

# Biotechnology, environmental forcing, and unintended trophic cascades

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**Abstract** A long ongoing discussion between scientists and policy decision-makers seems to have entered recently into a new phase. The consequences of release of transgenic crops into the environment are being discussed not only by scientists but also by farmers, environmental groups and politicians, while an increasing amount of data is becoming available at all biological scales, including the field level. However, data still rely on experiments designed to capture direct consumer–resource interactions. Here we argue that we should attempt to concentrate on the ecosystem functioning of soil biota under genetically-modified (GM) plants, because functional and mechanistic analysis of the multitrophic effects of GM plants on soil biota is still lacking. It is our opinion that we should avoid addressing taxa and soil communities separately, but link them at their functional level. We shall explain why, using examples from ecosystem services, allometric scaling, and soil food webs. The energy flow of any food web under stress incorporates several factors and pooled information on ecosystem services and on the different responses of soil invertebrates to induced perturbations in other trophic levels. Therefore, we will systematically focus on the complementarities of these approaches.

**Keywords** *Bacillus thuringiensis* (*Bt*) · Body size · Invertebrates · Maize · Microbial pools · Modeling · Plant–animal interactions · Risk-assessment · Transgenic plants

## Introduction

Species differ in their sensitivity to toxic compounds. The toxic mode of action is the way a compound works on a target organism, either ranging from a general mode of action like narcosis in a large suite of organisms, to specific interactions with target sites that are present within only few organisms, like the neurotoxic action of various insecticides. Since the late 1980s, species sensitivity distributions (so-called, SSDs) have been used as a methodology to take differences in toxicant sensitivity among taxa into account, and these results are used in most (probabilistic) environmental risk assessments (Posthuma et al. 2002). The input for SSDs mainly consists of single-species laboratory toxicity data, and represents either a concentration-value or a value for toxic stress. However, although the increasing amounts of data at all biological scales became a central feature in life sciences (including biotechnology), too often the real impact of computational research is diluted, because most papers tend to be published either in statistical journals which are not read by experimentalists, or in journals reporting novel discoveries which allow only very short methodological sections (Bourne et al. 2005).

The huge amount of data produced in the last decade has enabled a large number of reviews on effects of GM crops. During the last decade, 2,393 (!) reviews on effects of transgenic crops have been published (reported in [www.scopus.org](http://www.scopus.org), accessed January 10, 2009). In the case

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of transgenic *Zea mays*, engineered to express proteins from *Bacillus thuringiensis* (*Bt*) on single or grouped taxa, the 128 reviews reported in SCOPUS corresponded to only a small fraction of the entire peer-reviewed literature. Surprisingly, although most reviews are complete and carefully refer to many non-target taxa and taxon-related processes, no review addresses ecological networks. Potential effects outside specific genetic isolines are thus ignored. Therefore the relevant question is still to what extent agricultural practices like growing a transgenic crop will really affect the ecosystem services. Affecting ecosystems is inherent to mankind: examples are anthropogenic vegetation, migration of invasive species, and selection of cultivated species (Hultén 1970; Grime et al. 1988; Frenzel et al. 1992; Mulder 1999). Archaeobotanical studies of plant remains from protohistory and prehistory provide strong empirical evidence for genetic simplifications in crops such as spinach (Mulder 1999), maize (Fedoroff 2003), and rice (Ammann 2007). Any kind of culture manipulation, including transgenic cropping and most agricultural practices, is continuously affecting the dynamics of ecosystems.

Inputs of nutrients in terrestrial ecosystems primarily depend on plant–soil interactions and subsequent litter degradation. Therefore, the lack of knowledge on the extent to which ecosystem services might be affected by agricultural practices is supposed to be particularly evident underground. For instance, optimal growth conditions of bacteria and fungi are clearly reflected in the functional diversity of soil microbivores (Mulder et al. 2005a, 2006a). Thus, if GM plants affect the growth conditions for either bacteria or fungi (or both), secondary effects on other organisms at higher trophic levels could become detectable. Changes in available soil microbial resources always cause shifts in faunal interactions, making changes in soil biodiversity evident (Ammann 2005; Mulder 2006; Sánchez-Moreno et al. 2006; Powell 2007).

