Impact of genetically modified crops on rhizosphere microorganisms and processes: A review focusing on Bt cotton

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HIGHLIGHTS

- GM crops may impact nutrient cycling in the rhizosphere soil.
- GM crops do not adversely influence soil microbiological processes.
- Clay-humus complexes can protect *Cry* toxin in soils.
- Risk of gene transfer from GM crops to non-target organisms is minimal.
- Insufficient long-term experimental data restricts understanding of GM crop impacts.

Abstract

In recent years, the cultivation of genetically modified (GM) crops has become a topic of great interest, due in part to the considerable public controversy, which exists concerning their potential benefits or adverse effects. Since the development of the first GM crop about 25 years ago, a diverse range of new cultivars have been released into the environment which were developed by employing advanced molecular techniques to introduce new beneficial genes from a wide variety of sources. While GM crops have great potential for enhancing agricultural production, their potential impacts on soil biota are only partially understood and information on their long-term impact on soil biota is scant. Several recent studies have indicated that GM crops may cause changes in both the invertebrate and microorganism soil biota associated with these crops, with some laboratory-based experiments even revealing transfer of genes from GM plants to native soil bacteria. However, processes such as gene transfer and stable inheritance to subsequent generations remain unproven in natural soil systems. In addition, although significant research efforts have recently been directed towards understanding the effects of GM crops on soil biota, the wide variation in the scientific observations has often hindered an accurate understanding of the issues. Thus, this review collated and synthesized all available information

on the microbiological and biochemical effects of GM crops on soil biota with a special focus on GM Bt-cotton. The review also addressed the key issues associated with the use of GM crops including herbicide resistance, transgene flow and explored the plausibility of horizontal gene transfer in soil.

Keywords: Agricultural output; Bt cotton; Genetically modified plants; Soil ecosystems; Soil microorganisms

1. Introduction

Today genetically modified (GM) crops are commonly developed worldwide by deliberately introducing beneficial genes of one organism into another. When genes are transferred into agriculturally important plant crops, this genetic manipulation can provide consistent and substantial agronomic and economic benefits. For example, a gene that codes for insecticide toxin production in the subspecies of *Bacillus thuringiensis* (Bt), when genetically engineered into cotton, can allow the GM cotton plant to express the Bt toxin gene and produce insecticidal toxins to kill common lepidopteran pests such as the cotton bollworm (Palma et al., 2014; Tabashnik et al., 2002).

GM crops are generally classified, based on desirable traits, into four major groups (1) herbicide tolerant (HT), (2) insect resistant, (3) combined herbicide and insect resistant, and (4) viral disease resistant (Hails, 2000). These four groups account for 63, 15, 22 and < 0.1% of total GM crops, respectively (James, 2008). In 2008, worldwide 25 countries had approved the cultivation of GM crops (Liu, 2010), recently rising to 28 countries (Giri and Tyagi, 2016); which is likely to only increase in the future due to the need to increase agricultural production

globally. Likewise, the total worldwide cultivated area under GM crops increased from 1.7 million ha (Mha) in 1996 to 191.7 Mha in 2018 which translates to a 100-fold increase in acreage over the past 23 years (ISAAA, 2018).

Despite the substantial agronomic and economic benefits associated with the cultivation of GM crops, their use is still controversial because of considerable public concern and apprehension over potential environmental threats (Klümper and Qaim, 2014). The three main environmental and ecological risks associated with the use of GM crops are: (1) gene transfer from GM crops to wild relatives and related species, (2) development of herbicide, insect or virus tolerant or resistant crops, and (3) inadvertent detrimental impact on other non-target species and soil ecosystems (Liu, 2010; Tsatsakis et al., 2017). Even in the scientific community, controversy still exists regarding the cultivation of GM crops, with some researchers supporting the cultivation based on positive laboratory and field scale studies, while others are in strong opposition to the use of GM crops due to risks to mammals (Abbas, 2018). Supporters of GM crops often believe that it will aid in food security and minimize environmental degradation and also sustain agricultural production.

This review focusses on the collection and collation of information associated with the impact of GM crops on soil ecology and biodiversity. While ecological impacts of GM crops were initially confined to above ground effects, since early 2000 numerous studies have highlighted the potential influence of GM plants on below ground soil ecology and the associated microbial communities (Dunfield and Germida, 2001; 2004). Some research also suggests that GM plants may pose adverse effects to soil invertebrates (Bruinsma et al., 2003; Guan et al., 2016; Singh and Dubey, 2017).

One of the issues, in many of the studies conducted to date, was that the effects of GM crops on soil biological properties and available nutrient status were often transient, and thus their long-term impact on the soil ecosystem were difficult to quantify. This uncertainty in their impact on soil ecosystem and human health has since their inception, fueled public and scientific debate over their long-term risk. The aim of this current critical review is to help resolve this debate by providing a source of comprehensive information on the impact of GM crops on soil organisms and their influence on rhizosphere processes including nutrient availability and dynamics.

2. Status of GM crops

Following their commercial introduction in the USA in 1996, the cultivation of GM crops has spread rapidly. In 2002, GM crops covered 58 Mha, which had increased by a factor of 2.3 by 2009. Worldwide, the total area under GM crop cultivation increased by a factor of 80 between 1996 and 2009 (James, 2009), and by a factor of 110 between 1996 and 2017 (James, 2017). While initially GM crop cultivation occurred in a few large countries, namely the USA, Brazil, Argentina, India, Canada and China (in descending order of GM cultivated area), these crops are now being more widely grown in many developing countries worldwide (Table 1) with about 53% of the global GM crop areas cultivated in 19 developing countries.

