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Biological Control of Soil-borne Phytopathogenic Fungi and their Mycotoxins by Soil Fauna

A review

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Abstract. Yield loss through harmful fungi is a serious problem in crop production worldwide. Cereal residues like straw are frequently infected by *Fusarium* fungi, which produce mycotoxins like deoxynivalenol (DON). Mycotoxins lead to quality losses in cereal-based food and feed which endangers human and animal health. Especially under conservation tillage, when mulching techniques are applied to protect soil from erosion, run-off etc., residues should be efficiently degraded to protect the currently cultivated crop from fungal infection and mycotoxin contamination. The objective of this review is to give an overview on which role decomposing soil fauna plays in the fate of *Fusarium* fungi and there main mycotoxin DON in the soil system. Generally, soil fauna benefits from conservation tillage compared to conventional tillage. Results from experiments in the laboratory and field revealed that earthworms as primary and secondary decomposers as well as fungivorous collembolans and soil nematodes contribute to the ecosystem services of pathogen depression and toxin degradation with respect to *Fusarium* and DON. *Fusarium* seems to be an attractive food source. Furthermore, the mycotoxin DON does not cause any harm to the soil fauna tested. Key factors for the control of *Fusarium* development by antagonistic soil fauna are: (1) interaction with soil microorganisms; (2) interaction of soil fauna species; (3) soil texture; (4) residue exposure. Ecosystem services of antagonistic soil fauna are vital to crop production and the functioning of agroecosystems. They will be discussed in a broader context of soil health and conservation tillage.

Keywords: crop residue management, *Fusarium* infection, mycotoxins, ecosystem services, earthworms, collembolans, nematodes

FUSARIUM INFECTION AND TOXIN PRODUCTION: A SERIOUS PROBLEM IN AGRICULTURE

Conservation tillage as a measure of soil protection in agriculture offers several ecological and economic advantages for farmers like mitigation of erosion, improvement of water supply, decrease in energy expenses etc. compared to conventional tillage (Holland, 2004; Kassam et al., 2009). Usually under conservation tillage, residues of the preceding crop remain on or near the soil surface which promotes soil biodiversity (van Capelle et al., 2012) and stimulates decomposition processes by soil biota thus improving the humus balance of the soil (Berg and McClaugherty, 2003).

At first glance, increasing soil biological activity and preserving soil biodiversity through residue mulching (Tebrügge and Düring, 1999) are good news. However, a closer view reveals promotion of biological activity not being that positive in any case because pest organisms also benefit from straw amendment on the soil surface. A drawback of conservation tillage is the problem of an increasing infection risk for pests like soil-borne phytopathogenic fungi. Such fungi growing on crop residues endanger plant health of the following crop (Pereyra and Dill-Macky, 2008).

Worldwide, *Fusarium* head blight is one of the most important fungal diseases in small grain cereals. In temperate regions, this disease is mainly caused by species like *Fusarium graminearum*, *F. culmorum* and *F. avenaceum* (Nicholson et al., 2003; Parry et al., 1995; Wagacha and Muthomi, 2007). Tight crop rotations combined with a high ratio of cereals and maize is most risky regarding an infection. Each year, farmers suffer significant yield losses caused by *Fusarium* head blight (Leplat *et al*., 2013). Disservices are likely to increase through the survival of mycotoxin producing plant pathogenic fungi like *Fusarium* species on crop residues (Champeil *et al*., 2004). Farmers can minimize the risk of *Fusarium* head blight development by paying more care to the crop rotation scheme. A periodical interruption of the cultivation of susceptible crops with crops, which are not host of this plant disease, might be a strategy for risk limitation (Leplat *et al.,* 2013).

In addition to quantitative losses, a decline in quality of crop products and residues may result from contamination of grains and straw with mycotoxins. One of the most abundant mycotoxins, which are produced by the above mentioned *Fusarium* species, is the trichothecene mycotoxin deoxynivalenol (DON). Mycotoxins like DON persist during storage, are heat-resistent and are of major concern for human and animal health after consumption of contaminated food and feed, respectively (JEFCA, 2001). In the interest of ensuring public health protection, the European Commission set thresholds for certain mycotoxins including DON (Tab. 1) in foodstuffs like unprocessed cereals, pasta and bakery wares including specific maximum levels for food for infants and young children (EC, 2006a; 2007).

Tab. 1. Maximum levels for deoxynivalenol in selected foodstuffs as ruled by the European Commission (EC, 2006a).