### Domino-like effect cascades

The arising question is the extent to which *Bt* plants and transgenic crop residues affect not only the single taxa and their specific food chains, but the entire soil food web. There are at least two major reasons to expect top-down effect cascades on soil biota induced by *Bt* crops. One is that a genetic modification may result in a different crop management with a different exposure of the soil biota to crop protection agents (Ammann 2005). Another reason is the difference in the biochemistry of *Bt* cultivars in comparison to their non-*Bt* isogenic relatives (Flores et al.

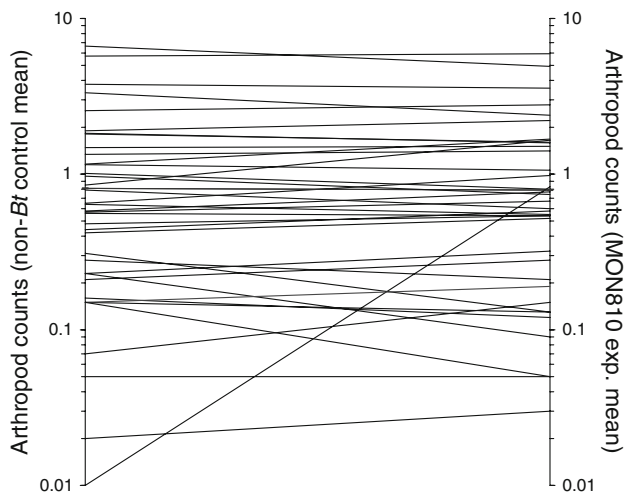
2005; Poerschmann et al. 2005). *Bt* maize produces insecticidal crystal protein (ICP) which protects the plant from herbivory. In natural circumstances this protein is never synthesized. Two main routes through which ICP reaches the soil were identified: (1) incorporation of plant residue containing *Bt* toxin into soil organic matter and (2) *Bt* toxin exudation from roots of living plants (Flores et al. 2005). It is also probable that pollen tasseling may be of minor contribution to the two abovementioned processes (Saxena et al. 2004).

As *Bt* maize is especially used for the cobs, there is a large input into the soil of plant material after harvesting. For instance, the rather slow degradation of the Cry1Ab protein could change the soil organic matter balance and the soil microbial activity (Baumgarte and Tebbe 2005). Biodegradation studies of *Bt* maize in soil show that the Cry1Ab protein can be present for long periods without losing its toxicity (Clark et al. 2005). Tapp and Stotzky (1998) reported the presence of *Bt* toxin for 234 days in sandy loams (kaolinite-rich ‘Kitchawan soils’). Such persistence of bound toxins from *Bt* could pose a potential hazard to non-target organisms.

Researchers dealing with the possible environmental risks of biotechnology must think about how to include soil detritus in ecological modeling. In contrast to soil organisms, detritus is organic but dead and difficult to be defined as entity (Mulder et al. 2005b; Dunne 2006). Besides phytophagous arthropods, which are per se expected to become affected by the *cry* gene if they have the appropriate receptors, empirical evidence for belowground effects at other trophic levels is controversial. Most reports of effects of *Bt* plants consider pollinating insects and collembolans. Aboveground macro-arthropods (like the spiders and coleopterans shown in Fig. 1) have been widely investigated. Still, plant biotechnology in general (and *Bt* maize in particular) shows divergent evidence in the open literature (reviewed, among others, by Icoz and Stotzky 2008). For instance, by comparing laboratory surveys with field data, we see that lepidopteran larvae fed with *Bt*-engineered plant tissues or pollen may show lethal effects, but only when abnormally high concentrations were used (Shelton and Sears 2001; Chapman and Burke 2006). However, these ecotoxicological studies are again based on a single consumer–resource chain, not on any food-web modeling.