Brazil is a typical example of a country which has embraced the use of GM crops, where the area under GM crop cultivation increased by almost 35% in 2009 compared to 2008 (James, 2009), with GM soybean having the highest cultivated area. Similarly, a rapid and continued expansion of GM crop cultivation also occurred in India with 8.4 Mha under GM cotton cultivation in 2009 (James, 2009). Presently, 11.4 Mha are under GM Bt-cotton cultivation with

an adoption rate of 93%, which accounts for about 36% of the area growing cotton globally (James, 2017). In the USA in 2009/2010, key GM crops as a proportion of total crop cultivated area included corn (86%), soybean and cotton (93% each), and sugar beet (95%) (James, 2009). Worldwide the major commercially grown GM crops are cotton (*Gossypium hirsutum* L.), canola or oilseed rape (*Brassica napus* L.) and corn or maize (*Zea mays* L.) and soybean (*Glycine max* L.) (James, 2017). While the global net economic benefits to farmers from growing GM crops were US$ 18.8 billion in 2012, the accumulated benefits during the period 1996 to 2008 were US$116.6 billion (Brookes and Barfoot, 2014).

3. GM crops and biodiversity

Agricultural biodiversity is demonstrated by the presence of a wide variety of genetic resources including crops, insects, livestock, soil biota, and wild relatives. Agricultural biodiversity thus consists not only of the diversity within species, but also the diversity between species and within agro-ecosystems (Thrupp, 1997). Many researchers believe that GM crops may pose numerous adverse effects to insects, plants, and the wider environment (Carpenter, 2011; Tsatsakis et al., 2017). Some of these threats may occur inadvertently due to the continuous application of chemicals to GM crops, which allows non-target weeds and insects to gradually develop chemical resistances. Similarly, threats such as gene flow or genetic contamination may occur through cross-pollination between GM and non-GM crops (Quist and Chapela, 2001). For cotton, Shi et al. (2006) specifically reported that the mortality of neonate larvae of cotton bollworm decreased after they had been fed with body and faeces extracts from the beet armyworm larvae which had previously been exposed to Bt transgenic cotton (*cry* toxin). All of these potential problems might lead to significant adverse impacts on agricultural

production systems where GM crops are widely cultivated in large amounts. A few examples of the potential risks posed by the widespread adoption of GM crops are briefly discussed in the following sub-sections.

3.1 Genetically modified Bt-crops

GM Bt-crops have remarkable potential to increase the yield of important agricultural crops because these crops often provide for a significantly higher level of protection against cotton bollworm (*Helicoverpa armigera)* with a consequential reduction in the number of insecticidal applications. In many regions of the world, the uptake of improved Bt cultivars has increased the productivity of cotton from 23 to 60%, and revolutionized cotton production (Koch et al., 2015; Sharma et al., 2006; Venugopalan et al., 2009). Upon expression of the gene, GM Bt crops produce a protein-like crystalline substance known as Bt-toxin (δ-endotoxin) commonly found in Bt bacterium, which has insecticidal properties. When produced within GM crops, the Bt toxin thus reduces crop damage due to insect attacks, because although the Bt protein is non-toxic in its free crystal form, it dissolves rapidly in the gut of insects (e.g., bollworm in cotton) at the prevailing high pH (pH $\approx$ 10.5). Following insect ingestion, the protein converts to a polypeptide toxin and causes toxemia and death of insects.

One key fact with GM Bt-crops is that the target insect pests, particularly the corn borer or cotton bollworm, may develop resistance to the Bt toxin over time, akin to how insects developing resistance to pesticides. Pesticide resistance is a major agricultural concern, which could lead to farmers increasingly spray more frequent and at higher pesticide levels to kill off troublesome insects. In the worst-case scenario, resistance could build to a level that sees the pesticide become totally ineffective against the target organisms. This is a very real concern;

with >500 insects showing resistance to various pesticides commonly used in agricultural practice (Andow, 2008). Thus, the cultivation of GM Bt crops is advantageous because it can reduce the use of broad-spectrum insecticides and also protect non-target insect diversity (Arshad et al., 2018).

One recommended way to mitigate the occurrence of Bt resistance in insects is for farmers to plant refuges of non-GM crops in the adjoining strips of the GM field. In the USA, Bt cotton growers plant either 20% of the area with a traditional cotton cultivar wherein they follow conventional pest control, or plant about 4% with a conventional cultivar without any pest control (Marra et al., 2002). These refuge crops are intended to maintain the diversity of vulnerable non-resistant insects and to increase their chances of breeding with Bt resistant insects with the purpose of decreasing the abundance of resistant insects (Andow, 2008; Watkinson-Powell and Alphey, 2017). Despite the growing of refuge crops being adopted in many other countries, including China and India, to slow down resistance buildup, target insects have still evolved to break the toxic effect of the *cry* protein in numerous instances. Such incidence of resistance was mainly due to the fact that the refuge growing practice did not work effectively in case of all the varieties and hybrids of GM cotton crops planted under different climatic conditions and cultivation practices (Tabashnik and Yves Carrière, 2019). Additionally, the above refuge practice may potentially transfer GM genes to the non-GM refuge crop over time. Thus, the effectiveness of insect refuges for enhancing agricultural production for both GM and non-GM crops over extended time periods still needs further investigation. It is also not known whether this approach can increase agricultural production by farmers in developing countries where the land available for crop cultivation is already small and the preparation of a separate fraction as an insect refuge may be a significant financial burden.