Foodstuff	Maximum level
Unprocessed durum wheat and oats	1.750 μg kg^{-1}
Unprocessed cereals other than durum wheat, oats and maize	1.250 μg kg^{-1}
Pasta	750 μg kg^{-1}
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500 μg kg^{-1}
Processed cereal-based foods for infants and young children	200 μg kg^{-1}

Farmers are urged to minimize the occurrence of *Fusarium* infection and toxins through good agricultural practice depending on the prevailing site and climatic conditions (EC, 2006b). Good agricultural practice covers all management measures including seeding/planting as well as pre-harvest and post-harvest strategies. Recommendations by the European Commission include principles, which prevent and reduce *Fusarium* toxin contamination in cereals, and risk factors, which have to be taken into account by farmers (EC, 2006b). Besides cultivation of less susceptible cultivars and chemical plant protection measures, residue treatment by mulching machinery is an appropriate method to reduce the risk of crop infection with *Fusarium*. Therefore, the farmer’s technical residue management promotes natural mechanisms of self-regulation in the soil system and maintains food and feed quality. Recently, Vogelgsang *et al.* (2011) reported from their on-farm experiments that small *versus* large and spliced *versus* intact residue pieces being more favourable for saprovor and fungivor soil organisms due to a larger surface and higher humidity. Accordingly, effective stimulation of depomposition in soil is a pivotal measure in order to significantly reduce the risk for *Fusarium* infection in agroecosystems dominated by cereal crops (Berg and McClaugherty, 2003; Stemann and Lütke Entrup, 2005).

SOIL INTRINSIC MECHANISMS OF *FUSARIUM* CONTROL

The increasing use of external mechanical and agrochemical inputs of the past has led to a decreasing use of internal beneficial biotic interaction (Beylich *et al*., 2010; Médiène *et al*., 2011). Currently, there is growing evidence that loss of soil biodiversity is a serious issue for arable soils under intensive cultivation. Threats to soil biodiversity implicate a decline in the provision of ecosystem services (Jeffery *et al.,* 2010) like pest control and toxin degradation, which both contribute to soil health. Recently, Médiène et al. (2011) presented an overview on potential options to support and to get used of ecosystem services provided by functional soil biodiversity under sustainable management of agroecosystems.

Competitive and antagonistic as well as mutualistic interactions between soil organisms are important drivers of self-regulation in soil. These interactions within and between soil biota communities imply soil intrinsic mechanisms of the control of harmful fungi. Repression and degradation of phytopathogenic soil fungi is an important ecosystem service, which is often neglected from an agricultural viewpoint.

According to Leplat *et al*. (2013), especially *F. graminearum* is a weak competitor within the soil fungal community. Other *Fusarium* species seem to have a better saprotrophic performance and may repress other microbial competitors depending on organic matter related conditions in soil and agricultural management measures (Fernandez et al., 2008). However, the mechanisms of fungal succession on crop residues as well as the diverse interactions between saprophytic fungi and within the microbial community during residue decomposition are complex, less understood and still to be analyzed (Leplat *et al*., 2013).

Tab. 2

Relative soil surface cover with *Fusarium* infected wheat straw and non-infected control straw after 5 and 11 weeks exposure to earthworm (*Lumbricus terrestris*) activity (data from Oldenburg et al., 2008). Different letters indicate significant differences within the column ($P<0.05$).

Straw type	Exposition time	Soil surface cover	
	weeks	%	(SD)
Contr./inf.	0	94.5 [a]	(1.4)
Control	5	58.5 [b]	(5.4)
	11	44.6 [c]	(9.8)
Infected	5	41.5 [d]	(4.5)
	11	24.4 [e]	(10.3)

Fungal feeding representatives of the soil fauna are obviously antagonistic to a *Fusarium* infection. Tab. 2 demonstrates an accelerated incorporation of wheat straw, which was infected with *F. culmorum*, into soil through the activity of the detritivorous earthworm species *Lumbricus terrestris* (Oldenburg *et al.,* 2008). The infected straw seemed to be much more attractive for *L. terrestris* than the non-infected control straw. This significant difference is in accordance with results by Moody *et al.* (1995) who found increased feeding activity of earthworms on fungal infected crop residues. Furthermore, Bonkowski *et al.* (2000) demonstrated food preferences of earthworms for 8 different soil fungal species. Three earthworm species including *L. terrestris* clearly preferred *Fusarium* species as food source. Apart from soil environmental conditions, consumption rates of earthworms are mainly determined by quality and palatability of the food source (Curry and Schmidt, 2007).

Microcosm studies under constant laboratory conditions with different earthworm species revealed detritivorous earthworms like *L. terrestris* as the main drivers with respect to *Fusarium* degradation on infected wheat straw (Wolfarth *et al.,* 2011). The role of geophagous earthworm species like *Aporrectodea caliginosa* is assessed to be minor in this context (Wolfarth *et al*., 2011).