### Importance of single traits

Food webs underly the energy and biomass flow in real ecosystems. Therefore, food-web modeling is becoming omnipresent across disciplines. Research on food webs is



**Fig. 1** Bipartite graph depicting two-node network characteristic of the averaged densities of arthropods in maize fields sampled by pitfalls. At the *left*, non-transgenic maize control plots; at the *right*, MON810 experimental *Bt* plots. Each crossing line represents one natural population with variable lengths of exposure to the *cry* gene. Atrazine and pendimethalin were applied to control weeds, and fields were fertilized at the rate of 95 kg/ha of  $P_2O_5$  and 112 kg/ha of nitrogen. The magnitude of the observed *Bt* effect was variable within the set. Original population data from Daly and Buntin (2005), functional guilds as provided in Wolfenbarger et al. (2008). The coefficient of variation (CV) of the insects is higher in the control plots than in the experimental *Bt* plots (139% and 123%, respectively), possibly due to collateral effects of atrazine and pendimethalin, but the CV of the spiders (keystone predator species) is the highest in the experimental *Bt* plots (45% vs. 28%). The lumping of all the intermediate and top predating arthropods (Anthicidae, Carabidae, Cicindelidae, Elateridae, Formicidae, Gryllidae, Staphylinidae, and Araneae) shows some congruent escalatory fashions to predation of most beetles. Escalated food-web interactions between intermediate or top species and basal species such as the herbivore Scarabaeidae, whose CV in the *Bt* plots is highly enhanced (77% vs. 57% in the control plots), might be expected as well

expanding to incorporate species' responses to disturbance and species' traits. Traits are variously defined, but essentially concern properties of species, populations and communities that govern habitat-response relationships. It is important to distinguish between 'response traits' that affect the response of organisms to biotic factors (Mulder and Elser 2009) and 'effect traits' that affect ecosystem services (Lavorel and Garnier 2002). Still, the actual role of effect traits is neglected and the number of studies that are not conceptual or review contributions remains low (Naeem and Wright 2003).

Within each trophic level of a natural community food web, specific ecosystem services are dependent upon combinations of traits (Table 1). Species are characterized by traits that *collectively* provide information about their life history. Implementation of trait-based studies, like those based on body size and growth rates, has to play a

key role, offering the comparison between microcosm studies and field conditions. Only theory-driven research will identify these properties across ecophysiological scales.

There is wide empirical evidence for an extremely effective synergy between microbes, plants and arthropods for nutrient-uptake (Yeates 2003; Wardle et al. 2004). In an ideal sequence of increasing resistance to the microbial breakdown occurring during decomposition processes, the residual resources of organic carbon are sugars < starch < hemicellulose and proteins < cellulose < lignins < suberins < cutins (Begon et al. 2006). Some more complex, refractory litter components can even resist microbial attack. *Bt* crops mostly show such remarkable changes in chemistry in their lignin patterns, with consequent pleiotropic effects and effects on symbionts and pathogens.

The different pathways of the subsequent decomposition process are supposed to reflect multitrophic effects under *Bt* plants due to biochemical consequences of the quality of *Bt* straw (resulting in higher microbial C:N biomass ratio and fungal–bacterial ratio). In Fig. 2 we try to summarize the extent to which, under standardized conditions like those of laboratory experiments, several non-target invertebrates show rather undetectable or confounding effects attributable to transgenic cropping (Bhatti et al. 2005; Cortet et al. 2007; Griffiths et al. 2006, 2007a), in contrast to non-parasitic soil nematodes (Wei et al. 2003; Griffiths et al. 2005, 2007b; Icoz and Stotzky 2008). To our knowledge, no complete study on GM plant–microbe–animal interactions has been published and this is a serious gap.

### Disentangling resource quality

Soil bacteria are among the few organisms whose population density and functional performance are clearly enhanced by the elemental value of plant residues. Measuring functional changes by metabolic fingerprinting techniques, although targeting only the cultivable part of the soil microflora (i.e., the heterotrophic, fast growing bacterial cells), revealed short, but robust, changes in the bacterial community when exposed to straw of *Bt* cultivars (Griffiths et al. 2006; Mulder et al. 2006b). Furthermore, most carbohydrates were positively correlated with the *cry* gene (Mulder et al. 2007). Bacteria and fungi react in different ways. In their recent survey, Icoz and Stotzky (2008) stated that “fungi appear to be the organisms most affected by Cry proteins in soil”, but referred only to Turrini et al. (2004) and Castaldini et al. (2005). Also, other studies on mycotoxins showed a reduction of pest damage in *Bt* plants (e.g., Wu 2006);

