highest populations of *A. avenae*. According to Lootsma and Scholte (1997b) it is also possible that the level of infection with *Fusarium culmorum* in our study was not as high as that detected by the authors. They detected an additive effect of both introduced animals when providing a high infection level of *Rhizoctonia solani* under wet and normal moisture conditions. Consequently, the hypothesis, that the species interaction of *A. saprophilus* and *F. candida* enhances the degradation of *Fusarium* biomass in infected wheat straw, can only be taken with caution. It should be tested again with straw of a higher infection level and / or a shorter experimental time span. As the statistical evaluation (RM ANOVA) did not show any significant effects of the factor "soil", the FPE degradation capability of the introduced soil fauna seemed also not to be directly affected by different soil texture. This result contradicts reports of varying effects of microarthropods on litter decomposition when substrates are placed in different soils (Seastedt, 1984).

With regard to mycotoxin degradation, DON concentration was reduced significantly in all treatments. According to RM ANOVA, the effect of the introduced soil fauna in degrading DON was significant. The sandy loam and silt loam treatments of the mixed species MCs showed the highest decrease (Fig. 3.1.2), but the decline was also significant in MCs of sandy and silt loam containing *A. saprophilus*. This confirms findings of Ishibasi and Choi (1991), who demonstrated the suppression of *Rhizoctonia solani* by *Aphelenchus avenae*. The reduction efficiency of *F. candida* was also considerable in sandy and silt loam, but differed significantly from the mixed species treatments and from *A. saprophilus*, whereas no statistical difference could be determined between treatments with *F. candida* and non-faunal control treatments.

These results strongly suggest that the degradation of DON is not only related to the effect of one single member of the soil fauna food web. As the degradation of DON in the present study was highest in treatments containing both nematodes and collembolans, it is obvious that the interaction of at least two or more players of the soil food web is promoting the reduction of DON. Sabatini and Innocenti (2001) pointed out that suppression of plant pathogens is not based on the effect of a single antagonistic organism but on the optimisation

and integration of different organisms and control agents. Thus, the initial hypothesis, that the interaction of *A. saprophilus* and *F. candida* enhances the degradation of DON in *Fusarium* infected wheat straw, is confirmed.

Not only the introduced soil fauna revealed statistical effects in reducing DON concentration, but also the given soil texture provided an environment which significantly influenced the degradation of the mycotoxin in infected wheat straw, as the results clearly showed. This confirms our third hypothesis, that the degradation efficiency of nematodes and collembolans is affected by soil texture. After 4 weeks in sandy loam and silt loam the reduction efficiency of the soil fauna was significantly higher than in clay loam treatments (Fig. 3.1.2).

Soil texture affects activity and life cycle of nematodes and collembolans (Yeates and Bongers, 1999; van Capelle et al., 2012). Coarse-structured soil promotes abundance and mobility of nematodes (Yeates and Bongers, 1999; Hohberg and Traunspurger, 2005). For the collembolan species *F. candida*, Domene et al. (2011) reported lower reproduction in soils with an increasing content of fine particles. Those findings can be confirmed in only some cases of the present study (Table 3.1.2).

Generally, soils managed by conservation tillage are regarded to have a higher soil biodiversity as well as enhanced biological activity compared to conventionally tilled soils (Holland, 2004; van Capelle et al., 2012). Conservation tillage contributes to a better ecosystem functioning by providing ecosystem services, such as preservation (maintenance of biodiversity), support (nutrient and hydrological cycles, soil formation, crop pollination) and regulation (control of climate, soil erosion, pests and diseases) (Kassam et al., 2009).

Reduced-till soils are usually chracterised by more organic matter and nutrients near the soil surface, resulting under certain conditions in a fungal-based decomposer system (Hendrix et al., 1986). As shown in the present study, soil texture is obviously an important factor for controlling the conditions of soil fauna in degradation processes. There is a dynamic balance between fungal and bacterial pathways, which nematodes are known to influence most. In the absence of microarthropods, fungal feeding nematodes become regulators of the system (Ruess and Ferris, 2004). This, however, was not true in our study where interacting collembolans and nematodes were most efficient. Especially in the case of tight crop rotations, where the time slot between harvest of the previous and sowing of the following

crop is short, interacting soil fauna and, in addition, soil microorganisms might enhance and accelerate the degradation of soil-borne phytopathogenic fungi and their mycotoxins as ecosystem services for crop protection.

Conclusions

According to the results of the present investigation collembolans and nematodes significantly contribute to mycotoxin degradation in wheat straw, especially in sandy and silty soils. We conclude that particularly interacting collembolans and nematodes play an important role in plant pathogen repression and mycotoxin degradation as ecosystem services. Accordingly, fungal feeding soil micro- and mesofauna might be able to promote compensation for the enhanced risk of fungal crop diseases and mycotoxin contamination of food and feed deriving from conservation tillage practices. In any case, soil texture matters in the provision of these ecosystem services by collembolans and nematodes.

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Chapter 4

4 Degradation performance of fungivorous soil organisms on mycotoxins

4.1 Regulation of the mycotoxin deoxynivalenol by *Folsomia candida* (Collembola) and *Aphelenchoides saprophilus* (Nematoda) in an on-farm experiment

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Abstract

A field experiment based on a minicontainer-system was conducted on decontamination as an ecosystem service provided by soil fauna (*Folsomia candida*, Collembola and *Aphelenchoides saprophilus*, Nematoda). The objective was to investigate if the introduced soil fauna is able to reduce the concentration of the mycotoxin deoxynivalenol (DON) in wheat straw. The minicontainers were filled with soil and either DON-contaminated or non-contaminated wheat straw. Soil fauna was introduced in different combination into the minicontainers (single-collembolan, single-nematode, mixed and a non-faunal control treatment) and placed into the topsoil (0-5 cm) of an arable field. Each treatment was replicated five times. After 2 and 4 weeks, soil fauna was counted and DON content was detected in soil and straw. Population density of *A. saprophilus* developed mostly when received DON-contaminated wheat straw. Individual numbers of *F. candida* was highest in the mixed, non-infected treatment after 4 weeks. DON concentration in remaining straw of the contaminated minicontainers was reduced in the single collembolan treatment and in the single nematode treatment after 2 weeks. In contrast, there was an increase of DON in the non-faunal control treatment and the mixed treatment. After 4 weeks DON concentration decreased throughout all treatments compared to the initial concentration. In soil, a DON concentration was measured throughout all treatments after 2 weeks, which was reduced significantly after 4 weeks. We conclude that nematodes and collembolans significantly contribute to the degradation of the mycotoxin DON in wheat straw and protect soil from DON contamination as an ecosystem service.

Keywords: Soil health, Conservation tillage, Ecosystem services, Soil biodiversity, Mycotoxin degradation

Introduction

Conservation tillage as a sustainable management measure has expanded rapidly in the past decade (Derpsch et al. 2010) to protect arable soils from erosion and compaction, to retain moisture and reduce production costs (Holland 2004; Kassam et al. 2009). Organic amendment techniques like mulching result in high biodiversity belowground (Hobbs 2007; van Capelle et al. 2012) and provide benefits to ecosystem services (Derpsch et al. 2010; Kassam et al. 2009). Many soil processes including organic matter decomposition, nutrient cycling, soil structure formation, pest regulation and bioremediation of contaminants are controlled by soil organisms and their fundamental interactions (Dominati et al. 2010).

However, a closer view reveals that promotion of biological activity is less favourable than it appears to be, since pest organisms also benefit from residual plant materials like straw on the soil surface. A drawback of conservation tillage 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). *Fusarium* head blight is one of the most important diseases in small grain cereals, such as wheat, causing significant yield loss and enormous economically damage to farmers all over the world (Leplat et al. 2013).

In addition to quantitative losses, a decline in quality of crop products may result from contamination with mycotoxins. Deoxynivalenol (DON) is one of the most frequently produced mycotoxin by *F. graminearum* and *F. culmorum* and therefore often detected in cereals (Curtui et al. 2005; Pestka 2007). It belongs to the structural group of trichothecenes all bearing a common tricyclic core structure (Audenaert et al. 2013). Mycotoxins like DON cause negative effects on eukaryotic cells like inhibition of protein synthesis. Furthermore, they persist during storage, are heat resistant and of major concern for human and animal health after consumption of contaminated food and feed, respectively (JEFCA 2001). Besides the emanating health threat of DON after consumption, there are increasing concerns about the importance of DON and other mycotoxins as potential environmental contaminants (Bucheli et al. 2008; Gautam and Dill-Macky 2012; Hartmann et al. 2008b). Leaching of mycotoxins from host tissue like infected plants or residual material must be considered as environmental threat since mycotoxins including DON were detected in soil, drainage water and soil water, which percolates to the ground water table (Hartmann et al. 2008a; Kolpin et al. 2014). Little is known about ecotoxicological consequences. However, it cannot be

excluded that pulsed exposure of DON has adverse effects on soil quality and soil biota as certain trichothecenes indicate insecticidal effects (Fornelli et al. 2004).

To reduce both, the infection risk of plant diseases by *Fusarium* species and the mycotoxin contamination of cultivated crops in arable systems an effective stimulation of decomposition of crop residues is highly important (Oldenburg et al. 2007; Stemann and Lütke-Entrup 2005). In general, decomposing soil fauna plays a major role in accelerating degradation of plant material (Brown et al. 2000). Fungi and bacteria are most responsible for organic matter breakdown. They are on their part greatly influenced by feeding activities of protozoans, nematodes, annelids and arthropods (Seastedt 1984). More recent studies revealed earthworms to be important actors in arable soil by degrading *Fusarium* biomass and DON from infected wheat straw (Oldenburg et al. 2008; Schrader et al. 2009; Wolfarth et al. 2011a, 2011b). In fact, there is a general consensus of the importance of microarthropods in decomposition and mineralization processes, especially of mites and collembolans as they account for about 95% of the total microarthropod number in soils (Seastedt 1984). Due to their notable contribution to decomposition processes in agroecosystems and their influence on fungal succession and the altering of soil saprotrophic fungal communities through grazing, collembolans are considered to play an important role in biological control of fungal plant pathogens (Klironomos et al. 1992; Klironomos and Kendrick 1995; Lartey et al. 1994).

Nematodes are also important regulators of residue decomposition (Ruess and Ferris 2004). Especially bacterial and fungal feeders are key components in decomposition processes and nutrient cycling, thus indirectly affecting primary production (Chen and Ferris 2000; Ruess and Ferris 2004). Many studies were conducted investigating the ability of fungivorous nematodes to control soil-borne plant pathogenic fungi (Gupta 1986; Hasna et al. 2008; Okada 2006).

In light of the fact that the members of soil micro- and mesofauna are strongly linked in belowground food webs, Wolfarth et al. (2013) examined the activity of nematodes and collembolans in terms of controlling plant pathogens and their mycotoxins under laboratory conditions and found that species interaction between the fungivorous nematode *Aphelenchoides saprophilus* and the collembolan species *Folsomia candida* significantly enhance the degradation of DON concentration in wheat straw. Microcosm experiments performed under laboratory conditions are a useful tool to study ecosystem processes or interactions between organisms. However, the relevance of laboratory results is always an open question and needs to be proved for their reliability under field conditions (Teuben and Verheuf 1992). Therefore, on-farm experiments based on minicontainers were performed with

fungal feeding representatives of mesofauna (collembolans) and microfauna (nematodes) to assess impacts of soil organisms on mycotoxin degradation under field conditions. The minicontainers were introduced directly into the topsoil of a cropping system of a commercial farm after harvest. In contrast to the laboratory study of Wolfarth et al. (2013) we used wheat straw with lower DON concentrations to get closer to field conditions.

The present study focused on the collembolan species *F. candida* and the fungal feeding nematode *A. saprophilus*. *F. candida* is a well-known model arthropod which is widespread and occurs in arable soils (Fountain & Hopkin, 2005). Based on these two species, this investigation contributes to the understanding of the functional role of collembolans and nematodes as key components in soils of agroecosystems. Our hypothesis was: collembolans and nematodes stimulate the degradation of the mycotoxin deoxynivalenol (DON) in contaminated wheat straw under field conditions thus playing an important role in decontamination and promoting soil health as an ecosystem service in reduced tilled fields.

Materials and methods

Environmental conditions and site description

A field experiment was established at Adenstedt near Hildesheim in the southwest of Braunschweig, Northern Germany (9°56'E 52°00'N, 196 m a.s.l.) in 2011. The climate conditions of this region are chracterised by a mean annual air temperature of 8°C and an average precipitation rate of 700 mm y^{-1}.

In 2011, the experiment was conducted in a winter wheat field where conservation tillage had been practised for more than 20 years. 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. The average air temperature during the experiment was 12.4°C whereas precipitation was 43 mm during the experimental time span.

Soil

Topsoil from the agricultural field described above was collected and stored at 4°C until further treatment. The soil was defaunated by freezing at -20 °C for 24h and thereafter thawed at room temperature for 24h. After two freezing-thawing cycles the soil was additionally autoclaved to eliminate all fauna, which was initially present. For details see Wolfarth et al. (2013). Seven days before filling the minicontainers (see below) the defaunation and

autoclaving procedure was finished. Afterwards, the soil was macroscopically cleared of organic plant residues like straw or roots and sieved (mesh size 2 mm). The C_{org} content of the soil was 1.2%. Finally, 100 g of autoclaved soil was moistened with 5 ml filtered (No. 42 Whatman filter paper) soil extract, derived from the same soil prior to defaunating and autoclaving, to reinoculate microbial populations (van Vliet et al. 2004). At the beginning of the experiment, the soil water content was 22% (w/w), equivalent to 59% of water holding capacity.