181

182 *3.2 Herbicide resistance*

183 One side effect of overuse of herbicides with target herbicide tolerant crops can lead to
184 the inadvertent development of herbicide resistant in the associated weeds. Thus, it is
185 increasingly evident that common weeds are becoming resistant to herbicides, especially with
186 repeated applications of glyphosate in areas where glyphosate-resistant GM soybean was
187 extensively grown (Warwick and Meziani, 2002). For example, in 2002, farm advisors in the
188 USA reported that a horse-weed species had become so resistant to herbicide that it required
189 between a 6 to 13-fold greater amount of herbicide to obtain a similar level of control as a non-
190 resistant horse weed species (Warwick and Meziani, 2002).

191 To date, >400 herbicide resistant weed species have been documented (Pretty, 2001)
192 from various GM crop growing parts of the world. For example, in Canada, oil seed rape
193 varieties quickly became resistant to three commonly used herbicides following the cultivation of
194 GM varieties for just four years (Orson, 2002), where it was assumed that gene transfer between
195 herbicide tolerant crops and associated weeds was responsible for the resistance (Orson, 2002;
196 Vencill et al., 2012).

197 GM crops may also potentially impact non-plant biodiversity and non-target organisms,
198 since the diversity of beneficial insects and arthropods are often impacted when feed GM crops
199 (Carpenter, 2011; Gatehouse et al., 2011). This is a serious concern for the pest management of
200 small farm holdings that depend on a greater diversity of complex predators and parasites to
201 minimize insect damage to the cultivated crops. The advantage of Bt proteins introduced into
202 GM crops is that they do not seem to hamper the establishment of natural enemies which prey on
203 insects and as such their use supports the conservation of natural enemies and reduced

insecticidal use (Romeis et al., 2019). Indeed, in areas of long-term Bt-cotton cultivation there was no occurrence of insecticidal resistance and no effect on non-target organisms (Rocha-Munive et al., 2018).

3.3 Transgene flow

Gene flow may occur when engineered plant genes are unintentionally transferred from a GM crop to wild relatives, non-GM plants or other organisms. The possibility and impact of gene flow relies on the local environmental conditions and the heterogeneity of crop types. The phenomenon of gene flow has been widely observed in the GM canola crop; where canola pollen could pollinate plants up to 800 m away (Coghlan, 2001). Thus, gene flow could be avoided by planting GM crops at a minimum isolation distance away from non-GM cultivars.

One of the key issues associated with the use of GM crops is the potential development of super weeds as a result of gene transfer from GM crops to wild relatives. For example, wild sunflower super weed that received insect-resistant genes from a GM sunflower became robust and produced about 50% higher seeds than the GM cultivar (Cummings et al., 2002). Sorghum is another crop which may cause gene flow as sorghum easily hybridizes with weedy relatives such as John grass, sugar beet, carrot, rye grass and white clover (Pretty, 2001). However, super weed development is not a direct threat to crop production and there are potentially bigger risks of gene flow across different farm scales, e.g., from large commercial farms to small nearby farms. Furthermore, the problem of gene flow may directly endanger the biodiversity in countries that are centers of the genetic origin for specific crops because the unwantedly transferred genes may contaminate the purity of original crop species.

4. Potential consequences of GM crops on soil organisms

Soil is a highly heterogeneous system in which interactions between the biotic and abiotic components continually occur. Therefore, the impact of GM crops on soil ecology must be understood from the perspective of the natural variability which already exists in soils. Since nutrient management practices, particularly carbon and nitrogen dynamics, and climate are the key factors that impact soil microbial diversity and ecological parameters, due consideration should be given to those factors while assessing the effect of GM crops on the biodiversity and functions of soils (Balser et al., 2010; Louis et al., 2016). It is likely that modifications in agricultural practices will have a much more profound impact on soil ecology than the modified genetic trait itself, e.g., decreasing tillage operations in herbicide tolerant crops will cause less soil disturbances than conventional management of multiple herbicide applications for suppressing weeds. The potential impacts of GM crops on soils include: (a) unwanted effects resulting from novel products produced by GM crops, e.g., Bt toxin, (b) increased soil pollution due to the increased use of new agrochemicals/molecules to manage GM crops, (c) greater risk to the established agro-ecosystem due to the introduction of novel practices associated with GM crops, (d) reduction in soil biological diversity and nutrient cycling, (e) persistence of GM crop residues in soil, and (f) occurrence of gene flow from GM crops to soil microorganisms.

One of the problems in attributing any observed effects specifically to GM crop use is that should issues arise at only a very low to modest level, it would be very difficult to detect them against the backdrop of the normal fluctuations of soil performance, for example, fluctuations due to tillage practices. Although soil is a dynamic system subject to constant change, it is able to maintain functions due to the diversity of microorganisms responsible for the underlying processes (Patra et al., 2005). In fact, most of the novel genes introduced into GM

crops were first developed from soil bacteria. Thus, since the interaction in soil ecosystems are so complex it is important to consider the threat of GM crops to soil microorganisms on case by case basis and to closely monitor areas of possible concerns.