**Table 1** Main clades in soil biota and their correlation with nutrient cycling (modified from Mulder 2006)

	Nutrient cycling
Bacteria and fungi	Catabolize fresh organic matter (FOM) Mineralize soil organic matter (SOM) Carbon sequestration (detritus)
Nematoda	Regulate the population of their microbial resource (bacterivore and fungivore nematodes) Fragment plant roots and transport FOM (fungivore and plant-feeding nematodes) Alter nutrient turnover in the rhizosphere
Acarina, Collembola, and Enchytraeidae	Regulate the population of their microbial prey (microphytophagous arthropods) and of the nematofauna (through nematode-feeding mites) Fragment plant roots and transport FOM (panphytophagous arthropods and enchytraeids) Alter nutrient turnover in the rhizosphere
Lumbricidae	Fragment plant roots and transport FOM and SOM Enhance microbial activity (positive feedback) Carbon sequestration (detritus, microorganisms) Bioturbation (ecosystem engineers)

A combination of single traits (adult body sizes, feeding-strategies) within different clades contributes to the entire decomposition process. Like other ecosystem services, the nutrient cycling process is not affected by single trophic levels

some dicots show an increased nodule formation (Ferreira et al. 2003).

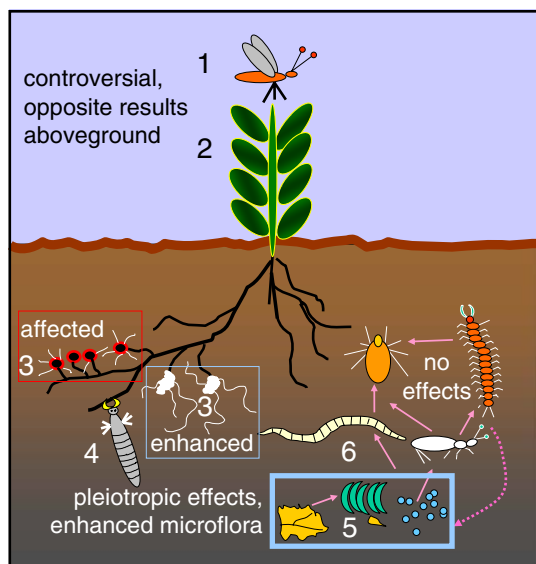
Comparable differences are reflected in shifted nutrient ratios, like those in the microbial C:N biomass ratio (Table 2; Escher et al. 2000; Mulder et al. 2006b, 2007). According to the ongoing multitrophic interactions, robust differences will occur in the soil microbial community after addition of transgenic plant material. Basal entities such as the soil microbes are either shortly enhanced (bacteria) or affected (fungi) by Cry proteins. Transgenic maize alters the fungal–bacterial biomass ratio, and consequently the decomposition process, possibly the most important ecosystem service in soil biota (Wardle et al. 2004; Mulder 2006). Articles involving soil nematodes (Perez-Moreno and Read 2001), oligochaetes (Hedlund et al. 2004) and insects (Klironomos and Hart 2001; Filser et al. 2002) have emphasized the importance of fungi in the facilitation of decomposition processes such as nitrogen recycling (Table 1; Jansa et al. 2002).

It is also known that some forms of disturbance in the facilitation of soil decomposition processes, such as manuring, affect the populations of larger soil invertebrate species more than the smaller ones (Mulder et al. 2008). These larger species belong to the fungivore oribatids, which handle different resources than the smaller (bacterivore) nematodes. This coexistence suggested that the mesofauna was partly responsible for keeping the populations of their (microbial) resource in check. Many insects feed on both the fungal tissues and the degraded plant residues (Fig. 3).

## Resource competition

Short-term resource competition (like bacterial population metabolizing carbohydrates) mentioned in the previous section supports a differentiation in the metabolic traits of the microbial community. Long-term competition is more difficult to determine. In agricultural soils, microbial communities cannot access groundwater and must rely on moisture available from precipitation. During the dry season, the soil's physical conditions may not allow commensurate increases in the rate of microbial carbon-degradation and in the degradation of ICPs not bound to colloids. These temporal trends within ecosystems, such as the decay of Cry1Ab, differences in the soils among ecosystems, in particular the silt fraction of loamy and sandy soils, and changes in resource quality have thus strong implications for almost all soil organisms.