Straw

Winter wheat (*Triticum aestivum cv.* Ritmo) was cultivated at an experimental site of the Julius Kühn Institute, Braunschweig (Germany). During flowering, winter wheat had been sprayed with 400 ml fungal spore suspension made of three strains of *Fusarium culmorum* for artificial infection as described by Wolfarth et al. (2013). For more details see Oldenburg et al. (2008) and Schrader et al. (2009). In straw harvested from infected plots deoxynivalenol (DON) reached a level of 155,667 ± 17,046 µg kg $^{-1}$. Finely chopped straw of approximately 1.5 mm length was used for the experiments. Winter wheat straw of *cv*. Ritmo cultivated at the same location, which was not artificially infected with *F. culmorum*, contained DON at a low level of 1,092 ± 95.08 µg kg $^{-1}$ and served as a control (in the following called "non-contaminated").

Soil fauna

The fungal feeding nematode species *Aphelenchoides saprophilus* was obtained from mass cultures with the mycorrhizal fungus *Laccaria laccata* (Scop.: Fr.) Cooke. Before introducing the nematodes to *L. laccata* for cultivation, the fungus was grown on Pachlewska agar at 17°C in darkness. A detailed description of this method is given in Ruess et al. (2002). Nematodes were extracted from agar for 24 h at room temperature by the Baermann funnel method.

The collembolan species *Folsomia candida* used for the experiment originated from laboratory mass cultures. *F. candida* was reared on a mixture of moist plaster of Paris and charcoal (9:1) and fed with brewer´s yeast and carrots twice a week. According to Pfeffer and Filser (2010) only young adults of the same age (12 – 14 days) and size were introduced to the experimental system after starving for 24 h.

Experimental design

For the experiment, the Eisenbeis minicontainer system (Eisenbeis et al. 1999) was used. This system consists of two components: A PVC-bar as carrier device for several minicontainers (MCs) (Fig. 4.1.1). More detailed descriptions and figures of the MC system are given in Eisenbeis et al. (1999). The MCs were used as experimental units. Each end of the cylindrically shaped MCs (16 mm long, 11 mm in diameter) was covered with nylon-gauze. A mesh size of 15 µm was chosen to enable an exchange of air and water with the surrounding soil but prevent soil fauna from escaping or immigrating. The MCs were filled with 150 mg of soil and 200 mg of finely chopped straw according to Eisenbeis et al. (1999). Small quantities of soil and straw were retained at the beginning and stored at –20°C until further processing for detecting the initial DON concentrations. After filling the MCs, the soil fauna was introduced into the MCs.

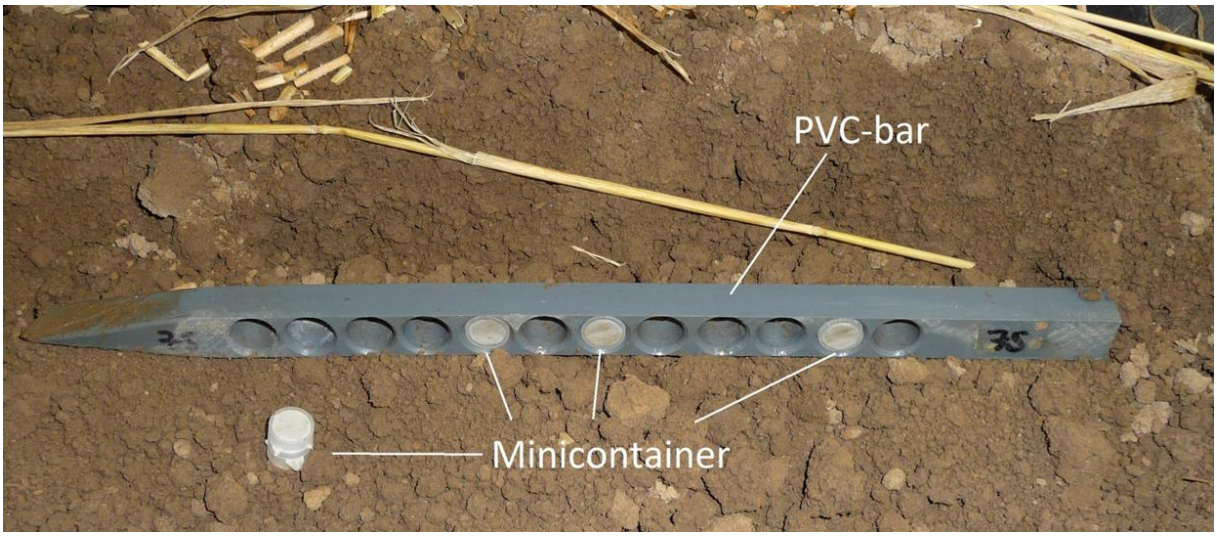

Fig. 4.1.1: PVC-bar with 4 minicontainers, horizontally introduced into the topsoil (0-5cm) of the experimental field, before covering with the surrounding soil.

The experimental system was divided into 2 straw treatments: *Fusarium*-infected straw *versus* non-infected straw. Furthermore, there were 4 different faunal treatments: single collembolan treatment (15 individuals per MC); single nematode treatment (50 individuals per MC); mixed treatment with collembolans (10 individuals) and nematodes (25 individuals) and one treatment without fauna as control.

The prepared MCs were placed into the openings of the PVC-bars. Each PVC-bar received 4 similarly assembled MCs to ensure an adequate amount of sampling material for faunal

extractions and analyses of DON concentrations. A total number of 80 PVC-bars were set up for the 2 straw treatments, 4 faunal treatments and 2 time treatments (see below) considering 5 replicates per treatment. Establishing the experiment in the field, the PVC-bars were placed in 4 lines of 40 m in length. Both the spatial interval between the PVC-bars in each line and the distance between the lines was 2 m to avoid wheeling tracks. The PVC-bars were randomly distributed within the lines and horizontally introduced into the topsoil (0-5cm) of the field in close contact to the surrounding soil. The experiment was carried out during a period of 2 and 4 weeks from mid-September to mid-October 2011.

Sampling and sample processing

After 2 and 4 weeks sampling, soil fauna was collected considering one MC for collembolan and one MC for nematode extraction from each PVC-bar. Collembolans were extracted for 10 days using a MacFadyen high-gradient extractor (MacFadyen, 1961). Nematodes were extracted at room temperature for 3 days by the Baermann funnel method. A particular description of soil fauna extraction is given in Wolfarth et al. (2013). Straw and soil samples were taken from 2 MCs of each PVC-bar (double samples for straw and soil). During sampling, great care was taken to mechanically separate adhesive soil from straw. Soil and straw samples were then stored at –20°C in readiness for analytical preparation.

All samples, including the parent materials at the start of the experiment, were treated by freeze-drying for 24 h. Straw was ground using a mixer mill (MM 400, Retsch GmbH, Haan, Germany). Samples of soil were manually homogenized with a mortar to obtain a fine powder (< 0.5 mm).

Determination of deoxynivalenol

The deoxynivalenol (DON) concentrations were analysed 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 100 mg for wheat straw and soil. The limits of quantification were 37 µg kg^{-1} for soil and non-infected straw and 74 µg DON kg^{-1} for infected wheat straw.

Statistics

The Kolmogorov-Smirnov test was applied to check data for normality. Also variance homogeneity was tested by using the Levene test. Data for population density of collembolans

were normally distributed with equal variances. For DON concentration and population density of nematodes, normality of data and variance homogeneity was violated. In these cases, a square root transformation was conducted to stabilise the variances and establish normality. After that, a univariate analysis of variance (ANOVA) was carried out to compare treatment effects of soil fauna (*F. candida, A. saprophilus*, Mix), DON contamination (contaminated, non-contaminated) and date (start as t_0, mid-term sampling as t_1 and end of the experiment as t_2) on DON concentration and population density. Bonferroni correction was chosen as adjustment level. All statistical analyses were done using the software package SPSS for Windows version 22.

Results

Individual density of soil fauna

Collembolans

According to ANOVA, significant effects were recorded for the factor "date" and the interaction of the factors "fauna", "contamination" and "date" (Table 4.1.1). The mean individual numbers of collembolans at the beginning, mid-term sampling and end of the experiment are shown in Table 4.1.2. After the first 2 weeks a decline of the mean individual number of collembolans was measured throughout all treatments. After 4 weeks the individual number of collembolans (single and mixed) increased twice compared to the 2 weeks date (Table 4.1.2). However, the differences of population density of collembolans were not significant.

Nematodes

Regarding the nematodes significant effects were recorded for the factors "contamination" and "date" (Table 4.1.1). The mean individual numbers of nematodes at the beginning, mid-term sampling and end of the experiment are given in Table 4.1.2. After 2 weeks of inoculation the individual density of *A. saprophilus* increased significantly throughout all treatments (Table 4.1.2). After 4 weeks, the individual numbers of nematodes were still considerably higher than the initial number but lower than after 2 weeks. In general, the individual density of nematodes was higher in minicontainers which received DON contaminated wheat straw (Table 4.1.2).

Table 4.1.1: Population density of collembolans and nematodes in minicontainers. *F*-values. *P*-values and degrees of freedom (df) of ANOVA on the effects of soil fauna (F) separated for collembolans and nematodes (*F. candida*, *F. candida* in mixture or *A. saprophilus*, *A. saprophilus* in mixture), DON contamination (C) (contaminated wheat straw, non-contaminated) and date (D) (start, mid-term sampling after 2 weeks and end of the experiment after 4 weeks).

		Collembolans		Nematodes	
	df	*F*	*P*	*F*	*P*
Fauna (F)	1	0.998	0.323	0.121	0.729
Contamination (C)	1	1.522	0.223	7.810**	0.008
Date (D)	2	12.125***	<0.001	94.291***	<0.001
F x C	1	2.643	0.111	0.062	0.804
F x D	2	1.182	0.315	0.427	0.655
C x D	2	0.087	0.055	2.161	0.127
F x C x D	2	4.079*	0.023	0.237	0.790

* P < 0.05; ** P < 0.01; *** P < 0.001

Table 4.1.2: Mean individual numbers of collembolans and nematodes (single species and mixed species treatment) per minicontainer with DON-contaminated or non-contaminated wheat straw after 2 and 4 weeks of inoculation compared to the initial number (start). Standard error is given in brackets.

	Collembolans				Nematodes			
	Single		Mixed		Single		Mixed	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
Start	15	15	10	10	50	50	25	25
Contaminated	9 (1)	18 (3)	9 (3)	10 (3)	1251 (419)	386 (133)	1147 (166)	415 (123)
Non-contaminated	8 (1)	17 (5)	6 (2)	28 (7)	661 (109)	243 (41)	743 (60)	185 (45)

DON concentrations in wheat straw

Regarding the DON concentration, ANOVA revealed significant effects for the factors "fauna", "contamination" and "date" as well as for all their interactions (Table 4.1.3). In contaminated wheat straw an initial DON concentration of 155,667 µg kg^{-1} was determined (Fig. 4.1.2).

Table 4.1.3: Deoxynivalenol (DON) concentration in wheat straw and soil. *F*-values, *P*-value*s* and degrees of freedom (df) of ANOVA on the effects of soil fauna (F) separated for collembolans and nematodes (*F. candida, F. candida* in mixture or *A. saprophilus, A. saprophilus* in mixture), DON contamination (C) (contaminated wheat straw, non-contaminated) and date (D) (start, mid-term sampling after 2 weeks and end of the experiment after 4 weeks). No data (-) indicate a DON concentration in soil below quantification limit (< 37 µg kg^{-1}) after 4 weeks.

	Straw			Soil		
	df	*F*	*P*	df	*F*	*P*
Fauna (F)	3	16.002***	<0.001	3	3.819*	0.020
Contamination (C)	1	2646.935***	<0.001	1	233.467***	<0.001
Date (D)	2	224.679***	<0.001	-	-	-
F x C	3	17.279***	<0.001	3	3.816*	0.020
F x D	6	11.430***	<0.001	-	-	-
C x D	2	180.557***	<0.001	-	-	-
F x C x D	6	11.496***	<0.001	-	-	-

* $P < 0.05$; *** $P < 0.001$

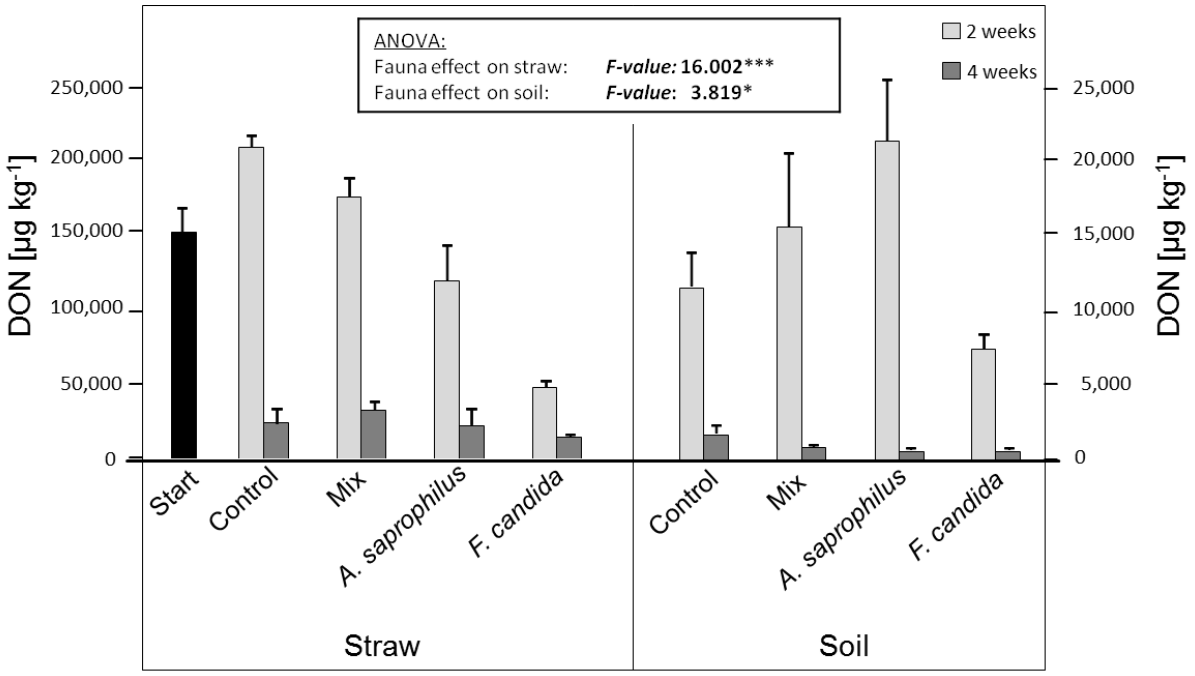

Fig. 4.1.2: Mean (+SE) concentrations of DON (deoxynivalenol) (µg kg^{-1}) in contaminated winter wheat straw and soil of minicontainers inoculated with soil fauna species *F. candida* (Collembola), *A. saprophilus* (Nematoda), both species (Mix) or without soil fauna (control) at the beginning (start) and after 2 and 4 weeks (number of replicates: n = 5), (* $P < 0.05$; *** $P < 0.001$).