4.1 Impact of GM crops on soil bacteria

Genetically modified plants can potentially alter soil microbial communities and hence vital ecosystem functions, including carbon cycling, nutrient solubilization, and the occurrence of soil-borne plant disease (Beura and Rakshit, 2013; Sarkar et al., 2009). However, it is not clear whether these impacts are directly due to the newly introduced gene or indirectly due to the modification of the rhizosphere chemistry of the GM plants (McGregor and Turner 2000). Many constituents of soils, especially colloidal particles including clay minerals and humic substances, have high affinity to adsorb biological molecules such as DNA and proteins originating from soil microorganisms (Cai et al., 2007; Cai et al., 2008; Kunito et al., 2016). Growing scientific evidence demonstrates that soil can safeguard such biomolecules from biological erosion (Cai et al., 2007; Morrissey et al., 2015) and consequently, the soil colloid-mediated protection mechanism might enable soils to retain concerned specific molecule's genetic and toxic properties for a long time (Cai et al., 2007). One good example of recent concern is the retention of antibiotic resistant genetic information in soil particles (Fahrenfeld et al., 2014; Bech et al., 2014; Burch et al., 2014). Similarly, the biomolecules responsible for carrying the toxicity and/or genetic information of GM crops can be retained by soil particles for long time (Cai et al., 2008; Crecchio and Stotzky, 1998).

The magnitude of the impact of GM crops on non-target soil biota entirely depends on the nature of recombinant proteins (i.e., its wide range of activity) and the degree of GM

exposure. Like all plants, GM plants also exude root exudates into the soil, where, the decomposition of GM plant residues also releases recombinant biomolecules into the soil. The potential impacts of horizontal gene transfer from GM crops on soil microbial diversity and microbial processes are illustrated in Fig. 1. Since the soil stability and persistence of the recombinant proteins (e.g., Bt toxin) is an important factor that dictates the degree of impact on non-target soil biota, Cai and co-workers (2008) extensively studied the persistence of Bt transgenes in soil. They found that the occurrence of montmorillonite clay coated by hydroxyl aluminum complexes in the soil provided protection for DNA against degradation by DNase I. This greater stability of DNA was mainly attributed to the conformational change of bound DNA and the soils higher adsorption capacity for DNase I. However, very little information is available on the fate and transformation of other Bt proteins, which may be released into the soil environment via a different GM crop species. It was assumed that the introduction of bacterial genetic material into plants might increase the probability of gene transfer from GM plants to soil bacteria (Stotzky, 2008), but there is currently insufficient evidence to support this. For instance, while a significant shift in the microbial communities residing in the rhizosphere of GM potato was found at crop harvest in one season, the effect was not present the season after (Dröge et al., 1998). Similarly, Lottmann and colleagues (Lottmann et al., 1999; Lottmann et al., 2000; Lottmann and Berg, 2001) extensively studied the effects of GM potato plants on the microbial composition of the potato rhizosphere and geocaulosphere under field trials. Although, the GM potatoes had been modified to produce T4-lysozyme (i.e., a bacteriolytic enzyme to gain resistance against *Erwinia carotovora* subsp *atroseptica*), they found that the microbial community shift occurring naturally in the soil simply outperformed any microbial effects resulting from T4-lysozyme exposure (Lottmann et al., 1999). Likewise, although cultivation of

296 opine producing *Lotus corniculatus* cv. Rodeo plants did not significantly change the total
297 cultivable bacteria in the soil, the opine utilizing bacterial population did increase in the
298 rhizosphere more than in the bulk soil (Heuer et al., 2002). Furthermore, Guyon et al., (1993)
299 reported that opine producing GM crops specifically promoted the growth of opine degrading
300 *Agrobacteria* in the soil.

301 Other studies have also confirmed a shift in soil biota constituents in response to GM
302 crop cultivation. For example, Siciliano and Germida (1998) observed a significant variation in
303 the microbial groups present in the rhizosphere of glyphosate-resistant and unmodified isogenic
304 canola (rape) varieties. In another field experiment, Dunfield and Germida (2001) examined the
305 diversity of bacterial communities in eight commercial canola varieties over two years at four
306 different field locations and surprisingly found that neither canola variety nor soil type affected
307 the total soil bacterial population. However, significant differences in fatty acid methyl ester
308 (FAME) and the community level physiological profile (CLPP) analyses of soil microorganisms
309 were found, where soil type had greater influence than canola variety. In such studies, soil
310 heterogeneity and/or variations in the nutritional status of GM crops make understanding the
311 apparent impact of GM crops on soil microorganisms difficult unless appropriate controls are
312 included to clearly delineate GM crop-induced effects from soil heterogeneity-induced effects
313 (Donegan et al., 1999; Escher et al., 2000; Hopkins et al., 2001).

314 Most of the studies conducted to date have involved culture dependent methods to
315 investigate the effect of GM crops on soil biota and microorganisms. However, this approach has
316 serious limitations because almost 99% of soil microorganisms are not culturable in the
317 laboratory. Gyamfi et al., (2002) examined the dominant *Pseudomanas* communities in the
318 rhizosphere of oil seed rape using the 16S rRNA molecular technique and found that there was

319 little variation in *Pseudomanas* populations of both oil seed rape and its wild relatives, and any
320 effects due to the GM trait were minimal compared to changes caused by plant growth stage.
321 Similarly, no significant difference in the diversity of bacterial communities under Bt and non-Bt
322 maize crops was observed using molecular techniques such as single-strand conformation
323 polymorphisms (SSCPs), phospholipid fatty acid (PLFA) profiling and CLPP (Baumgarte and
324 Tebbe, 2005; Griffiths et al., 2006). Likewise, comparison of differential C substrate utilization
325 patterns and DNA fingerprinting approaches (e.g., amplified ribosomal DNA restriction analysis
326 (ARDRA), ribosomal intergenic sequence analysis (RISA), and enterobacterial repetitive
327 intergenic consensus polymerase chain reaction (ERIC-PCR)), for microbial communities of
328 pink pigmented facultative methylotrophs available in the rhizoplane of Bt cotton did not differ
329 relative to non-Bt cotton (Balachandar et al., 2008; Zhang et al., 2015).