Occurrence and abundance of microbes and soil invertebrates offer valuable information on ecosystem functioning and ecosystem services. Figure 3 captures the ambiguity of earthworms, correctly considered “ecosystem engineers” due to their ability to change their soil habitat or even create new habitats for other organisms through various activities (Brown 1995; Scheu 2003; Schrader et al. 2008). However earthworms are known for a weak correlation between their occurrence and the soil quality (Römbke et al. 2005; Schrader et al. 2008). This in contrast to other soil invertebrates, for which body size remains determinant for their spatially-scaled predatory activity. Micro-arthropods are usually 1–5 orders of magnitude



**Fig. 2** Graphic representation of challenging gaps in the assessment of ecological risks of *Bt* plants. Redrawn from a picture by Heikki Setälä (modified with author’s permission). *Notes:* (1) Pollinators fed with unlikely high concentrations of *Bt*-engineered plant tissues or pollen show lethal effects, but only by amounts much greater than those expected in the field (Shelton and Sears 2001; Chapman and Burke 2006; Johnson et al. 2007). (2) *Bt* crops show remarkable changes in their chemistry, such as in their lignin patterns, with consequent pleiotropic effects and shifts in the soil nutrient quality (Table 2). (3) Plant pathogens are affected by roots exudates from *Bt* crops; nodule formation, nitrogen-fixing bacteria and mycorrhizal lengths are enhanced (Icoz and Stotzky 2008). (4) Browsing, sucking and engulfing arthropods could be affected by the crystal protein encoded in the *cry* gene (Fig. 1); more research is necessary to recognize possible effects. (5) Microbial activity, such as soil respiration, and functional diversity, like metabolic efficiency, are enhanced, at least for a short time, although almost no effects were detected in protozoans (Icoz and Stotzky 2008, but see also Griffiths et al. 2005, 2007b). (6) No effects seem to be detectable at higher trophic levels, like *Bt* effects on collembolans, mites and woodlice (Icoz and Stotzky 2008). However, these assessments rely on single food-chains, not on complete food webs. We strongly recommend food-web modeling

**Table 2** Results of the chemical analysis of the straw obtained from four maize cultivars (% of dry weight)

Cultivar	Isoline	Microbial C:N ratio
‘Novelis’ MON810	I (transgenic)	24.1
‘Valmont’ event 176	II (transgenic)	32.8
‘Nobilis’	I (conventional)	17.2
‘Prelude’	II (conventional)	18.7

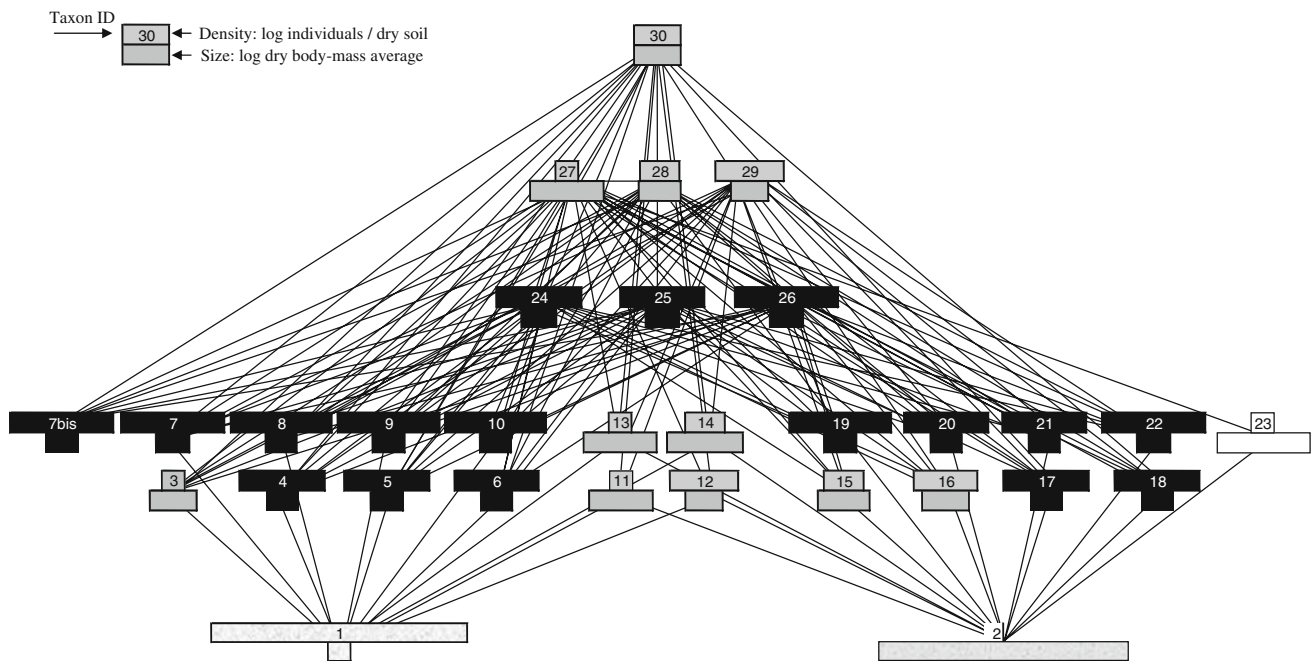
Methodological details in Mulder et al. (2006b). The respective isolines of the transgenic *Bt* cvs. Novelis (MON810) and Valmont (Event 176) are the non-*Bt* (conventional) cvs. Nobilis and Prelude. Note the increased  $C_{mic}:N_{mic}$  ratio in the *Bt* cultivars. Increases in the  $C_{mic}:N_{mic}$  ratio indicate long-term decreases in nutrient quality and availability to microorganisms (Joergensen and Emmerling 2006)