Compared to the initial concentration of DON, an increase of DON content was measured for the non-faunal control treatment (214,040 µg kg-1) and the mixed treatment (180,780 µg kg-1) after 2 weeks (Fig. 4.1.2). In the single species treatment containing collembolans a reduction of ca. 70% DON content was recorded whereas in the nematode treatment DON was reduced by ca. 20%. Compared to the non-faunal control, the DON concentration was significantly lower in all three faunal treatments in the following order: mixed > nematode > collembolan.

After 4 weeks there was a decrease of DON concentration throughout all treatments compared to the initial DON content of contaminated wheat straw (Fig. 4.1.2). Highest reduction was found in the minicontainers containing *F. candida* where DON content was reduced by 90%. Only in this case the final DON concentration was below the control treatment without animals, for which a reduction of the DON content of 85% was found. The same decline of 85% was measured in minicontainers containing *A. saprophilus*. DON in straw of the mixed treatment was reduced by 77% (Fig. 4.1.2).

The DON concentration of the non-contaminated wheat straw (initially 1,092 µg kg^{-1}) decreased throughout all different treatments (10-50%) after mid-term sampling and continued to decrease until the end of the experiment (data not shown).

DON concentrations in soil

Regarding DON concentration in soil, ANOVA recorded significant effects for the factors "fauna" and "contamination" as well as for their interaction (Table 4.1.3). At the beginning of the experiment the DON content of soil was below the quantification limit (37 µg kg^{-1}). After 2 weeks a DON concentration about one order of magnitude below the concentration in straw was measured in the soil of DON contaminated minicontainers throughout all treatments (Fig. 4.1.2). The highest concentration was measured in straw of the nematode treatment (21,246 µg kg^{-1}), followed by the mixed treatment (15,746 µg kg^{-1}) and the non-faunal control treatment (11,539 µg kg^{-1}). The lowest DON concentration (7,213 µg kg^{-1}) was determined in minicontainers with collembolans which was about 40% below the non-faunal control (Fig. 4.1.2).

After 4 weeks the DON concentration in soil was reduced (> 90%) significantly throughout all faunal treatments compared to the concentration after 2 weeks in the following order: mixed > nematode > collembolan. (Fig. 4.1.2). The DON concentrations of the faunal treatments were about 45-75% below the final DON concentration in the control treatment without animals.

In the soil of the non-contaminated treatments a low DON concentration was measured throughout all treatments after 2 weeks (159-245 µg kg^{-1}). After 4 weeks DON concentration was below the quantification limit (37 µg kg^{-1}).

Discussion

In case of the population density, a consistent development of the mean individual number of nematodes was observed, which was also supported by the statistical analysis (Table 4.1.1). Higher numbers of *A. saprophilus* were counted in the DON-contaminated minicontainers after 2 and 4 weeks compared to non-contaminated minicontainers, regardless of which faunal treatment (single or mixed). This result leads to the assumption that the nematode species *A. saprophilus* benefited from the *Fusarium*-infected and DON-contaminated wheat straw as it may have been nutrient enriched (Hendriksen 1990). In contrast to the population density of nematodes, the development of the mean individual number of collembolans was rather inconsistent. Since *F. candida* is known to feed not only on fungi but also to prey on

nematodes (Lee and Widden 1995; Kaneda and Kaneko 2008) the collembolans might possibly influenced the population of *A. saprophilus,* especially in non-infected treatments after 4 weeks where the lowest individual number of nematodes and the highest number of collembolans were found. In general, the population of collembolans tended towards a higher number of *F. candida* after 4 weeks.

Regarding DON concentration in straw, ANOVA revealed significant effects of the introduced soil fauna on the degradation of DON (Table 4.1.3). The findings of the current study are partly in line with the laboratory investigation of Wolfarth et al. (2013), who also detected an increase of DON concentration in control treatments without soil fauna but also in the single species minicontainers with collembolans and nematodes after 2 weeks. In case of the non-faunal control treatments, we assume that favourable moisture conditions within the minicontainers induced fungal activity of *Fusarium culmorum* which resulted in an enhanced production of DON during the first 2 weeks of the experiment, whereas the mycotoxin production in the single species treatments could be repressed by *F. candida* and *A. saprophilus* in the present study. Nevertheless, collembolans and nematodes in the mixed treatments could not inhibit the production of DON under field conditions after 2 weeks. These findings contradicts laboratory results from Wolfarth et al. (2013), who demonstrated, that in particular the interaction of the collembolan species *F. candida* and the nematode species *A. saprophilus* significantly contributed to the degradation of DON in wheat straw. It remains an open question why the DON concentration could not be reduced in the mixed treatment under field conditions after 2 weeks.

At the end of the experiments (after 4 weeks) a significant reduction of DON in wheat straw throughout all treatments was measured, whereas even in the non-faunal control treatment DON was reduced to a greater extend. This leads to the assumption that microbial activity plays an important role during the degradation process of mycotoxins. Also in case of the faunal treatments it seems likely that DON applied with *Fusarium*-infected straw was metabolized in an interactive process between the introduced soil fauna and soil microorganisms. Furthermore, there is evidence that biological control of pollutants is not based on single effects but on reciprocal effects of different control organisms, which are already inhabitants in soils (Sabatini and Innocenti 2001). Obviously, the activity of *F. candida* and *A. saprophilus* in single species treatments was dominating the degradation of DON during the first 2 weeks of the experiment, whereas after 4 weeks the decomposition process became rather microbiologically influenced. In terms of nutrient cycling and

decomposition collembolans and nematodes are considered to be very important. By stimulating microbial growth fungal feeding microarthropods may enhance decomposition indirectly (Beare et al. 1992) which could be suggested for the present study in case of DON.

Since the results demonstrated a single species effect of *F. candida* and *A. saprophilus* in promoting the decontamination of DON, the initial hypothesis that collembolans and nematodes are able to stimulate the degradation of the mycotoxin deoxynivalenol in contaminated wheat straw under field conditions can be confirmed. It has to be noted that under field conditions the single species effects were highest whereas under laboratory conditions the interaction of both species caused the highest reduction in DON concentrations (Wolfarth et al. 2013). This result supports the need to test laboratory findings in the field (Teuben and Verheuf 1992).

In soil concentrations of DON were below the quantification limit at the beginning of the experiment. After 2 weeks, DON was found in soil of all treatments, containing contaminated wheat straw. Whether DON was leached from the contaminated straw into the surrounding soil was not analysed but could be hypothesised for the present study. After 4 weeks the mycotoxin was reduced significantly (85 – 98%) in all minicontainers. Accordingly, results from Wolfarth et al. (2013), who detected DON concentrations at a similar order of magnitude after 2 and 4 weeks in sandy loam and silt loam, are confirmed. Compared to the non-faunal control treatment the DON concentration in soil of the mixed species treatment was reduced for about 50% and even more (70-75%) in the single species treatments of *F. candida* and *A. saprophilus*, respectively, after 4 weeks. Obviously, also time seems to be an important factor concerning the degradation of DON. Nevertheless, the present results indicate a decontamination effect of both introduced soil fauna species (*F. candida, A. saprophilus*) not only in wheat straw but also in soil. This ecosystem service contributes to mitigate the potential risk of mycotoxins as an environmental threat as emphasised by Hartmann et al. (2008a) and Kolpin et al. (2014).

Conclusions

The results of the present investigation show an accelerated degradation of the mycotoxin DON in *Fusarium*-infected wheat straw and soil under field conditions in presence of soil faunal activity. Against the background of mycotoxins as environmental pollutants induced by agricultural runoff or infiltration, the degradation performance of the introduced soil fauna (*F. candida, A. saprophilus*) must be considered as a crucial ecosystem service. In particular,

since infected plant material is known to be a source of mycotoxins to the environment as a potential threat (Kolpin et al. 2014). Furthermore, collembolans and nematodes are recognised to be part of a functional assemblage of biological regulators (Kibblewhite et al. 2008) developing their potential under conservation agriculture even more. Future studies should consider a more complex community of soil biota and a longer sampling period in fields with naturally contaminated crop residues to quantify the ecosystem service of DON degradation related to farming practice.

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4.2 Mycotoxin contamination and its regulation by the earthworm species *Lumbricus terrestris* in presence of other soil fauna in an agroecosystem

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Abstract

Background and aims

In 2011 and 2013, mesocosm-studies were conducted in the field to assess the degradation performance of soil fauna (earthworms: *Lumbricus terrestris*, collembolans: *Folsomia candida* and nematodes: *Aphelenchoides saprophilus*) on mycotoxin-contaminated crop residues. The aim of the study was to investigate the potential of anecic earthworms in the regulation of the mycotoxin deoxynivalenol (DON) and whether the degradation capacity is influenced by the presence of collembolans and nematodes.

Methods

After 4 and 8 weeks, DON concentrations in residual straw and in soil samples of all faunal treatments (containing earthworms, collembolans and nematodes in different combination) and the non-faunal control treatments were determined using an ELISA-method.

Results

After 4 weeks, the DON concentration in straw decreased in all treatments: faunal treatments 2011: 97-99%; 2013: 78-94%; non faunal treatments 2011: 88%; 2013: 68%. After 8 weeks a further decline of DON concentrations was measured in all faunal treatments (2011: 58-91%; 2013: 50-86%). DON concentration of the non-faunal treatments increased during the final four weeks. In soil the DON concentration was below quantification limits (<0.037 mg kg^{-1}).

Conclusion

This study revealed *L. terrestris* as the driver of the degradation process. The presence of collembolans and nematodes did not affect its degradation capacity. Earthworms contribute to a sustainable control of mycotoxins in wheat straw, thus reducing the risk of environmental pollution as an ecosystem service.

Keywords: Soil health, Ecosystem services, Plant pathogen control, Mycotoxin degradation, Conservation tillage, Functional soil biodiversity

Introduction

Many soil processes and functions, including organic matter decomposition, nutrient cycling, soil structure formation, pest regulation and bioremediation of contaminants are controlled by soil organisms and their fundamental interactions (Dominati et al. 2010). Although soil biota provide a variety of ecosystem services, there are also disservices (Zhang et al., 2007). For example, fungal plant pathogens survive on crop residues, from where they may infect the subsequent crop causing specific plant diseases (Goswami and Kistler, 2004). In this context, it is estimated that over 40% of the pre-harvest yield of the eight most important crop species is lost due to disease and damage by pest organisms, and the global food production losses due to plant disease have been estimated at 10% (Cheatham et al., 2009). The fungal disease complex Fusarium head blight (FHB) is one of the most noxious diseases worldwide and causes significant yield losses in cereals such as wheat, oat and barley (Leplat et al., 2013; Vogelgsang et al., 2011). It is caused by several *Fusarium* species such as *Fusarium graminearum, F. culmorum* and *F. avenaceum* which are found predominantely under European climate conditions (Leplat et al., 2013). During the infection of host plants, the *Fusarium* species mentioned above produce mycotoxins leading to a decline in quality of crop products (Leslie and Summerell, 2013). One of the best known *Fusarium* mycotoxins is the trichothecenes class which includes deoxynivalenol (DON) (Leslie and Summerell, 2013). Deoxynivalenol is the mycotoxin most frequently produced by *F. graminearum* and *F. culmorum* and therefore often detected in cereals (Leslie and Summerell, 2013; 2005; Pestka, 2007). An acute health risk of DON is given after consumption of large amounts of DON within a short time frame, resulting in vomiting and feed refusal for animals and gastroenteritis with vomiting for humans (Bianchini et al. 2015). A chronic exposure to DON causes growth retardation, alters immune function and interferes with reproduction and development (cited in Bianchini et al. 2015). Furthermore, DON causes negative effects on eukaryotic cells including the inhibition of protein synthesis (Rotter et al., 1996). In addition to the health threat of DON, there is an increasing number of reports about the importance of DON and other mycotoxins as potential environmental contaminants (Bucheli et al. 2008; Gautam and Dill-Macky 2012; Hartmann et al. 2008b). Leaching of mycotoxins from host tissue like infected plants or residual material has been considered an environmental threat since mycotoxins including DON were detected in soil, drainage water and soil water which percolates to the ground water table (Hartmann et al. 2008a; Kolpin et al. 2014). The ecotoxicological consequences are so far unknown. Nevertheless, it cannot be excluded that the exposure of DON has adverse effects on soil quality and soil biota. For example, Fornelli

et al. (2004) reported insecticidal effects induced by certain trichothecenes Schrader et al. (2009) demonstrated the take-up of DON by earthworms (*L. terrestris*) after feeding on *Fusarium*-infected crop residues and the incorporating of DON into their tissue (gut tissue and body wall), which might have unfavourable effects unknown to date..