Direct feeding is not the only way earthworms contribute to a reduction of *Fusarium* biomass. Another aspect seems to be the induction of a priming effect by mucus secretion (Binet *et al.,* 1998; Kuzyakov *et al.,* 2010). Cutaneous mucus of earthworms contains highly bioavailable compounds, which enhance microbial activity (Brown, 1995). The significant reduction of *Fusarium* biomass in remaining straw, which was incorporated into soil by *L. terrestris* but not consumed, supports this assumption (Wolfarth *et al.,* 2011). The interaction between earthworms and soil micoorganisms should be considered as an important factor in reducing *Fusarium* biomass. Besides earthworms other fungi feeders within the soil faunal community also contribute to repression and degradation of *Fusarium* infestation on crop residues. Collembolans play an important role in decomposition processes by grazing on fungi and bacteria. According to Klironomos and Kendrick (1995) and Lartey *et al.* (1994), collembolans significantly promote a biological control of fungal plant pathogens. For instance, *F. culmorum* is a palatable food source for the collembolan species *Folsomia candida* and *Folsomia fimetaria* (Larsen et al., 2008). Sabatini and Innocenti (2000) found *F. culmorum* to be a food source even adequate for reproduction in case of *Mesaphorura krausbaueri*. These collembolan species are quite common in arable soils.

Nematodes are very abundant in agricultural soils (Yeates and Bongers, 1999) and show a wide range of feeding types (Yeates *et al*., 1993). They play critical roles in controlling turnover and structure of soil microbial communities (Yeates, 2003). It has been shown that fungal feeding nematodes are able to control soil-borne plant pathogenic fungi. Roessner and Urland (1983) demonstrated a clear repression of *F. culmorum* by the fungivorous nematode species *Aphelenchoides hamatus*. Further studies revealed that *Aphelenchus avenae* significantly reduces plant pathogens like *Fusarium moniliforme* (Gupta, 1986) and *Fusarium oxysporum* (Okada, 2006).

Just recently, Wolfarth *et al.* (2013) demonstrated that the interaction of fungivorous collembolans (here: *Folsomia candida*) and nematodes (here: *Aphelenchoides saprophilus*) significantly reduce *F. culmorum* in wheat straw remaining on the soil surface compared to treatments with collembolans and nematodes being separated.

SOIL FAUNAL DIVERSITY PROMOTES MYCOTOXIN REDUCTION

Compared to studies on soil faunal repression of phythopathogenic *Fusarium* species on crop residues less research has been done on DON degradation by soil fauna. Results from laboratory studies by Oldenburg *et al.* (2008) and field experiments by Wolfarth *et al.* (2011) demonstrated a significant reduction of DON contents in remaining wheat straw by the detritivorous earthworm species *L. terrestris* to 2 orders of magnitude after 2 to 3 months. In the non-earthworm control a DON degradation of only 1 order of magnitude was detected by microbial activity (Oldenburg *et al*., 2008; Wolfarth *et al*., 2011). The analysis of the DON concentration in gut content and casts of *L. terrestris* gave clear evidence that earthworms degrade DON during food digestion probably supported by mutualistic gut microflora. The DON content in the earthworm gut was significantly lower compared to remaining straw (Schrader et al. 2009) and it was below quantification limit in earthworm casts (Oldenburg *et al*., 2008; Wolfarth *et al*., 2011).

First results on *Folsomia candida* and *Aphelenchoides saprophilus* by Wolfarth et al. (2013) indicate collembolans and nematodes being able to reduce DON concentrations of *Fusarium*-infected wheat straw significantly compared to the non-faunal control (Tab. 3). The interaction of both species is apparently most efficient (Tab. 3) like in case of *Fusarium* biomass reduction. Furthermore, soil texture is a key factor, which controls the provision of toxin degradation as ecosystem service provided by both soil faunal taxa. A high reduction in DON concentration of 94% was found in sandy and silty soils compared to a rather small reduction of about 40% in clayey soils with respect to interacting collembolans and nematodes (Tab. 3).

Tab. 3.

Relative reduction of deoxynivalenol in *Fusarium*-infected wheat straw in presence of nematodes (*Aphelenchoides saprophilus*), collembolans (*Folsomia candida*) and interaction of both fungal feeding species regarding different soil texture after 4 weeks soil faunal activity (data from Wolfarth et al., 2013). Different letters indicate significant differences within the row (P<0.05).

	Nematodes	Collembolans	Interaction	Non-faunal control
Sand	90%	67%	92%	83%
Silt	79%	88%	95%	65%
$\text{Mean}_{\text{Sand+Silt}}$	85% ^{b}	77% ^{c}	94% ^{a}	74% ^{c}
Clay	6%	34%	39%	20%

Conservation and promotion of soil faunal diversity through good agricultural practice enhance rapid residue decomposition including degradation of *Fusarium* species and their toxins from the preceding crop. Obviously, such a sustainable management improves or at least maintains soil health in arable land.