larger than their faunal prey, while grazing nematodes are 5–10 orders of magnitude larger than their microbial prey, in terms of average mass of bacterial cells (Mulder et al. 2005b). These distinctly separate pathways imply that micro-arthropods like the Scutacaridae (top species) feed on varying compartments at lower trophic levels (intermediate species) at different points in time, linking belowground compartments into a larger soil and litter food web. Icoz and Stotzky (2008) recognized that fungivore nematodes were more abundant in fields with *Bt* maize, whereas bacterivore nematodes were more abundant in non-*Bt* maize fields. It is in fact the relative contribution of bacterivore nematodes like *Acrobeloides* that matters (Griffiths et al. 2006, 2007b).

Two simple categories like “smaller invertebrates” (nematodes) and “larger invertebrates” (micro-arthropods and enchytraeids) provide in Fig. 4 proxies to measure the extent to which species of a given body-size (*S*), are interacting within soil community food webs. In fact, if community food webs are size-structured so that large taxa eat primarily small ones, then differences between food webs in the body-size distribution reflect differences in how energy flows through trophic links, which is related to the web trophic structure. According to Peters (1983), the metabolic rate of an individual organism is a power law of its body size ( $\propto S_{ind}^\eta$ ). In each food chain, if the production of the resource *r* is  $k_1 \times D_r \times S_r^\eta$  and the production of the consumer, *c*, of *r* is  $k_2 \times D_c \times S_c^\eta$ , assuming all the population density (*D*) of *r* is consumed by *c*, then  $\alpha \times k_1 \times D_r \times S_r^\eta = k_2 \times D_c \times S_c^\eta$ , where  $\alpha$  is the efficiency by which *c* converts *r* into its own production, and the exponent  $\eta$  is claimed to be close to  $3/4$  (Peters 1983; Brown et al. 2004; Hendriks and Mulder 2008). These authors have elaborated theories to relate aspects of food-web structure like consumer-to-resource body-size ratio and some reproductive parameters to allometric scaling. It is our meaning that more comparable linkages between (community) response trait and effect traits might lead to predictable effects of environmental change on ecosystem services (Mulder and Elser 2009).