330 In contrast to the above studies which have shown little or no influence of GM crops on
331 microbial communities, other studies have reported that GM crops have considerable effects on
332 soil microbial communities. For example, under greenhouse conditions Bt corn had a
333 significantly lower level of mycorrhizal colonization than non-Bt corn, as detected by
334 Denaturing Gradient Gel Electrophoresis (DGGE) analyses of 16S rRNA genes (Castaldini et al.,
335 2005). Similarly, adverse impacts on fungal diversity and communities of methanogenic archaea
336 and methanotrophic bacteria were also observed in soils during the initial phase of root decay for
337 Bt rice when measured using terminal restriction enzyme fragment length polymorphism (T-
338 RFLP), DGGE and RT-PCR (Han et al., 2013; Lu et al., 2010). In direct contrast, a Bt maize
339 field trial showed greater total microbial activity, higher rhizosphere microbial diversity and
340 enriched community structure compared to the non-Bt cultivar (Velasco et al., 2013) when using
341 bacteria- and phylum-specific PCR-DGGE and PCR cloning techniques (Velasco et al., 2013).

Mandal et al. (2019) also reported significantly higher counts of beneficial soil microbes and enzymatic activities, *viz.* dehydrogenase, alkaline phosphatase and fluorescein di-acetate hydrolysis, in a Bt-cotton-soybean cropping system than other systems of Bt- and non Bt-cotton crops. While Mandal et al. (2019) enumerated only the culturable soil microorganisms, nevertheless, the higher microbial activities, especially the enzymatic activities, at all Bt-cotton growth stages indicated that labile carbon fractions in the rhizospheres of Bt-cotton was the main factor governing microbial activities of Vertisol (Mandal et al., 2019).

4.2 Impact on other soil dwelling organisms

Very few studies have evaluated the potential impact of GM crops on soil organisms essential for the decomposition of organic residues and nutrient cycling. Griffiths et al. (2000) reported that the reduction in soil protozoan population was transient when the soil was grown with GM potatoes expressing lectins. Similarly, Donegan et al. (1997) found that during leaf litter decomposition nematode population structure and density varied in GM tobacco plants, which was attributed to changes in carbon content between GM and non-GM plant leaves (Donegan et al., 1997). In another study, while growth of Bt rice had no significant impact on the nematode abundance and community composition, it did strongly influence trophic connection within nematode communities (Liu et al., 2018). Another study involving cyst nematode resistant GM potato showed that GM lines effected the fungal PLFA profile (Cowgill et al., 2002), where the ratio of fungal to bacterial PLFA provided a measure of the differences in the relative abundance of bacteria and fungi in response to the GM potato crop (Cowgill et al., 2002).

5. Effect of *Bacillus thuringiensis* on soil

Bacillus thuringiensis (Bt) is a common soil bacterium found all over the world (Martin and Travers, 1989). The bacterium is widely used commercially as a bio-control agent for the control of insect pests in arable crops and consequently many of the GM crops cultivated today contain pesticidal genes from Bt. Particularly, Bt cotton has been grown commercially in various parts of the world to control lepidopteron insects. While the vegetative cells of Bt are well adapted to thrive in the gut of susceptible insects (Raymond, 2017; Yara et al., 1997), the Bt endospores can also survive in a wide range of soils and environmental conditions except at below pH 4.8 (Dulmage and Aizawa, 1982; Saleh et al., 1970). Otherwise the existence of Bt in soils is largely dependent on existing soil microbial communities which actively competes with the introduced Bt species and tends to competitively diminish overall Bt populations (Akiba et al., 1977). For example, 12 - 16 months after inoculation of Bt, a 100-fold reduction in the Bt population compared to soil bacilli was observed (Pruett et al., 1980). After 135 days of inoculation of the soil with *Bacillus thuringiensis* var. *galleriae*, the viable spores of Bt reduced considerably to 24% of the initial spores and also a negligible insecticidal activity was observed (Pruett et al., 1980).

For the Bt toxin to be more broadly effective in a soil a critical factor is the distribution of the Bt organism. Studies using an antibiotic resistant marked Bt strain showed very limited movement of Bt through the soil, with no downward movement beyond 6 cm and lateral movement beyond 10 m outside the experimental site, indicating both limited mobility and low potential for genetic exchange (DeLucca et al., 1981; Martin and Reichelderfer, 1980; Meadows, 1993).