Among fungivorous soil organisms, there are representatives of the soil fauna which are obviously antagonistic to a *Fusarium* infection. Oldenburg et al. (2008) and Schrader et al. (2009) demonstrated an accelerated incorporation of *Fusarium*-infected and DON-contaminated wheat straw into soil through the activity of the detritivorous earthworm species *Lumbricus terrestris*. The earthworms seemed to be more attracted to the contaminated straw than to the non-contaminated control straw. An increased feeding activity of earthworms was also reported by Moody et al. (1995) when earthworms fed on fungal infested crop residues. Furthermore, microcosm field-studies with earthworms of different ecological groups revealed anecic detritivorous earthworms like *L. terrestris* as the main drivers with respect to *Fusarium* degradation and DON reduction in contaminated wheat straw (Wolfarth et al. 2011a). The influence of the endogeic species *Aporrectodea caliginosa* was reported to be minor (Wolfarth et al. 2011a).

Besides earthworms, fungi feeders within the soil faunal community also contribute to repression of *Fusarium* infestation and the degradation of mycotoxins (e.g., DON) in crop residues. Due to their notable contribution to decomposition processes in agroecosystems and their influence on fungal succession and the altering of soil saprotrophic fungal communities through grazing, collembolans are considered to play an important role in the biological control of fungal plant pathogens (Klironomos et al. 1992; Klironomos and Kendrick 1995; Lartey et al. 1994).

Nematodes are very abundant in agricultural soils (Yeates and Bongers 1999) and most of the soil-inhabiting nematode species are involved in beneficial ecosystem processes (Neher, 2001). In general, microbial grazing of nematodes results in greater metabolic activity and alters microbial communities, thus regulating rates of decomposition and nutrient mineralization. Particularly bacterivorous and fungivorous nematodes play important roles in controlling soil microbial activity and enhancing the availability of plant nutrients. It has been shown that fungivorous nematodes are able to control soil-borne plant pathogenic fungi like *Fusarium moniliforme* (now known as *F. verticilliodes*) (Gupta 1986) and *Fusarium*

oxysporum (Okada 2006). Recently, Wolfarth et al. (2013) examined the activity of nematodes and collembolans in terms of controlling plant pathogens and their mycotoxins under laboratory conditions and found that species interaction between the fungivorous nematode *Aphelenchoides saprophilus* and the collembolan species *Folsomia candida* significantly enhance the degradation of DON concentration in wheat straw. However, more complex investigations under field conditions on a larger scale concerning three different members (earthworms, collembolans and nematodes) of the soil food web in terms of mycotoxin regulation are missing up to now. Furthermore, the presence and potential outcome of this faunal combination under field conditions are not yet known.

Therefore, mesocosm-based field studies were conducted to contribute to the understanding of the linkage between earthworms (*L. terrestris*), collembolans (*F. candida*) and nematodes (*A. saprophilus*) and their potential role in providing ecosystem services like mycotoxin degradation in agroecosystems. The introduced species of the present study (*L. terrestris*, *F. candida* and *A. saprophilus*) are well known, widespread and occur in arable soils. Moreover, they serve as bioindicators and represent essential elements of the soil food web as fungal feeders.

Our hypotheses were: (1) Anecic earthworms contribute to the regulation of the mycotoxin deoxynivalenol (DON) in contaminated wheat straw (2) The degradation efficiency of anecic earthworms is influenced by the presence of collembolans and nematodes.

Materials and methods

Environmental conditions and site description

The field experiments in 2011 and 2013 were conducted 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}.

In 2011 and 2013, an experiment was conducted, respectively, in a winter wheat field where conservation tillage had been practised for more than 20 years (machines used: disc harrow, grubber, ring roller). 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 14% clay, 80% silt and 6% sand resulting in a silt loam. In 2011, the average air and soil temperature during the experiment was 10.7°C and 10.4°C respectively, whereas precipitation

was 45 mm during the experimental time span of 8 weeks. In 2013, the average air and soil temperature was 12.5°C and 11.4°C, whereas precipitation was 153 mm during the same experimental time span.

Soil

Topsoil of the Ap horizon was collected from the agricultural field described above and stored at 4°C until further treatment. Approximately ten days before filling the mesocosms, the soil was defaunated by freezing at -20 °C for 24h and thereafter thawed at room temperature for 24h. After two freezing-thawing cycles, the soil was additionally autoclaved to eliminate all organisms, including microorganisms, which were initially present. For details see Wolfarth et al. (2013). Afterwards, the soil was macroscopically cleared of organic plant residues like straw or roots and sieved (mesh size 2 mm). The C_{org} content of the soil was 1.2% and total N was 0.13%. To reinoculate microbial populations, 100 g of autoclaved soil was moistened with 5 ml filtered (No. 42 Whatman filter paper; W.O. Schmidt Laborbedarf GmbH, Braunschweig, Germany) soil extract, derived from the same soil prior to defaunating and autoclaving (van Vliet et al. 2004). At the beginning of the experiment in both years, the soil water content was 22% (w/w), equivalent to 59% of water holding capacity.

Straw

Winter wheat (*Triticum aestivum cv.* Ritmo) was cultivated at the site of the Julius Kühn-Institute, Braunschweig (Germany) on two experimental field plots of 4 m^2 each in 2011 and 2013 to gain the DON-contaminated and control straw required for the experiments. To promote *Fusarium* infection and DON production in winter wheat, one plot had been spray-inoculated with 400 ml fungal spore suspension made of three strains of *Fusarium culmorum* at flowering in June of both years as described by Wolfarth et al. (2013). For more details see Oldenburg et al. (2008) and Schrader et al. (2009). Winter wheat straw harvested from the second plot which was not artificially inoculated with *Fusarium culmorum* (in the following called "non-contaminated") served as a control. The concentration of deoxynivalenol (DON) in straw harvested from the inoculated plot was 318.56 ± 41.33 mg kg^{-1} in 2011. At harvest in 2013, a lower DON content of 40.63 ± 11.04 mg kg^{-1} was determined in the wheat straw from the inoculated plot due to weaker infection level of the plants.

DON content in the control straw resulting from natural *Fusarium* infection was low (2.54 ± 0.42 mg kg^{-1} and 0.40 ± 0.15 mg kg^{-1} in 2011 and 2013, respectively). The aim was to gain high differences in the DON concentration level between the control straw and the

contaminated straw which was fully achieved. Both straw types were chopped to approximately 1-2 cm length which 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.

Soil fauna

The detritivorous earthworm species *Lumbricus terrestris* (L.t.) was purchased from a commercial supplier (Superwurm e.K., Düren, Germany). Adult individuals (160) of *L. terrestris* were adapted to the soil conditions for 10 days by keeping them in plastic jars at 15°C (±1°C) containing the soil described above. During adaption, non-contaminated wheat straw served as food substrate. Before they were placed into the experimental mesocosms, the earthworms were washed with water to remove adhesive residues and mucus.

The collembolan species *Folsomia candida* (F.c.) used for the experiment originated from own laboratory mass cultures. *F. candida* was reared on a mixture of moist plaster of Paris and charcoal (9:1) and fed with brewer´s yeast and carrots twice a week. According to Pfeffer and Filser (2010) only young adults of the same age and size were introduced to the experimental system after starving for 24 h.

The fungal feeding nematode species *Aphelenchoides saprophilus* (A.s.) was obtained from own laboratory mass cultures fed with the mycorrhizal fungus *Laccaria laccata* (Scop.: Fr.) Cooke. Before introducing the nematodes to *L. laccata* for cultivation, the fungus was grown on Pachlewska agar of own production at 17°C in darkness. A detailed description of this method is given in Ruess et al. (2002). Nematodes were extracted from agar for 24 h at room temperature by the Baermann funnel method.

Establishment of the mesocosms

The experimental units (mesocosms) were cylinder-shaped bags made of nylon-gauze of 12 cm x 40 cm in size (diameter x height). A mesh size of 15 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, 4.8 kg of soil were filled in each mesocosm resulting in a 25 cm high soil column with a bulk density of 1.30 g cm^{-3}, which represented real field conditions. Portions of 8 g air-dried wheat straw per mesocosm were moistened by spraying with water (3 ml) and added as a layer in close contact with the soil

surface. This amount of straw is equivalent to 7 t ha^{-1} which represents common mulching conditions under reduced tillage.

Experimental design

The experiment was carried out during a period of 4 and 8 weeks (2 sampling dates). In 2011 the experimental time span was from the beginning of September to the beginning of November, in 2013 from mid-August to mid-October. In total, there were 100 mesocosms in each experimental year. One set of 50 mesocosms was applied with DON-contaminated straw and another set (50 mesocosms) received non-contaminated straw. Each set was divided into two subsets (25 mesocosms) for two sampling dates (see above). Five treatments with five replicates were prepared for each treatment: first treatment with *L. terrestris* (2 individuals), second treatment with *L. terrestris* (2 individuals) and *F. candida* (250 individuals), third treatment with *L. terrestris* (2 individuals) and *A. saprophilus* (1500 individuals), fourth treatment with *L. terrestris* (2 individuals), *F. candida* (250 individuals) and *A. saprophilus* (1500 individuals) and a fifth treatment without soil fauna. The collembolans were counted from petri dishes with an exhauster and introduced below the straw layer on the soil surface of the mesocosms. Nematodes were extracted from the culture dishes (Baermann funnel method, see below) and transferred into water, from where they were counted with a disposable pipette and introduced below the straw layer on the soil surface of the mesocosms. The number of collembolans corresponds to a density of 22 000 individuals m^{-2} (Tischler, 1965) whereas the individual number of introduced nematodes is equivalent to 130 000 individuals m^{-2} which is based on data from Stinner and Crossley (1982). Additional small quantities of soil (100 g) and straw (50 g of contaminated and non-contaminated straw, respectively) were taken from the 0-5 cm layer of the mesocosms, retained and stored at –20°C until further processing for detecting the initial DON concentrations. The mesocosms were supplied on average with approximately the same earthworm biomass per treatment. The mean earthworm biomass was 7.3 – 8.2 g per mesocosm. After the introduction of soil fauna, the nylon-gauze of each mesocosm was closed with a clip which kept the straw in place and prevented soil fauna from escaping and entering.

To prepare the establishment of the experiment in the field, 100 pits were drilled 30 cm deep in the ground with a hydraulic soil driller. The pits were placed in four lines of 50 m in length. The interval between each pit was 2 m whereas the distance between each line was 5 m, to avoid drilling in wheeling tracks. Since we assume all abiotic and biotic conditions at the

experimental field to be homogeneous, a complete randomised design was used. The mesocosms were randomly introduced into the prepared pits in close contact to the surrounding soil whereupon the soil surface of the mesocosms was at the same surface level as the surrounding field.

Sampling and sample processing

At the end of the experiment, the mesocosms were cautiously excavated and transported to the laboratory. Collembolans and nematodes were extracted from soil samples, which were taken from the mesocosms by means of a corer (diameter: 4cm for collembolan extraction; 2 cm for nematode extraction; depth: 0-10cm). Collembolans were extracted for 10 days using a MacFadyen high-gradient extractor (MacFadyen 1961). Nematodes were extracted at room temperature for 3 days by the Baermann funnel method. A particular description of soil fauna extraction is given in Wolfarth et al. (2013). The remaining straw of the mesocosms was removed immediately. During sampling, great care was taken to mechanically separate adhesive soil from straw. Finally, the earthworms were removed, put into cold water to carefully clean off soil particles and weighed individually to determine their biomass for comparison with their initial biomass. We did not place the earthworms on filter paper in order to standardise their weight because gut voiding may weaken the earthworms and is not always recommended (Fründ et al., 2010). Soil samples were taken from the 0-5 cm layer of the mesocosms. Soil and straw samples were then stored at –20°C in readiness for analytical preparation.

All samples, including the parent materials at the start of the experiment, were treated by freeze-drying for 24 h. Straw was ground using a mixer mill (MM 400, Retsch GmbH, Haan, Germany). Dried samples of soil were manually homogenized with a mortar to obtain a fine powder (< 0.5 mm).

Determination of deoxynivalenol

The deoxynivalenol (DON) concentrations were analysed 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) and Schrader et al. (2009). The initial sample weight was 1 g for wheat straw and soil. The limits of quantification were 37 µg DON kg^{-1} for soil and non-contaminated straw and 74 µg DON kg^{-1} for contaminated wheat straw.

Statistics

The Kolmogorov-Smirnov test was applied to check data for normality. Variance homogeneity was tested by using the Levene test. Data for population density of collembolans and nematodes were normally distributed with equal variances. For the data sets of DON concentration and earthworm-biomass normality and variance homogeneity was violated. For the population density of collembolans and nematodes a univariate analysis of variance (ANOVA) was carried out to compare treatment effects of soil fauna (*L. terrestris* + *F. candida*, L.t + *A. saprophilus*, L.t + F.c + A.s), DON contamination (contaminated, non-contaminated) and sampling date (start, mid-term sampling [4 weeks] and end of the experiment [8 weeks]) on population density of collembolans and nematodes. Bonferroni correction was chosen as adjustment level. To assess the relationship of individual numbers of collembolans and nematodes of the different experimental years, the Pearson correlation coefficient (R) was calculated. The nonparametric Wilcoxon test was conducted for the data of earthworm biomass. In the case of DON, the nonparametric Kruskal-Wallis test was implemented to determine treatment effects of soil fauna, DON contamination and date on the DON concentration. For multiple comparisons within the factor "fauna" a Mann-Whitney test was carried out. All data are presented as arithmetic means + standard errors (SE). All statistical analyses were done using the software package SPSS for Windows version 22.