**Conclusions**

A stable ecosystem occurs when the sum of the negative feedbacks between components equals the sum of the positive feedbacks (Berryman 1991), where the classical feedbacks are these consumer–resource links. As soon as the micro-arthropods start to dominate, resources become exploited; only a resource depletion can force the final arthropod decrease. We briefly explained how fungi, bacteria and related consumers have to be monitored and modeled in synergy, because bottom-up (secondary) effects are expected in mites feeding on the nematofauna in



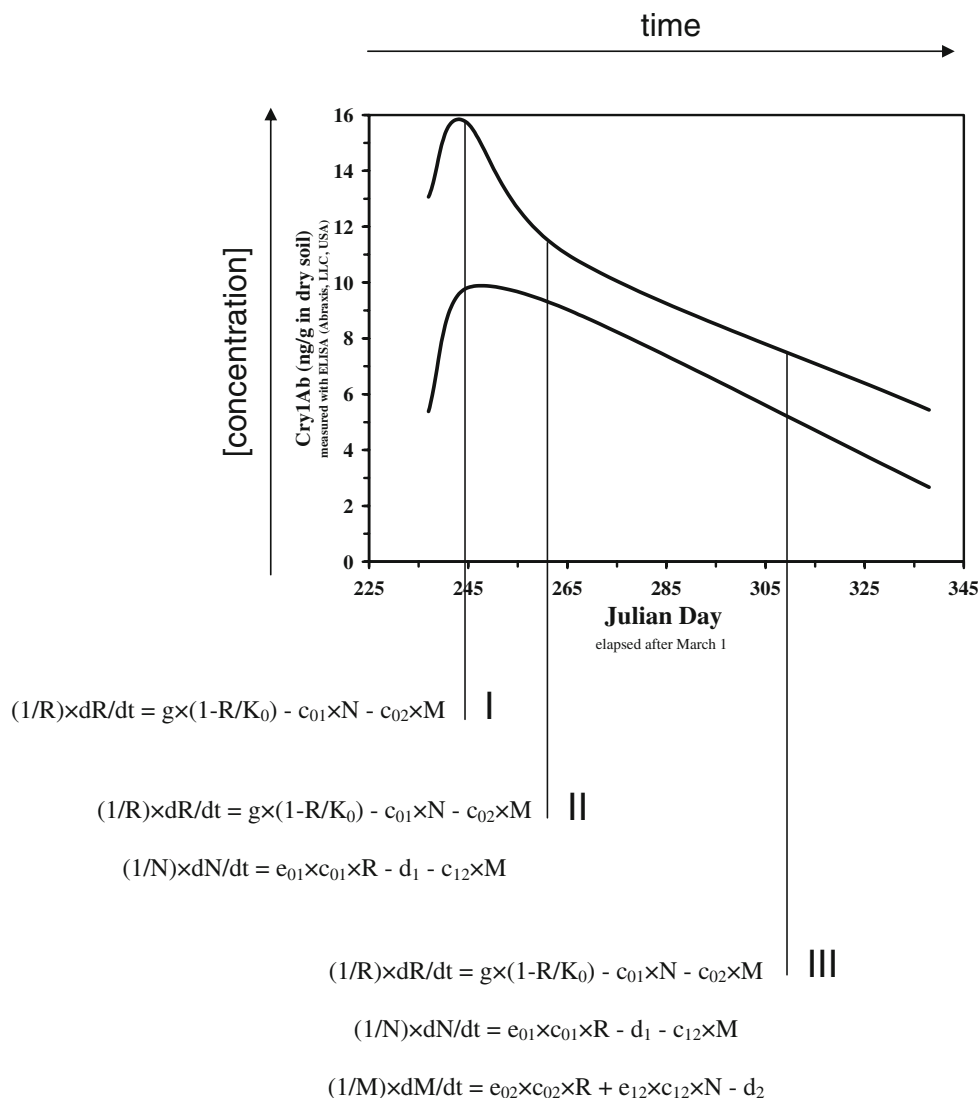
**Fig. 3** Different parameters to describe recurrent population patterns in an environment, in our case the population densities (*upper balks*), the body sizes (*lower balks*) and the trophic links for all occurring organisms and entities. This empirical network of an agro-ecosystem in the Netherlands depicts the two energy pathways from the basal resources at the bottom (1 = bacterial cells on the *left*; and 2 = fungi and detritus on the *right*) and thus shows the energetic setup in terms of size and density. The width of the *horizontal bars* shows for each taxon the relative rank of size average and density [the larger the bar, the bigger (more abundant) the organism]. The position of the bars reflect the trophic height of the populations; *black bars* show the soil