BENEFICIAL INTERACTION BETWEEN FARMERS AND SOIL FAUNA

In cropped agroecosystems soil-borne phytopathogenic fungi like *Fusarium* species and their production of mycotoxins like DON is controlled by farmers and soil fauna in a kind of "mutualistic interaction" (Fig. 1). It is the combination between anthropogenic top-down control by farmers through good agricultural practice and natural bottom-up control by soil fauna through their provision of ecosystem services. Both (farmers and soil fauna) are benefiting from each other because their activities promote soil health (Fig. 1). Combating measures and degrading activities control harmful fungi and improve or at least maintain quality and quantity of crop products and residues.

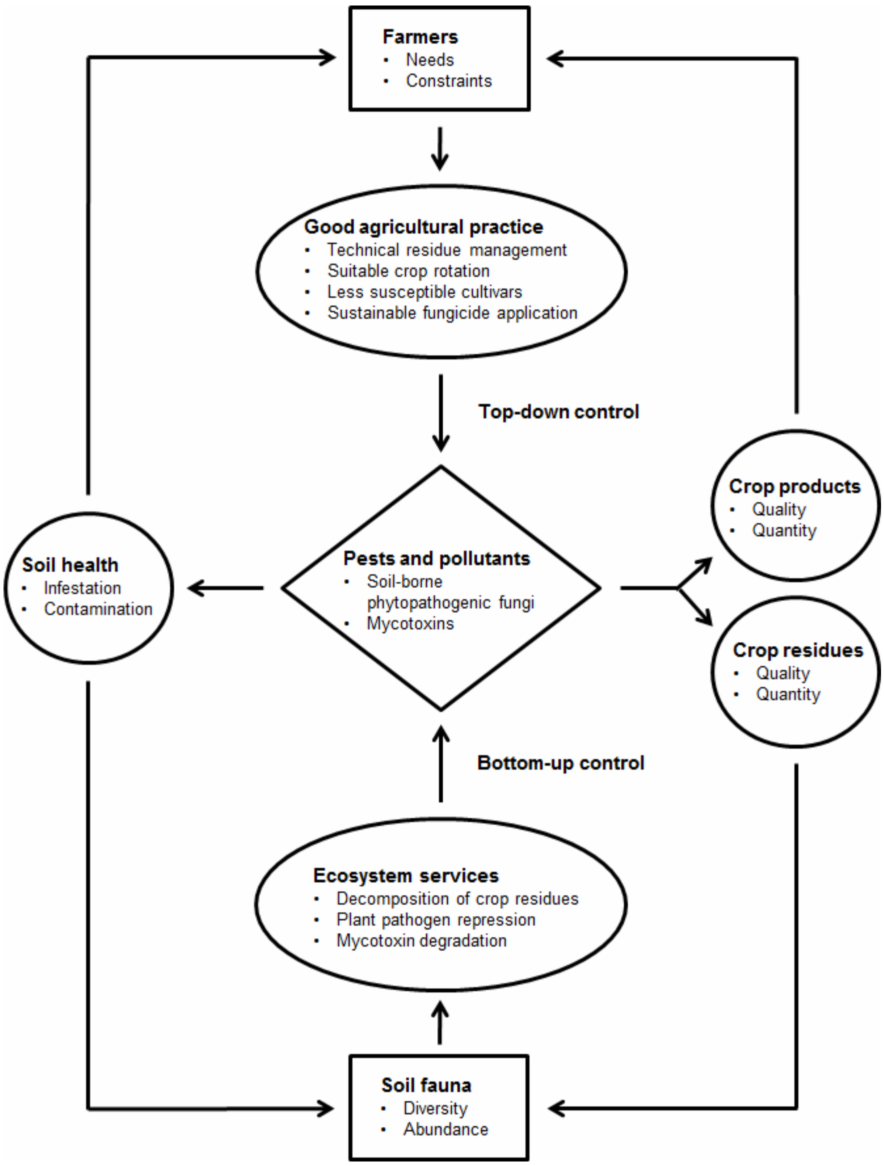

Fig. 1. Model of natural (bottom-up control) and anthropogenic (top-down control) interrelations in agroecosystems regarding ecosystem services and good agricultural practice for sustainable management of harmful fungi and their pollutants.

CONCLUSIONS

Up to now, it has been the objective of ecotoxicology to analyze dose response relations of pollutants on selected soil fauna species. However, converse studies i.e. impacts of soil faunal activities on toxic compounds are rare so far. Analyzing degradation potentials of soil fauna to plant pathogenic microorganisms and their environmentally risky substances is challenging in future lab and field studies. The delivery of ecosystem services by soil fauna and the application of good agricultural practice by farmers is a beneficial relation, which promotes soil health in arable fields as well as quality and quantity of agricultural products.

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