Results

Biomass and individual density of soil fauna

Earthworms

In 2011 a loss of 18 out of 160 individuals of *L. terrestris* was recorded resulting in a recapture rate of 89%. The number of losses was equally distributed among all treatments. After 4 weeks there was a reduction of the initial earthworm biomass throughout all mesocosms (-2% to -12%) (Fig. 4.2.1) which was significant for the contaminated treatments ($U = 2.017$; $P = 0.044$). Only in the non-contaminated treatments containing collembolans and nematodes did the biomass of *L. terrestris* increase (+8%) (Fig. 4.2.1). After 8 weeks, the reduction of earthworm biomass ranged between -2 and -8% or the biomass increased (L.t. + A.s.) compared with the initial biomass. According to the Wilcoxon test this reduction was significant for the non-contaminated treatments ($U = 2.201$; $P = 0.028$).

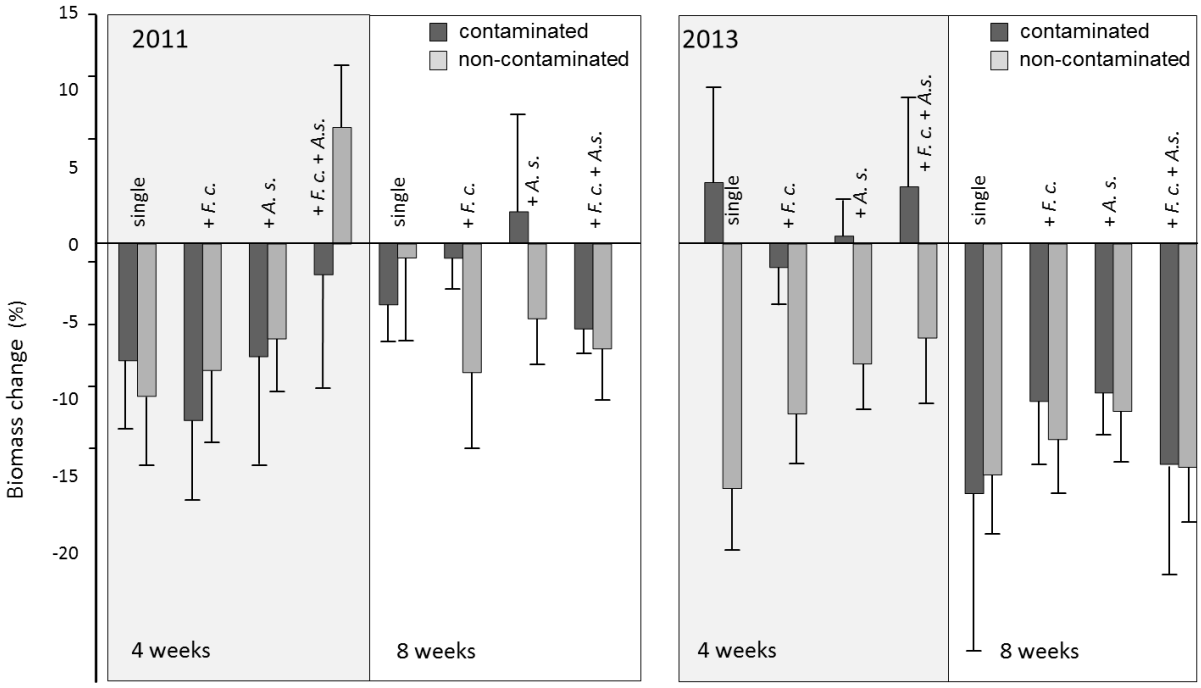

Fig. 4.2.1: Mean relative change of biomass (±SE) of *L. terrestris* when fed on DON-contaminated or non-contaminated wheat straw in both experimental years (2011 and 2013) after 4 and 8 weeks separated into different faunal treatments: *L. terrestris* (single), *L. terrestris* and collembolans (+ F.c.), *L. terrestris* and nematodes (+ A.s.) or all three species in mixture (+ F.c. + A.s.).

In 2013 only 4 out of 160 individuals of *L. terrestris* were missing at the end of the experiment, which is a recapture rate of 98%. The number of losses was equally distributed among all treatments. After 4 weeks, a decline (-2%) of the average earthworm weight per mesocosm compared with the initial earthworm biomass was measured only in treatments with collembolans receiving contaminated wheat straw. For the remaining contaminated treatments an increase (1 to 4%) of the mean earthworm weight per mesocosm could be documented. For the non-contaminated treatments with collembolans and nematodes, respectively, as well as for the non-contaminated single species treatment, this reduction was significant (U = 3.845; P < 0.001) (Fig. 4.2.1). After 8 weeks the decline of earthworm biomass (-10 to -16%) was significant in all treatments (contaminated: U = 3.920; P < 0.001; non-contaminated: U = 3.920; P < 0.001) (Fig. 4.2.1) compared with the initial biomass.

Table 4.2.1: Mean (+SE) individual number of collembolans and nematodes per mesocosm when fed on DON-contaminated or non-contaminated wheat straw in both experimental years (2011 and 2013) at the beginning of the experiments (Start) and after 4 and 8 weeks separated in different faunal treatments: ("+L.t." = collembolans or nematodes + earthworms; "+ L.t.+A.s." = all three species in mixture; "+ L.t.+F.c." = all three species in mixture), (number of replicates: n = 5). A.s. = *Aphelenchoides saprophilus;* F.c. = *Folsomia candida*; L.t. = *Lumbricus terrestris*.

	Collembolans				**Nematodes**			
	+ L.t.		**+ L.t. + A.s.**		**+ L.t.**		**+ L.t. + F. c.**	
Individual number	**4 weeks**	**8 weeks**	**4 weeks**	**8 weeks**	**4 weeks**	**8 weeks**	**4 weeks**	**8 weeks**
Start	250	250	250	250	1500	1500	1500	1500
2011								
Contaminated	**3791** ±1232	**6316** ± 2088	**4574** ± 1808	**6923** ± 2076	**14572** ± 3417	**5343** ± 1541	**5630** ± 1956	**4457** ± 1400
Non-contaminated	**1130** ± 496	**1604** ± 642	**1224** ± 319	**3062** ± 1291	**4602** ± 1424	**2766** ± 671	**10894** ± 2964	**2742** ± 657
2013								
Contaminated	**1873** ± 774	**1389** ± 297	**1046** ± 433	**3732** ± 1050	**17963** ± 3495	**4205** ± 1282	**5673** ± 1573	**4220** ± 1204
Non-contaminated	**1898** ± 280	**2287** ± 650	**1287** ± 223	**4799** ± 665	**9542** ± 3131	**8726** ± 2919	**4589** ± 1277	**6784** ± 1376

Collembolans

The initial numbers of collembolans per mesocosm (start, after 4 weeks and after 8 weeks) are given in Table 4.2.1. In 2011, ANOVA revealed significant effects for the factor "contamination" (F = 13.392; P = 0.001). The individual numbers of Collembolans per mesocosm in both treatments (contaminated and non-contaminated) increased after 4 weeks and continued to increase until the end of the experiment after 8 weeks. However, the population density in the contaminated treatments was significantly higher than in the non-contaminated mesocosms. In 2013, significant effects were recorded for the factor "date" (F = 12.548; P = 0.001) as well as for its interaction with "fauna" (F = 13.344; P = 0.001). Compared with 2011, a divergent pattern of the population density of collembolans was observed in 2013 (Table 4.2.1). The increase of the individual numbers of collembolans of the contaminated treatments was not as high as in 2011. The development of population density of collembolans in the non-contaminated treatments was quite similar and not significantly different compared with the contaminated treatments (Table 4.2.1).

A strong correlation (R = 0.979) of the mean individual numbers of collembolans in the non-contaminated treatments between 2011 and 2013 was calculated, whereas the mean individual numbers of *F. candida* in contaminated treatments of both experimental years correlated weakly (R = 0.584).

Nematodes

The initial numbers of nematodes per mesocosm (start, after 4 weeks and after 8 weeks) are presented in Table 4.2.1. In 2011 significant effects were recorded for the factor "date" (*F* = 13.185; *P* = 0.001) and, interaction of the factors "fauna" and "contamination" (*F* = 8.218; *P* = 0.007) and the interaction of all three factors (*F* = 6.55; *P* = 0.015). After 4 weeks of field exposure, the individual density of *A. saprophilus* increased significantly throughout all treatments (Table 4.2.1). After 8 weeks the mean individual numbers of nematodes were significantly lower compared with the population density after 4 weeks. In 2013, ANOVA revealed significant effects for the factors "fauna" (*F* = 8.364; *P* = 0.007) and "date" (*F* = 4.987; *P* = 0.033) as well as for their interaction (*F* = 5.288; *P* = 0.028) and the interaction of the factors "contamination" and "date" (*F* = 6.094; *P* = 0.019). Again the highest number of *A. saprophilus* was found in the contaminated treatments with additional *L. terrestris*. After 8 weeks the population density of nematodes of the contaminated mesocosms was lower than that of the non-contaminated treatments (Table 4.2.1).

The population density of nematodes in non-contaminated treatments of both experimental years correlated negatively (R = -0.743), whereas a strong positive correlation (R = 0.997) of *A. saprophilus* in the contaminated treatments between 2011 and 2013 was calculated.

DON concentrations in contaminated wheat straw and in soil

Regarding the DON concentration in wheat straw, the Kruskal-Wallis test revealed significant effects for all three factors ("fauna", "contamination" and "date") in both experimental years (Table 4.2.2).

Table 4.2.2: *H*-values, *P*-values and degrees of freedom (df) of Kruskal Wallis test on the effects of soil fauna (F) *(L. terrestris*, L.t. + *F. candida*, L.t. + *A. saprophilus* or L.t. + F.c. + A.s. in mixture), DON contamination (C) (contaminated, non-contaminated wheat straw) and date (D) (start, mid-term sampling after 4 weeks and end of the experiment after 8 weeks) on the DON concentration in wheat straw.

	df	2011		2013	
		H	***P***	***H***	***P***
Fauna (F)	4	12.443*	0.014	11.914*	0.018
Contamination (C)	1	59.823***	<0.001	70.889***	<0.001
Date (D)	1	15.025***	<0.001	6.726*	0.010

* $P < 0.05$; *** $P < 0.001$

In 2011 the initial DON concentration of contaminated wheat straw was 319 ± 41 mg kg^{-1} (Fig. 4.2.2). After 4 weeks a significant reduction of DON concentration was measured throughout all mesocosms, whereas the decline in the faunal treatments was higher (97-99%) compared to the DON reduction in the non-faunal control treatments (88%). After 8 weeks a further decrease of the DON concentration was determined only in mesocosms containing soil fauna (58-91%). In the non-faunal control treatments an increase of DON concentration was determined compared with the DON concentration after 4 weeks (Fig. 4.2.2). The DON concentration of the control treatments differed significantly from the treatments containing soil fauna. But no statistical differences could be determined between the faunal treatments.

In 2013 the reduction of DON concentration was similar to that in 2011. The initial DON concentration of 41 ± 11 mg kg^{-1} in contaminated wheat straw was reduced significantly throughout all treatments. The decline of DON concentration in the faunal treatments was

higher (78-94%) compared with the non-faunal control treatments, where DON was reduced by 68% (Fig. 4.2.2). After 8 weeks, DON concentration continued to decrease in mesocosms containing soil fauna (50-86%), whereas DON content of the non-faunal control treatments increased considerably compared with the DON concentration after 4 weeks. The control treatments differed significantly from the treatments containing soil fauna. But no statistical differences could be determined between the faunal treatments.

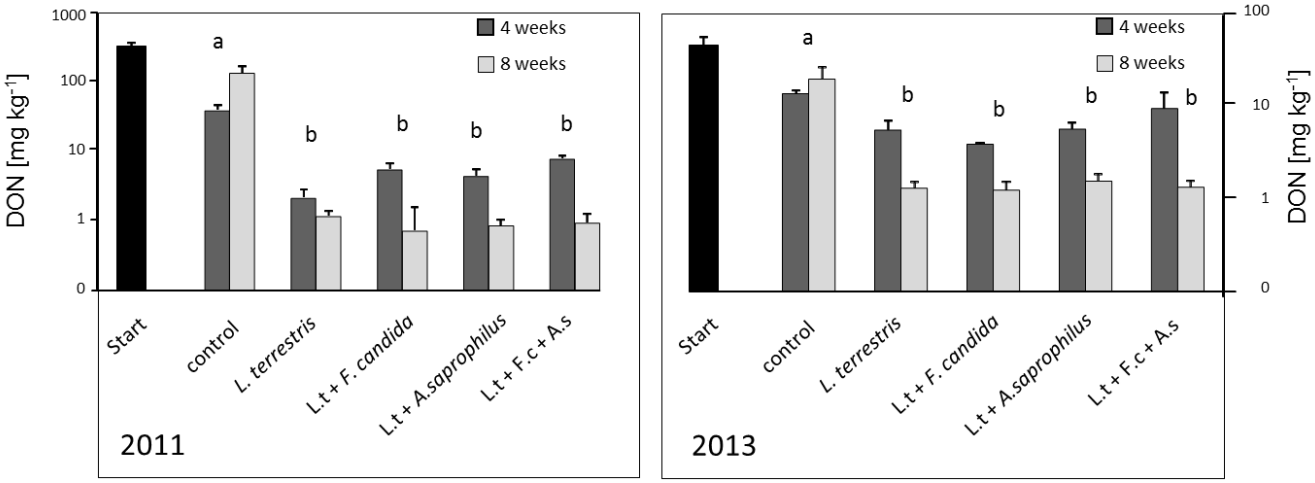

Fig. 4.2.2: Logarithmic design of mean (+SE) concentrations of DON (deoxynivalenol) (mg kg^{-1}) in contaminated wheat straw of mesocosms inoculated with soil fauna species *L. terrestris*, *L. terrestris* and collembolans (L.t + *F. candida*), *L. terrestris* and nematodes (L.t + *A. saprophilus*) or all three species in mixture (L.t. + F.c. + A.s.) after 4 and 8 weeks of inoculation separated for the different experimental years (2011 and 2013) (number of replicates: n = 5). Note the difference in the scale between 2011 and 2013. Different letters indicate bars to be significantly different ($P < 0.05$).