invertebrates belonging to the microfauna, *gray bars* those belonging to the soil mesofauna, and *white bars* those belonging to the soil macrofauna (all taxa numbered as in Mulder 2006). From *top* to *bottom*: top species have only resources, intermediate species have resources and consumers, basal species have only consumers. Microbes are the only true basal species in detrital soil food webs. Taxa belonging to the microfauna and to the mesofauna can be easily assigned to either the intermediate or the top species, but most taxa belonging to the macrofauna (here, lumbricids) have an ambiguous and confounding trophic position (*right*)

addition to direct *Bt* effects. We believe that differences can be ascribed to a form of bacterial antagonism, a mechanism which can take the form of either niche exclusion at the root surface (induced systemic resistance of the plant) or competition for nutrients required for bacterial growth and activity (cf. Feldgarden et al. 2003; van Loon and Glick 2004). The biodiversity richness of soil bacteria is enormous and prokaryotes respond to environmental perturbations in the same way as most eukaryotes do, though potentially much faster (Øvreås 2000). Our technique of metabolic fingerprinting showed, in contrast to precedent studies reviewed in Icoz and Stotzky (2008), significant responses of the microbial resources to the *cry* gene and demands further investigation at higher trophic levels. In the entire community food web, unintended effects might become magnified.

According to Naeem and Li (1997), a stable ecosystem behaves in a predictable way despite disturbance. Although their experimental data on relationships between biodiversity and ecosystem services rely on manipulated diversity (Daly and Buntin 2005), we might reverse their

statement and claim that a reliable prediction model is a kind of statistical insurance against possible failure of certain ecosystem services. This makes a synthetic, more integrative analysis urgent to understand, predict and wisely manage new biotechnological resources. Large soil invertebrates are often present in high numbers, show a high species diversity, and are easy to count. Their numerical abundance provides good information of the diversity and the stability of soil biota. Still, a functional and mechanistic analysis of the multitrophic effects of GM plants on soil biota such as the proposed one is lacking. The sensitivity of a soil community food web to rhizosphere changes under transgenic crops and the energy flow of any food web under stress incorporate several pooled information on the ecosystem services and the different responses of *all* soil invertebrates to perturbations in other trophic levels. New data and novel analyses always invigorate old debates, but we believe that actual transgenic plant–arthropod interactions should be modeled in such dynamic ways. Our plea for a much improved focus on the multitrophic interactions (the “missing links”)



**Fig. 4** Fundamental plant–microbe–animal interactions are structured in a new model. For illustrative purposes, we model induced changes in the biomass of soil organisms through time using coupled differential equations. As soon as *Bt* maize straw reaches the soil or is plowed under into the rhizosphere (min vs. max Cry1Ab curves), the heterotrophic respiration starts with the decomposing process, making more nutrients available. Let *R* be the biomass of all microbial resources in a soil community food web with growth rate enhanced by carbohydrates (soil-specific Eubacteria), separate all faunal biomass into two body-size categories, microfauna and mesofauna, and let their aggregate biomass be *N* (nematodes) and *M* (micro-arthropods). Short-term effects of Cry1Ab are most evident in the Phase I. Nematodes feed mainly on *R* (and on other small invertebrates), and larger soil arthropods feed on *R* and *N* (and larger invertebrates

besides lumbricids, see Fig. 3). Medium-term effects become most evident in the microfauna during Phase II and long-term effects in the microfauna and mesofauna (Phase III). Such a model allows for a certain omnivory and may be formalized in the equations embedded in the figure, where *K*<sub>0</sub> is the logistic carrying capacity of *R*, *c*<sub>*ij*</sub> is the rate of consumption on soil compartment *i* by soil compartment *j*, *e*<sub>*ij*</sub> is the ecological efficiency of trophic energy transfer from compartment *i* to compartment *j*, and *d*<sub>1</sub>, *d*<sub>2</sub> are death rates (per unit biomass, per unit time) of nematodes (*N*) and micro-arthropods (*M*) respectively. The balance between *M* and *N* (when *M* > *N*, micro-arthropods dominate; when *M* < *N*, nematodes dominate) reflects microbial carbon-to-nitrogen ratios under *Bt* straw, which tend to be higher than under conventional straw (Table 2)

under GM plants aims to establish trait-based protocols for ecological risk assessments.

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