5.1 Effect of Bacillus thuringiensis and Bt-toxin on soil microflora

Studies concerning the influence of Bt on soil microorganisms are scant, and inconsistent (Addison, 1993). For example, while enhancement of soil microbial populations were reported after 2-4 weeks when using a Bt formulation consisting of *Bacillus thuringiensis* subsp. *galleriae* and *Bacillus thuringiensis* subsp. *kurstaki* (Petras and Casida, 1985; Pruett et al., 1980), Atlavinyte et al. (1982) reported a decline in bacterial and actinomycete populations as well as an increase in fungal population following the addition of *Bacillus thuringiensis* subsp. *galleriae* (Krieg et al., 1983; Visser et al., 1994). Thus, there are clear contradictions regarding the efficacy of Bt on non-target microflora. A three-year continuous field trial with Bt cotton observed no significant changes in fungal community diversity and population in the rhizosphere of Bt-cotton compared to conventional cotton (Xie et al., 2016). Similarly, Qi et al. (2018) found no significant changes in bacterial communities in Bt cotton when compared to the non-Bt cultivar. Zhaolei et al. (2018) observed a rapid decline in the concentration of the Bt protein without any significant changes in the microbial community structure and diversity. Li et al. (2018) also reported that the cultivation of Bt cotton vis-à-vis conventional varieties did not significantly affect soil bacterial population dynamics, and indicated that soil factors such as pH greatly influenced the microbial community. Indeed, no trace of the Bt protein (*Cry*1Ac) was detected in fields one year after Bt cotton cultivation and crop residue incorporation (Zhang et al., 2019).

Saxena and Stozky (2000) conducted studies on the secretion of Bt toxin from the roots of Bt corn into the soil and detected the Bt toxin in root exudates at 7, 15 and 25 days after seed germination. However, the toxin was only detected under sterile conditions and under non-sterile conditions it was rapidly hydrolyzed by microbial proteases. No evidence of the Bt toxin was

found in the soils grown with non-Bt corn (Table 2). The detection of the Bt-toxin after a certain period indicated some protection of the toxin in clay–humus structures under both sterile and non-sterile soil conditions.

5.2 Fate of Bt toxin in soil

It is expected that the Bt toxin would be rapidly adsorbed and tightly bound to soil clays, which would protect the Bt toxin from degradation, while keeping insecticidal activity intact (Crecchio and Stotzky, 1998). In fact, compared to the free protein, the presence of various humic acid functional groups strongly influenced the binding of the Bt toxin to soil constituents including clays, where the humic acid-bound Bt toxin was highly recalcitrant to microbial degradation (Crecchio and Stotzky, 1998). Indeed, Koskella and Stozky (1997) reported that the free toxin from *Bacillus thuringiensis* subsp. *kurstaki* or *Bacillus thuringiensis* subsp. *tenebrionis* could be utilized by *Proteus vulgaris, Enterobacter aerogenes* and a diverse microbial culture isolated from soils, but not when the Bt toxin was bound to montmorillonite clay mineral (Table 3). In addition to soil clay contents, soil organic matter may also influence the accumulation of the Bt toxin in soils. Thus, while there is considerable evidence that the accumulation of the Bt toxin in soils may potentially pose a risk to non-target soil organisms, in short term studies the Bt toxin, either free or bound, had no adverse influence on the growth and development of soil biota (Rui et al., 2005; Saxena and Stotzky, 2001).

5.3 Impact of Bt cotton on soil microbial and biochemical indicators

Since Bt cotton is the most cultivated and commercially released GM crop worldwide, the possibility for Bt efflux into the soil environment is relatively high with possible entry routes

433 into the soil either through root release and/or residue decomposition during crop growth (Sarkar
434 et al., 2009). The Bt toxin is present in every major part of Bt cotton plants including leaves,
435 stems and roots, with the highest Bt toxin production in the roots during the latter growth stages
436 of the plants (Sarkar et al., 2009).

437 Soil microorganisms may thus come into close contact with the *Cry* toxin produced
438 from GM Bt plants at various developmental stages. Although Bt is naturally present in the soil,
439 growing GM Bt corn, for example, may increase the concentration of the Bt toxin in agricultural
440 systems; up to 0.25 g ha^{-1} for soils and up to 650 g t^{-1} in the Bt corn plants excluding grains
441 (Blackwood and Buyer, 2004; Sarkar et al., 2009). However, the slight effects due to a particular
442 GM crop trait on plant-associated microorganisms might often be practically over-shadowed by
443 the developmental stages of the crop themselves. For example, using a high-throughput
444 sequencing technique, Pan et al. (2018) showed that developmental stages had a significant
445 influence on shaping the phyllosphere micro-biota of Bt cotton which was indistinguishable
446 from the effect of *Cry1AC* gene itself.

447 Limited information is currently available on the effects of Bt cotton on soil
448 microbiological and biochemical indicators (Mandal et al., 2019; Mina et al., 2011; Sarkar et al.,
449 2008; Zhou et al., 2016). The known effects of GM cotton on the rhizospheric microorganisms
450 and processes measured using various tools have been summarized in Table 5. A pot culture
451 study comparing Bt cotton and a corresponding non-Bt isogenic line (Fig. 2) revealed a
452 significantly higher ($P < 0.05$) microbial biomass carbon (MBC), microbial biomass nitrogen
453 (MBN), microbial biomass phosphorus (MBP) and microbial quotient (MQ) in the Bt
454 rhizospheric soil (Sarkar et al., 2009). This study also found that soil enzymatic activities;
455 comprising nitrate reductase and phosphatases; were greater in the rhizospheric soil of Bt cotton