In both years and in all soil samples, the DON concentration in soil was below the quantification limit (0.037 mg kg^{-1}).

Discussion

Results of both experimental years concerning the earthworm biomass are mostly in line with previous investigations (Oldenburg et al. 2008; Schrader et al. 2009; Wolfarth et al. 2011a; Wolfarth et al. 2011b), where a higher decline of earthworm body weight was recorded when fed on non-contaminated control straw compared to earthworms receiving *Fusarium*-infected wheat straw. This finding applies to 11 out of 16 cases in the present study (2011 and 2013). Furthermore, the body weight loss for the introduced earthworms was less than 20 % in every treatment of both experimental years (Fig. 4.2.1), which indicates appropriate experimental conditions with high validity of the results (Fründ et al. 2010). A heterogeneous development

was observed with regard to the population density of collembolans and nematodes (Table 4.2.1). The development of individual numbers of collembolans and nematodes was possibly also influenced by different weather conditions during both experimental seasons. In general, the average air and soil temperature during the experimental time span in 2011 was approximately 2°C lower than during the experiment in 2013 and there was less precipitation. However, the mean individual numbers of collembolans in the non-contaminated treatments of 2011 correlated strongly positive (R = 0.979) with the mean individual numbers of collembolans in the non-contaminated treatments of 2013. This correlation suggests that collembolans might develop better in non-contaminated treatments. In contrast, for *A. saprophilus,* the individual numbers of nematodes in the contaminated treatments of 2011 correlated strongly with the mean individual numbers of nematodes in the contaminated treatments of 2013, leading to the assumption that nematodes benefited from contaminated wheat straw.

With regard to mycotoxin degradation in wheat straw, the Kruskal Wallis test revealed a significant effect of the introduced soil fauna in reducing DON in both experimental years (Table 4.2.2), but no statistical differences could be detected between the different soil fauna treatments (Fig. 4.2.2). Wurst et al. (2012) emphasised that across different size classes the composition of community and the traits of key species appear to be significant contributors of soil processes and function. In this context, our first hypothesis that earthworms contribute to the regulation of the mycotoxin deoxynivalenol (DON) in contaminated wheat straw can be confirmed. *L. terrestris* as an anecic detritivorous earthworm species has obviously been the driver of the degradation process and seemed to be the crucial factor in reducing DON in a cropping system with reduced tillage conditions.

In contrast to a previous investigation (Wolfarth et al. 2013), where significant interaction effects concerning the reduction of DON were found by the introduced collembolans and nematodes under artificial laboratory conditions, the current results are based on field situations. On account of this, the distinct combination of species of the soil meso- and microfauna (*F. candida, A. saprophilus*) seemed not to be pivotal considering the mycotoxin degradation in contaminated crop residues. Hence, our second hypothesis that the degradation efficiency of earthworms is influenced by the presence of collembolans and nematodes must be declined. Furthermore, these results are also in line with findings of Wolfarth et al. (2015),

who reported the activity of *F. candida* and *A. saprophilus* in single species treatments to dominate the degradation of DON in finely ground wheat straw under field conditions.

With regard to the DON concentration in the soil of the mesocosms we cannot confirm findings of other authors (Hartmann et al., 2008 a; 2008b; Kolpin et al. 2014), who detected DON in soil which was leached from infested plant material. Concentrations of DON in soil of all treatments were below the quantification limit (<37 µg kg^{-1}) in both experimental years. Consequently, we suppose that *L. terrestris* reduced DON concentration in wheat straw directly by litter decomposition and by incorporating infected plant material into the soil. On the other hand a considerable amount of DON in straw and potentially also in soil was most likely degraded due to metabolic interactions between earthworms and soil microorganisms (Oldenburg et al. 2008; Schrader et al. 2009; Wolfarth et al. 2011a). In fact, there is a general consensus that earthworm activity like casting and litter collection increase population densities of beneficial microbes by providing improved conditions of microclimate and habitat structure (Brown et al. 2000; Elmer and Ferrandino 2009; Schrader and Seibel 2001). These beneficial microbes may help to decrease the DON concentration by one or more different mechanisms, which are not yet understood. Moreover, there is evidence that biological control of pollutants is not based on single effects but on reciprocal effects of different soil organisms which are already inhabitants in soils (Sabatini and Innocenti, 2001). For instance, the mineralisation of DON into a less toxic compound (3-keto-DON) by soil bacteria was reported by Shima et al. (1997) and Ikunaga et al. (2011). Furthermore, Elmer (2009) showed that earthworms may increase the population density of fluorescent pseudomonads and filmentous actinomycetes which have been implicated in disease suppression (Mazurier et al. 2009; Lemanceau and Alabouvette 1993).

According to the present results earthworms revealed a great potential to reduce the risk of environmental pollutants as an important ecosystem service for soil health. Doran and Zeiss (2000) characterised healthy soil as a living, dynamic system whose functions are mediated by the diversity of living organisms to sustain biological productivity, promote environmental quality, and maintain plant and animal health.

Conclusions

The results of the present investigation show that *L. terrestris* significantly enhances the degradation of the mycotoxin DON in *Fusarium*-infected wheat straw under field conditions.

The degradation performance of the introduced earthworms must be considered as a crucial agroecosystem service for wheat production, which is of increasing relevance since nutrient mobilization and turnover and biotransformation of organic pollutants are essential from the perspective of global change and sustainable soil fertility (Thiele-Bruhn et al. 2012). In the last 15 years the percentage of arable land used to grow maize has increased by almost 50% in Germany (Federal Statistical Office 2010). This trend is leading towards less diverse and shorter crop rotations (Karlen et al. 1994), which increases diseases risks induced by soil and straw borne pathogens like *Fusarium* species. Against this background, studies on plant pathogen repression and mycotoxin regulation on maize stubbles or maize residues by earthworms should be taken into account in the near future. Furthermore, investigations under practical plant production conditions should also be considered, studying the effects of the natural inhabiting fungivorous soil fauna in an agricultural field after a *Fusarium*-infection of the cultivated crop.

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Chapter 5

5 The role of fungivorous soil organisms in repression of fungal plant pathogens

5.1 Repression of the soil borne plant pathogen *Fusarium culmorum* by soil organisms in an agroecosystem

Unpublished results

Abstract

In 2011 and 2013, several field studies were conducted to investigate biocontrol and interaction effects of key stone species (*Lumbricus terrestris*, earthworms; *Folsomia candida*, collembolans and *Aphelenchoides saprophilus*, nematodes) of the soil food web on the plant pathogenic fungus *Fusarium culmorum* in wheat straw. The soil fauna was introduced in minicontainers (collembolans and nematodes) and mesocosms (earthworms, collembolans and nematodes) in different numbers and combinations. The soil fauna was exposed to either *Fusarium*-infected or non-infected wheat straw. Minicontainer (in 2011) and mesocosms (in 2011 and 2013) were established in the topsoil of a winter wheat field after harvest. After 2, 4, and 8 weeks, biomass of *Fusarium culmorum* was detected in samples of soil and wheat straw using double antibody sandwich (DAS)-ELISA method. Furthermore, collembolans and nematodes were counted and biomass of earthworms was determined.

In minicontainers, the content of Fusarium biomass was reduced significantly throughout all treatments after 2 weeks. After 4 weeks of minicontainer exposure *Fusarium* biomass decreased significantly in treatments containing collembolans (98%) and nematodes (97%) in single culture.

After 4 weeks of mesocosms inoculation, *Fusarium* biomass was reduced significantly in all treatments: faunal treatments 2011: 97-98%; 2013: 48-54%; non-faunal treatments 2011: 95%; 2013: 14%. After 8 weeks *Fusarium* biomass of the faunal treatments was below the quantification limit in 2011. In 2013, a further decline of *Fusarium* biomass was measured (76-85%) but the highest content of *Fusarium* biomass was still found in the non-faunal treatments.

The results demonstrate the potential of earthworms, collembolans and nematodes as biocontrol agents. Furthermore, the introduced soil fauna contribute to a sustainable control of fungal plant pathogens in wheat straw, thus reducing the risk of plant diseases as an important ecosystem service for soil health.

Keywords: Plant pathogen repression, Biocontrol, Soil health, Ecosystem services, Functional soil biodiversity

Introduction

Most fungi are decomposers that feed on decaying organic material and play critical roles in the decomposition of organic polymers of soils. Besides their important contribution to primary decomposition in forests, where litter contains high concentrations of complex polymers, they also play an important role in arable soils by breaking down plant residues (Leplat et al., 2013), which were left at the soil surface for example as a measure of conservation tillage. Among decomposing soil borne fungi, fungal plant pathogens colonise on crop residues and 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). It is estimated that over 40% of the preharvest yield of the eight most important crop species is lost due to disease and pest damage and the global food production losses to plant disease have been assessed at 10% (Cheatham et al., 2009). The fungal disease complex Fusarium head blight (FHB) is one of the most noxious diseases worldwide, which causes significant yield losses in maize and cereals such as wheat, oat and barley (Parry et al., 1995; Pereyra and Dill-Macky, 2008, Vogelgsang et al., 2011). Fusarium head blight is caused by several *Fusarium* species. Under European climate conditions *Fusarium graminearum, F. culmorum* and *F. avenaceum* are found predominantly (Leplat et al., 2013).

Fungal feeding representatives of the soil fauna are obviously antagonistic to a *Fusarium* infection. Oldenburg et al. (2008) and Schrader et al. (2009) demonstrated an accelerated incorporation of *Fusarium*-infected and DON-contaminated wheat straw into soil through the activity of the detritivorous earthworm species *Lumbricus terrestris*. The contaminated straw seemed to be much more attractive for *L. terrestris* than the non-contaminated control straw. Those findings are in line with results by Moody et al. (1995), who found increased feeding activity of earthworms on fungal infested crop residues. Besides earthworms other fungi feeders within the soil faunal community also contribute to repression of *Fusarium* infestation and the degradation of mycotoxins (DON) in crop residues. Collembolans are considered to play an important role in biological control of fungal plant pathogens due to their notable contribution to decomposition processes in agroecosystems and their influence on fungal succession and the altering of soil saprotrophic fungal communities through grazing (Klironomos et al. 1992; Klironomos and Kendrick 1995; Lartey et al. 1994). Larsen et al. (2008) even demonstrated that *F. culmorum* is a palatable food source for the collembolan species *Folsomia candida* and *Folsomia fimetaria*.

Furthermore, nematodes play critical roles in controlling turnover and structure of soil microbial communities (Yeates 2003). It has been shown that fungivorous nematodes are able to control soil-borne plant pathogenic fungi like *Fusarium moniliforme* (Gupta 1986) and *Fusarium oxysporum* (Okada 2006). However, exploring the interaction effect between key stone species of different functional groups concerning pathogen repression under field conditions has been neglected up to now.

Therefore, field experiments were performed to assess the impact of the anecic earthworm *L. terrestris*, the collembolan species *Folsomia candida* and the fungivorous nematode *Aphelenchoides saprophilus* on plant pathogen repression under on-farm conditions. The hypotheses were: (1) the anecic earthworm species *L. terrestris* contribute to the control of the plant pathogenic fungus *Fusarium culmorum* in infected wheat straw under field conditions; (2) *F. candida* and *A. saprophilus* reduce the biomass of the soil-borne plant pathogenic fungus *Fusarium culmorum* in infected wheat straw under field conditions; (3) the degradation efficiency of *L. terrestris* is influenced in the presence of collembolans and nematodes.

Material and methods: Minicontainer

In 2011 a field study based on minicontainers (Eisenbeis et al. 1995; 1999) was conducted in a winter wheat field in Northern Germany. The procedure and a detailed site description are given in capture 4.1.

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) and Schrader et al. (2009). Samples of 100 mg of wheat straw and soil were taken for the assay. The limits of quantification were 78 µg FPE kg^{-1} for all samples.

Statistics

A general linear model with negative binomial distributed errors was performed for the data set of *Fusarium* biomass. Analysis of variance was carried out to assess the effects of the factors “date”, “fauna” and “infection”. All two- and three-way interactions of these factors were included. To assess treatment effects of the significant factor “fauna”, pairwise comparisons were conducted. All statistical analysis was done in R (R Development Core

Team, 2014) [using libraries lme4 (Bates et al., 2013), nlme (Pinheiro et al., 2013) and MASS (Venables & Ripley, 2002)].

Results: Minicontainer

FPE concentrations in wheat straw of minicontainer

The statistical analysis revealed significant effects on Fusarium biomass in wheat straw for all factors including their interaction (Table 5.1.1).The initial concentration of Fusarium biomass in infected wheat straw was 3365 µg kg-1 (Fig. 5.1.1). After 2 weeks FPE content was reduced significantly in all treatments (Fig. 5.1.1). In minicontainers containing F. candida, Fusarium biomass was reduced by 93%. In treatments containing A. saprophilus a reduction of 91% was recorded. The decline of FPE content of the mixed treatments was 92% whereas the highest reduction was measured in minicontainers without animals with Fusarium biomass reduced by 94%. But no significant differences could be determined between all faunal treatments (Fig. 5.1.1).

Table 5.1.1: *Fusarium* biomass (FPE) in what straw of the minicontainers. Degrees of freedom (Df), NULL Deviance, Residual Deviance and *P*-values of ANOVA on the effects of soil fauna (collembolans: *F. candida*, nematodes: *A. saprophilus*, mixed species), *Fusarium* infection (*Fusarium*-infected wheat straw, non-infected), date (2 weeks and 4 weeks).

	Df	Deviance Residual	Df	Residual Deviance	P
NULL-model			119	4928.4	
Date (D)	2	2022.41	117	2906.0	<0.001
Fauna (F)	3	19.16	114	2886.8	<0.001
Infection (I)	1	2624.91	113	261.9	<0.001
D x F	6	22.69	107	239.2	<0.001
D x I	2	80.70	105	158.5	<0.001
F x I	3	10.86	102	147.7	0.012
D x F x I	6	12.89	96	134.8	0.045

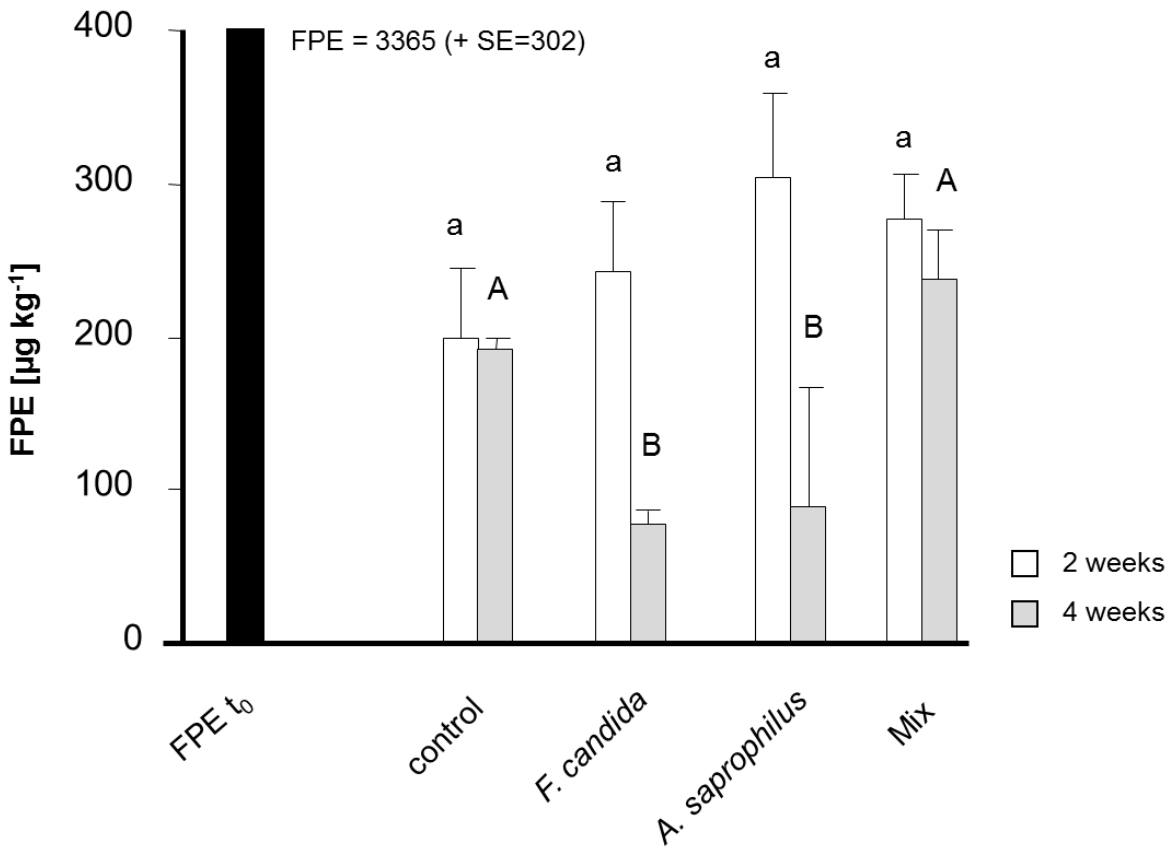

Fig. 5.1.1: Mean (+SE) concentrations of FPE (*Fusarium* Protein Equivalents) (µg kg^{-1}) in infected winter wheat straw in minicontainers inoculated with soil fauna species *F. candida* (Collembola), *A. saprophilus* (Nematoda), both species (Mix) or without soil fauna (control) at the beginning (t_0) and after 2 and 4 weeks. Different letters indicate bars to be significantly different ($P < 0.05$); small letters refer to the bars of 2 weeks and capital letters to the bars of 4 weeks.

After 4 weeks a further decrease of *Fusarium* biomass resulted in a decline of 98% for collembolan treatments compared to the initial FPE content. In minicontainers containing nematodes, *Fusarium* biomass was reduced by 97% after 4 weeks. In mixed treatments a reduction of 93% was determined. No further decrease was recorded for the control treatments without animals. Significant differences could be detected between the non-faunal control treatments and the single faunal treatments containing collembolans or nematodes, but no statistical differences were found between the control treatment and the mixed treatments (Fig. 5.1.1).

In the non-infected wheat straw the initial concentration of *Fusarium* biomass was below the quantification limit of 78 µg kg^{-1}.

FPE concentrations in soil

In the soil of the minicontainers, *Fusarium* biomass was below the quantification limit of 78 µg kg^{-1}.

Material and methods: Mesocosms

In 2011 and 2013 a mesocosm-study was conducted in an agricultural system where conservation tillage has been practised since more than 20 years. A detailed site description is given in chapter 4.2. The establishment as well as the materials and methods are also described detailed in capture 4.2.

Determination of straw cover

The area of straw covering the soil surface of the mesocosms was determined by scanning top view photographs of the mesocosms at the start, after 4 (mid-term sampling) and 8 weeks (end of the experiment) of field exposure. The specific areas of uncovered soil and those covered with straw were evaluated with the colour analysis program WinRHIZO 2002 (Régent Instruments Inc.).

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) and Schrader et al. (2009). Samples of 1g of wheat straw and soil were taken for the assay. The limits of quantification were 78 µg FPE kg^{-1} for all samples.

Statistics

For the data set of *Fusarium* biomass a general linear model (GLM) with negative binomial distributed errors was conducted. Analysis of variance was carried out to assess the effects of the factors “date”, “fauna” and “infection” on the *Fusarium* biomass in wheat straw of 2011 and 2013. All two- and three-way interactions of these factors were included. To assess treatment effects of the significant factor “fauna”, pairwise comparisons were conducted. For the data set of straw cover a linear model (LM) was performed. Furthermore, analysis of variance was carried out to assess the effects of the factors “date”, “fauna” and “infection” on the straw cover of the mesocosms in 2011 and 2013. Initially, all two- and three-way interactions between these factors were included, but the model was then simplified. Non-

significant three-way interactions were removed for the straw cover data of 2011. For the data set of 2013, non-significant three-way interactions and non-significant two-way interactions were removed. All statistical analysis was done in R (R Development Core Team, 2014) [using libraries lme4 (Bates et al., 2013), nlme (Pinheiro et al., 2013) and MASS (Venables & Ripley, 2002)].

Results: Mesocosms

Straw cover of the mesocosms

In 2011 statistical analysis revealed significant effects on the incorporation of straw for the factors "date" and "infection" (Table 5.1.2). In presence of soil fauna, the straw cover (infected or non-infected) on top of the mesocosms decreased significantly. In infected mesocosms, wheat straw was incorporated more efficiently into the soil by *L. terrestris*, as the straw cover was reduced by 12% (4 weeks) and 21% (8 weeks) respectively. Non-infected straw was incorporated to a lesser extend (4 weeks: –8%; 8 weeks: –13%) (Fig. 5.1.2).

Table 5.1.2: Straw cover of the mesocosms in 2011. Degrees of freedom, *F*-values and P-values of ANOVA on the effects of soil fauna (*L. terrestris (*L.t.), L.t. + *F. candida* (F.c.), L.t. + *A. saprophilus* (A.s.), L.t. + F.c. + A.s.), *Fusarium* infection (*Fusarium*-infected wheat straw, non-infected), date (4 weeks and 8 weeks).

	Df	F-value	P-value
Date (D)	1	17.9294	<0.001
Fauna (F)	3	0.2595	0.854
Infection (I)	1	15.5085	<0.001
D x F	3	2.7890	0.049
F x I	3	1.8759	0.144

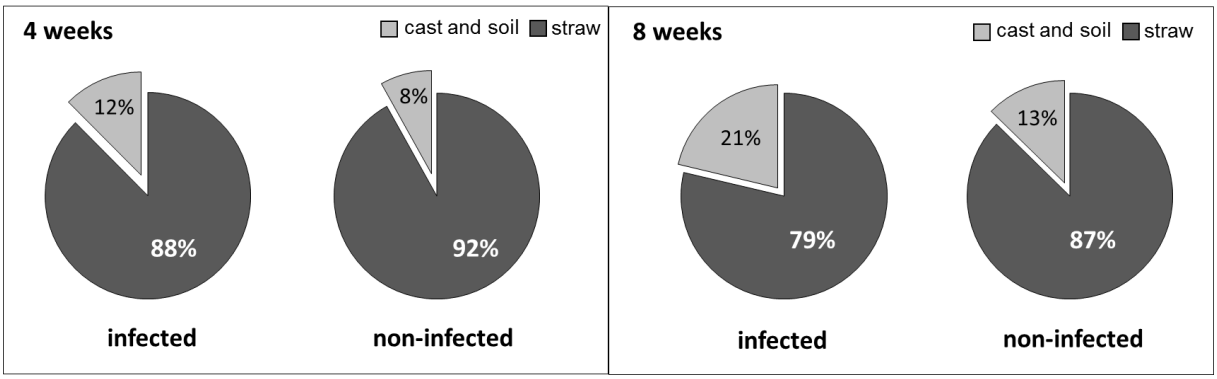

Fig. 5.1.2: Mean cover (%) of straw of all faunal mesocosms in 2011 after 4 and 8 weeks in the infected and non-infected treatments.

In 2013 significant effects on the incorporation of straw were recorded only for the factor "infection" (Table 5.1.3). The surface cover of infected straw in faunal treatments was reduced by 27% through earthworm activity after 4 and 8 weeks respectively. The surface cover of non-infected straw was reduced to a lesser degree (4 weeks: –18%; 8 weeks: –22%) (Fig. 5.1.3).

Table 5.1.3: Straw cover of the mesocosms in 2013.Degrees of freedom, *F*-values and P-values of ANOVA on the effects of soil fauna (*L. terrestris* (L.t.), L.t. + *F. candida* (F.c.), L.t. + *A. saprophilus* (A.s), L.t. + F.c. + A.s.), *Fusarium* infection (*Fusarium*-infected wheat straw, non-infected), date (4 weeks and 8 weeks).

	Df	F-value	P-value
Date (D)	1	1.1468	0.2879
Fauna (F)	3	0.3718	0.7736
Infection (I)	1	19.0890	<0.001

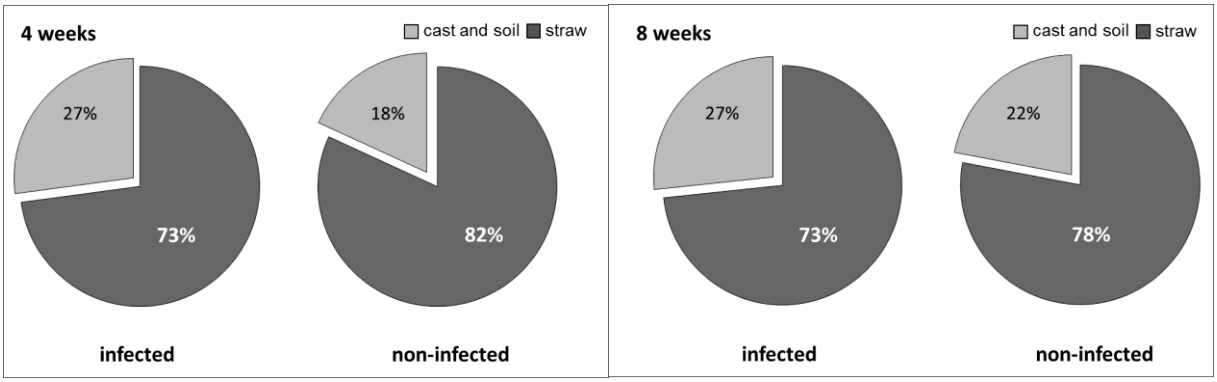

Fig. 5.1.3: Mean cover (%) of straw of all faunal mesocosms in 2013 after 4 and 8 weeks in the infected and non-infected treatments.

There was no reduction of straw coverage in treatments without soil fauna hence the soil surface cover remained 100%.

FPE concentrations in wheat straw of mesocosms

In 2011, ANOVA revealed significant effects for all factors as well as for their interactions (Table 5.1.4). The initial concentration of *Fusarium* biomass in infected wheat straw was 6,511.43 ± 172 µg kg^{-1} (Fig. 5.1.4). After 4 weeks considerable reduction of *Fusarium* biomass could be determined throughout all faunal treatments (97-98%), whereas *Fusarium*

biomass of the non-faunal control treatment was reduced least and differed significantly from all other treatments containing L. terrestris and L. t with *F. candida* in mixture (Fig. 5.1.4). After 8 weeks FPE content of the infected wheat straw of all faunal treatments was below the quantification limit of 78 µg kg-1.

Table 5.1.4: *Fusarium* biomass in wheat straw of the mesocosms in 2011. Degrees of freedom, NULL Deviance, Residual Deviance and *P*-values of the analysis of deviance on the effects of soil fauna (*L. terrestris*, L.t. + *F. candida*, L.t. + *A. saprophilus*, *L.t. + F.c. + A.s.*), *Fusarium* infection (*Fusarium*-infected wheat straw, non-infected), date (4 weeks and 8 weeks).