than in the unmodified isogenic line (Sarkar et al., 2009). Similarly, nitrification and potential N mineralization in the soil under Bt cotton crop were greater than the non-Bt isogenic line (Sarkar et al., 2009). However, the soil total organic carbon (TOC) contents showed no significant difference between the Bt and non-Bt cotton crops (Sarkar et al., 2009). In another study conducted under similar agro-climatic conditions, Mina et al. (2011) found that enzymatic activities; such as alkaline phosphatase, nitrate reductase and urease; did not significantly change ($P<0.05$) under Bt compared to non-Bt cotton cropping in field trials. However, the authors did report a significantly greater dehydrogenase activity in the soil under Bt-cotton than the unmodified isogenic line (Mina et al., 2011). The authors also observed higher numbers of soil fauna in the Bt cotton rhizosphere than the non-Bt cotton rhizosphere. Both these studies (Mina et al., 2011; Sarkar et al., 2009) concluded that the cultivation of Bt cotton did not pose any threat to the ecosystem functions of the soils, which was subsequently confirmed by several other studies (Kumari et al., 2015; Mina and Chaudhary, 2012; Singh et al., 2013; Velmourougane and Sahu, 2013; Velmourougane and Blaise, 2014). A subsequent field-based study also confirmed some positive impacts of Bt cotton based cropping systems on soil microbiological properties over non-Bt cotton based cropping systems (Mandal et al., 2019).

Kumari et al. (2015) reported that the presence of non-Bt cotton residues in the soil resulted in a significantly higher population of micro-flora and MBC than Bt cotton residues. However, when the interactive effect of crop varieties and soil types was investigated at various crop growth stages, the effect of Bt cotton residues on the soil micro-flora population was not significant (Kumari et al., 2015). The cropping pattern of Bt cotton could also influence its effect on soil microorganisms. For example, the population of soil bacteria, fungi, and actinomycetes

were enhanced by 60, 14 and 10%, respectively; in comparison to Bt cotton in isolation when peanut was grown as a cover crop between the Bt cotton rows (Singh et al., 2013).

In addition, the pattern of nutrient application strongly influenced soil dehydrogenase activity (total oxidative metabolic activity) under Bt cotton cultivation (Mina et al., 2011). For example, the application of urea along with farmyard manure (FYM) resulted in a greater level of dehydrogenase activity and N availability in soils under Bt cotton when compared to the application of urea alone (Singh et al., 2013; Singh and Ahlawat, 2014a). In practice, the introduction of a legume and organic manure combination to a Bt cotton–wheat system was shown to be a sustainable management approach for coping with the instability of GM hybrid adoption scenarios in south Asian countries (Singh and Ahlawat, 2014b)**.**

Sarkar et al. (2008) studied the nutrient (N and P) availability and dynamics in a sandy loam when a Bt cotton (cv. MRC-6301Bt) crop and its non-isogenic line were grown to maturity under pot culture. They found that the total inorganic-N (ammonium-N + nitrate-N) in the soil was reduced by 14%, whereas the available P was enhanced by 8% due to Bt cotton cultivation (Table 4) as well as a remarkable interactive influence of sampling time and Bt/non-Bt treatments (Sarkar et al., 2008). In contrast, in a field experiment, Mina et al. (2011) found 17 and 3.5% reductions in dehydrogenase activity and heterotrophic respiration, respectively, in the soil of Bt cotton compared to non-Bt cotton isoline. Kumari et al. (2015) also observed a 7.5% reduction in dehydrogenase activity due to Bt cotton residue incorporation into the soil. It was reported that Bt cotton might limit the supply of inorganic N, but enhance P-solubilization in soils (Sarkar et al., 2008). However, as discussed previously above, for many GM traits, the effects of Bt cotton on soil microbial and biochemical indicators were not as pronounced as other variable soil factors, crop uptake phenomena or prevailing ecological conditions.

5.4 Consequence of Bt cotton on nutrient dynamics and C cycling in soil

Cultivation of GM crops predominantly influences soil biogeochemical processes, particularly nutrient cycling in the soil ecosystem, by either modifying rhizosphere chemistry or through the products of the plant's introduced gene, i.e. the *Cry* toxin in case of Bt cotton (Fig.1). Rhizosphere dwelling microorganisms, their biomass and activity also influence nutrient mineralization in the root zone of GM crops. The genetically aided promotion of root characteristics; including root density and length; can lead to higher production of root exudates and the amount of easily bioavailable C and N in the soil under GM crops compared to conventional cultivars (Beura and Rakshit, 2013). For example, a 12-13% decrease in available soil N due to Bt cotton (preferably because of higher N uptake) compared to non-Bt isoline was reported (Beura and Rakshit, 2013). Thus, GM crops have a strong influence on soil nutrient cycling (Motavalli et al., 2004). However, no clear information is available as to whether root exudates directly cause the differences in soil nutrient cycling under GM crops or other non-targeted physiological changes such as content of starch, soluble N, proteins, carbohydrates, lignin in the plant parts are actually responsible (Icoz and Stotzky, 2008).

Available soil P is mainly regulated by interactions between plants and soil biota (Kennedy, 1998) and in the rhizosphere, both plant roots and associated microorganisms are influenced by the prevailing soil physico-chemical properties. Thus, amendments of organic acids to soils and organic acid release through root exudates play a significant role in P availability (Koyama et al., 2000; Lopez-Bucio et al., 2000). Likewise, changes in the composition and amount of root exudates in plants resulting from the expression of novel genetic traits may have a direct influence on soil P transformation, and/or indirect effects on P availability through shifts in the

524 community and activity of rhizosphere dwelling microorganisms. For example, P availability in
525 the soil improved due to alterations of rhizospheric environments under Bt cotton (Mina and
526 Chaudhary, 2012; Shen et al., 2006).