	Df	Deviance Residual	Df	Residual Deviance	P
NULL-model			135	18431.9	
Date (D)	2	13324.7	133	5107.3	<0.001
Fauna (F)	4	207.8	129	4899.5	<0.001
Infection (I)	1	45503.0	128	396.5	<0.001
D x F	8	93.8	120	302.7	<0.001
D x I	2	108.9	118	193.9	<0.001
F x I	4	14.5	114	179.4	0.006
D x F x I	8	38.4	106	140.9	<0.001

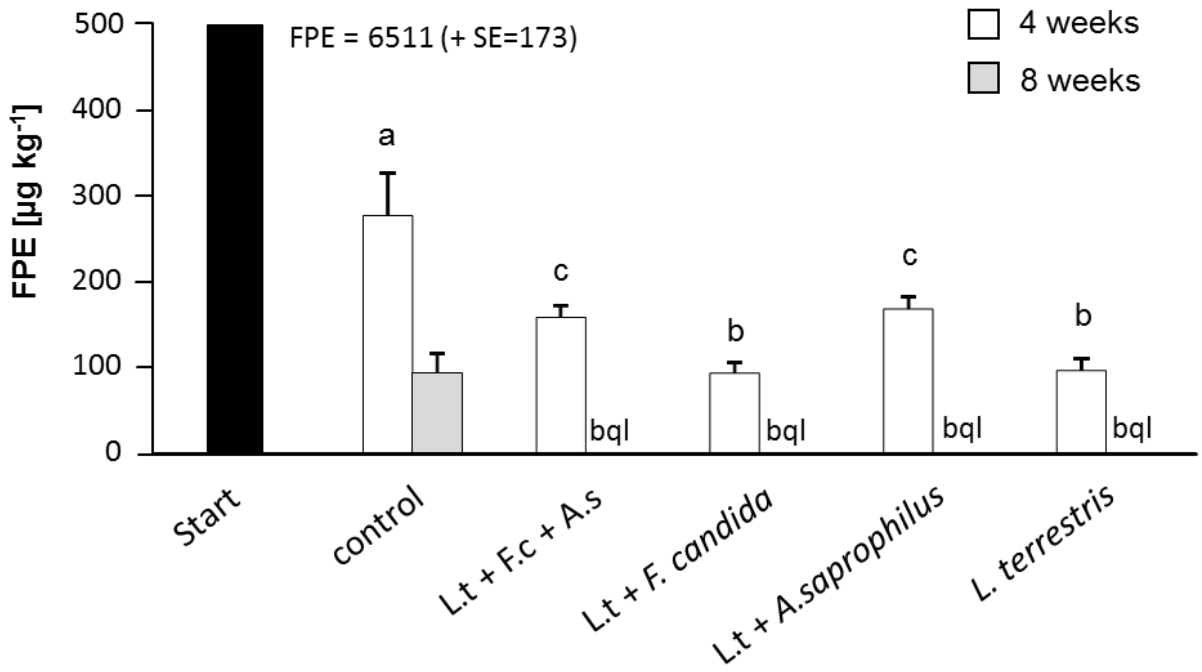

Fig. 5.1.4: Mean (+SE) concentrations of FPE (*Fusarium* Protein Equivalents) (µg kg^{-1}) in infected winter wheat straw of the mesocosms 2011 inoculated with soil fauna species *L. terrestris* (L.t.), L.t. + *F. candida* (F.c.), L.t. + *A. saprophilus* (A.s.), L.t. + F.c. + A.s. or without soil fauna (control) at the beginning (start) and after 4 and 8 weeks. Different letters indicate bars to be significantly different ($P < 0.05$). bql = below quantification limit.

Regarding FPE data of 2013, significant effects for all factors as well as for their interaction were determined (Table 5.1.5). The initial concentration of *Fusarium* biomass in infected wheat straw was 25,932 ± 926 µg kg^{-1}. After 4 weeks the concentration of *Fusarium* biomass was reduced significantly in all treatments (Fig. 5.1.5). However the FPE concentration in non-faunal treatments was reduced to a lesser extent (14%) compared to the reduction of the mesocosms containing soil fauna (48-54%). During the final 4 week a further decline of *Fusarium* biomass was measured (76-85%) but the highest concentration of FPE was still found in the non-faunal treatments (Fig. 5.1.5).

Table 5.1.5: *Fusarium* biomass in wheat straw of the mesocosms in 2013. Degrees of freedom, NULL Deviance, Residual Deviance and *P*-values of the analysis of deviance on the effects of soil fauna (*L. terrestris* (L.t.), L.t. + *F. candida* (F.c.), L.t. + *A. saprophilus* (A.s.), L.t. + F.c. + A.s.), *Fusarium* infection (*Fusarium*-infected wheat straw, non-infected), date (4 weeks and 8 weeks).

	Df	Deviance Residual	Df	Residual Deviance	P
NULL-model			147	7987.7	
Date (D)	2	2015.8	145	5971.9	<0.001
Fauna (F)	4	28.0	141	5943.9	<0.001
Infection (I)	1	5376.9	140	566.9	<0.001
D x F	8	35.7	132	531.3	<0.001
D x I	2	301.3	130	230.0	<0.001
F x I	4	50.1	126	179.9	<0.001
D x F x I	8	28.0	118	151.8	<0.001

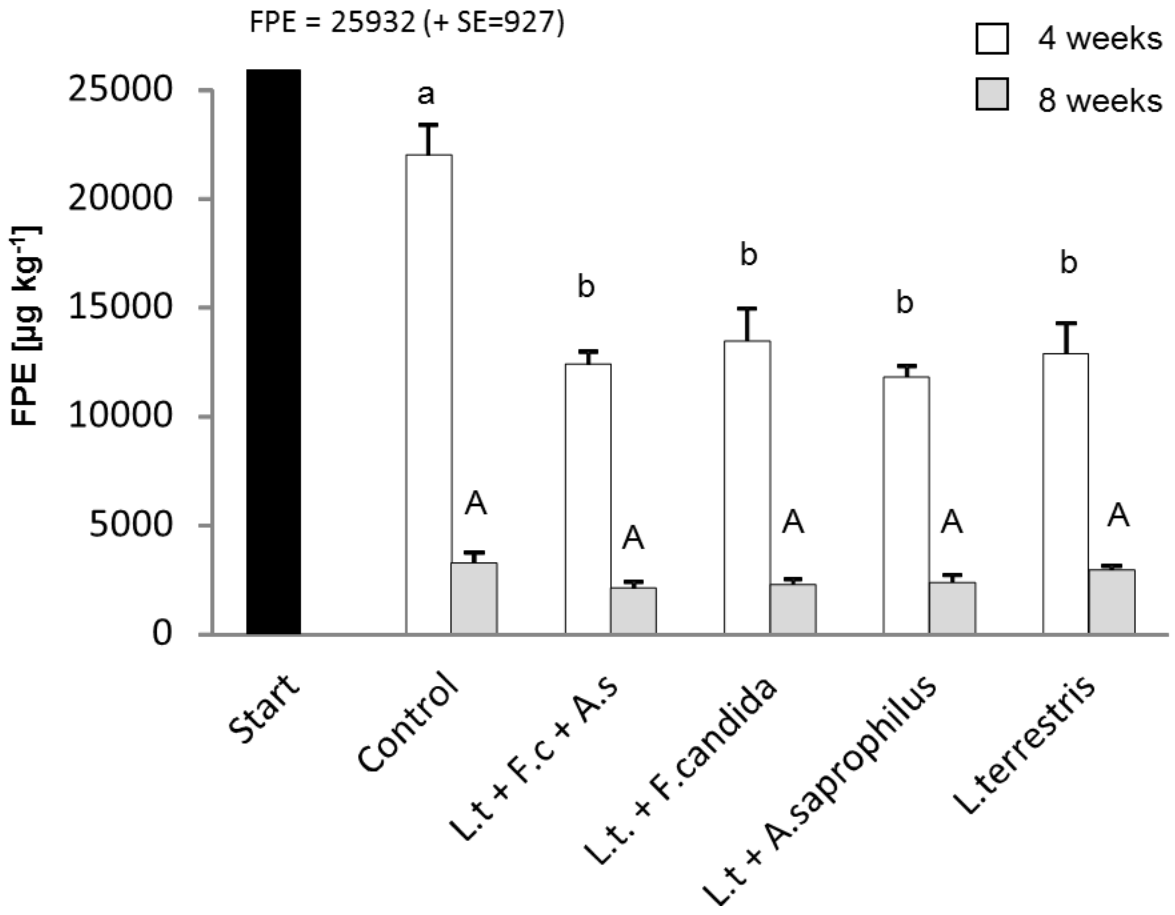

Fig. 5.1.5: Mean (+SE) concentrations of FPE (*Fusarium* Protein Equivalents) (µg kg^{-1}) in infected winter wheat straw of the mesocosms 2013 inoculated with soil fauna species *L. terrestris* (L.t.), L.t. + *F. candida* (F.c.), L.t. + *A. saprophilus* (A.s.), L.t. + F.c. + A.s. or without soil fauna (control) at the beginning (start) and after 4 and 8 weeks. Different letters indicate bars to be significantly different ($P < 0.05$). Small letters refer to the bars of 4 weeks and capital letters to the bars of 8 weeks.

FPE concentrations in soil

In the soil of the mesocosms no contents of *Fusarium* biomass could be detected.

Discussion: Minicontainer and mesocosms

The results of the minicontainer-study with collembolans and nematodes (2011) revealed a considerable reduction of the soil-borne fungus *F. culmorum* after 2 weeks (Fig. 5.1.1). Since there were no significances between the different treatments it must be supposed that the introduced soil fauna did not contribute to the degradation process during the first 2 weeks of the experiment. A faunal impact of collembolans and nematodes on the degradation of *Fusarium* biomass in wheat straw was first measured after 4 weeks. The single species treatments of *F. candida* and *A. saprophilus*, respectively, differed significantly from the non-faunal control and the mixed treatment. Possibly, the production of mycotoxins by the fungus

and the high content of DON during the first 2 weeks (see chapter 4.1, Fig. 4.1.2) repelled the introduced collembolans and nematodes from the infected wheat straw. This possible connection between DON-concentration and *Fusarium* biomass reduction is observed for the first time and can be explained by Böllmann et al. (2010) who demonstrated that fungi with poisonous metabolites were significantly less grazed by *F. candida*. Like it is already assumed for the investigation in chapter 3, microbial activity most likely caused most of the degradation occurring during the first 2 weeks of the experiment. Only after 4 weeks, when DON concentration in wheat straw was notably reduced (see chapter 4.1, Fig. 4.1.2), the degradation of *Fusarium* biomass was influenced by the activity of collembolans and nematodes (Fig. 5.1.1). This result confirms reports of Lagerlöf et al. (2011) who showed that introduced fungivorous nematodes significantly reduce pathogenic fungi. Furthermore, Shiraishi et al. (2003) indicate the potential of the collembolan species *F. hidakana* for the use as a biological control agent against fungal plant pathogens and Sabatini and Innocenti (2001) demonstrated a significant reduction of *Fusarium*-induced disease severity due to feeding activity of collembolans. The initial hypothesis that *F. candida* and *A. saprophilus* enhance the degradation of the biomass of the soil-borne plant pathogenic fungus *F. culmorum* in infected wheat straw can be confirmed but only in the case of single species treatments.

Regarding the results of the straw cover of the mesocosm-studies, a similar trend was measured in both experimental years: The *Fusarium*-infected wheat straw was incorporated faster into the soil by the earthworms than the non-infected control straw after 4 weeks as well as after 8 weeks (Fig. 5.1.2 and Fig. 5.1.3). These results are in line with Oldenburg et al. (2008) and Wolfarth et al. (2011) who reported a more efficient incorporation of *Fusarium*-infected wheat straw into the soil by the earthworm species *L. terrestris*. However, the introduced collembolans and nematodes did not influence the incorporation activity of the earthworms as the statistical analysis revealed no faunal effects on the straw cover of the mesocosms (Table 5.1.2 and Table 5.1.3).

In 2011, the *Fusarium* biomass of the infected wheat straw of the mesocosms was reduced significantly in all faunal treatments after 4 weeks. The differences between the faunal treatments on the degradation of *Fusarium* biomass (Fig. 5.1.4) after 4 weeks leading to the careful assumption that *F. candida* might have influenced the degradation performance of *L. terrestris* more positive than *A. saprophilus*. But this result should be considered with caution, as it could not be verified in 2013 (Fig. 5.1.5). After 4 weeks there were no differences

between faunal treatments after and after 8 weeks there were no differences between the faunal treatments and the non-faunal control treatment in 2013. These results rather suggest that *L. terrestris*, as an anecic detritivorous earthworm species has been the driver of the degradation process in reducing *Fusarium* biomass in a cropping system with reduced tillage conditions. The initial hypothesis that *L. terrestris* contribute to the control of the plant pathogenic fungus *Fusarium culmorum* in infected wheat straw can therefore be clearly confirmed. Whereas the contribution of the soil meso- and microfauna (*F. candida, A. saprophilus*) considering fungal degradation in infected crop residues seemed to be minor. Confirming the results of the present investigation, Bertrand et al. (2015) also revealed a great potential of *L. terrestris* to be an effective biocontrol agent for the fungal-induced eyespot disease on winter wheat.

The results of the present study showed the significant contribution of earthworms to the biocontrol of fungal plant diseases as an important ecosystem service for soil health and plant production in agroecosystems

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig und auf Grundlage der derzeit gültigen Fassung der Promotionsordnung des Fachbereichs VI der Universität Trier angefertigt habe. Es wurden nur die in der Arbeit ausdrücklich benannten Quellen und Hilfsmittel benutzt. Wörtlich oder sinngemäß übernommenes Gedankengut habe ich als solches kenntlich gemacht. Ergebnisse und Beiträge anderer Beteiligter sowie Autoren und Co-Autoren sind als solche gekennzeichnet. Die Arbeit hat in gleicher oder ähnlicher Form noch keiner anderen Prüfungsbehörde vorgelegen oder wurde von dieser als Teil einer Prüfungsleistung angenommen.

Goslar, Dezember 2016

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DANKE ANTON.
…für Lachen, Leben und Liebe!

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