527 Similarly, increases in both macro- and micro-nutrient availability were observed in the
528 rhizosphere soil of GM alfalfa due to a greater root exudation of low molecular organic acids by
529 GM alfalfa compared to the non-GM crop (Tesfaye et al., 2003). In another study, a strong non-
530 linear relationship between available P and root parameters suggested that the higher availability
531 of P in GM crops might not be solely due to variation in root exudates, but might have also been
532 due to variations in rhizospheric microorganisms (Cabugao et al., 2017). For Bt cotton, relative
533 to its non-isogenic cultivar, available N and K contents were lower due to the higher nutrient
534 demand of the Bt plants relative to its non-Bt counterparts (Sarkar et al., 2008), where Bt cotton
535 seemed to limit N and K soil availability while increasing P availability (Sarkar et al., 2008).
536 Efflux of root exudates from GM crops also influenced soil C pools by enhancing the C
537 fractions; including MBC in the rhizosphere of Bt cotton (Velmourgane and Sahu, 2013). In
538 addition, high soil enzymatic activities and enhanced beneficial microbial populations in the
539 rhizosphere of Bt cotton might positively affect the soil available nutrient contents (Mandal et
540 al., 2019). However, it was also observed that irrespective of the nutrient status, there were
541 significant interaction effects between soil types and Bt crop at different growth stages (Beura
542 and Rakshit, 2013).

543

544 **6. Impact of genetically modified microorganisms on soil biota**

545 Since field scale addition of GM microorganisms to soils has been very limited, the
546 impact of GM microorganisms on soil biota has also been less well studied. The ecological

consequences of GM microorganism addition to the soil have primarily considered the initial capacity of the introduced GM microorganisms to survive competition with native soil microorganisms (Doyle et al., 1995). It is only once the newly introduced microorganisms have exhibited successful competition and growth, that they might cause a shift in the native structural and functional microbial community (Doyle et al., 1995). Such a microbial community shift could then be achieved via a gene transfer mechanism to the native bacteria and subsequent biomass turnover. One limitation of current research in this area is that most of the changes in native bacterial community and biomass turnover were only observed in *in-vitro* research which might not be reliable extrapolated to field conditions. Moreover, only a temporary variation in the native microbial community may occur after inoculation of GM microorganisms into the soil (DeLeij et al., 1995).

A brief account of possible benefits and limitations of GM crops has been presented in Table 6. It is clearly evident that an inadequate number of studies have been conducted concerning the impact of functionally modified bacteria on native soil microorganisms. For example, *Rhizobium leguminosarum*, which was modified with a Bt gene in order to achieve protection against Sitona *(Sitona discoides)* weevil, surprisingly had a higher ability to compete for nodule sites on pea (*Pisum sativa cv* Meteor) roots than the wild *Rhizobium* strain (Giddings et al., 2000). While the development rates of the GM strain and the wild type were similar in the *in vitro* culture, when applied into the soil of growing pea plants the GM strain had a better ecological benefit than the wild type strain (Giddings et al., 2000). However, the authors did suggest that this effect might not be because of the transgene function directly but as a result of the variation in the random sites of insertion of the new gene (Giddings et al., 2000).

7. Horizontal gene transfer

By and large the mechanisms of gene transfer from crop plants to microorganisms and the resulting shaping of the root microbial community are unknown (Fitzpatrick et al., 2018). A few mechanisms were however suggested for the pollen hybridization process amongst suitable plant species (Nielsen et al., 1998). Whether such mechanisms could explain the gene transfer from GM plants to soil microorganisms requires more extensive research. In contrast, various mechanisms for horizontal gene transfer (HGT) amongst bacterial species have been previously described including transformation, transduction and conjugation (Crisp et al., 2015). Of these, only transformation and conjugation are considered here as being plausible for gene transfer between GM and unmodified bacterial species. In case of transduction, the gene transfer involves the participation of a virus or viral vector, which is considered the least plausible scenario for gene transfer from GM plants to soil microorganisms because of the extremely heterogeneous soil environment.

7.1 Gene transfer through natural transformation

Transformation is widely recognized as the most plausible pathway for genes to be transferred from one bacterial species to another in the soil ecosystem. In this mechanism, the competent bacterium may take up naked DNA from the adjoining environment (Dröge et al., 1998), where the conditions for competency vary between bacterial species and the naked DNA, can be derived from either the chromosomal or plasmid DNA released from living or dead microorganisms (David et al., 2016). However, there are many barriers to transformations in the soil and the rates of transformation could be extremely challenging to measure (Nielsen et al., 1998). While many reports have indicated that DNA could persist in the soil under certain

conditions for months to years, this also depends on the prevailing environmental conditions (Gebhard and Smalla, 1999; Nagler et al., 2018). For example, a greater level of DNA persistence can be expected in a soil with higher clay content and lower temperature than that with lower clay content and higher temperature. Today many more species of bacteria are capable of transformation than was previously thought (Havarstein, 1998). For example, Demanechee et al. (2001) showed that in soil microcosms under natural conditions HGT through transformation was possible between *Pseudomonas fluorescens* and *Agrobacterium tumefaciens*, but the same was not observed under *in vitro* conditions.

Experimental attempts to transfer genes from GM crops to soil microorganisms were largely unsuccessful. For example, by screening a massive 4000 bacterial colonies, Gebhard and Smalla (1999) found that there was no gene (kanamycin) transfer from GM sugar beet to the native soil bacteria. Although, the authors did qualify this result by reporting that the possibility of identifying transformation was hampered by the higher natural incidence of kanamycin resistant bacteria in the native soil environment (Gebhard and Smalla, 1999).

To date most experimental attempts to demonstrate HGT from GM crops to soil microorganisms have mainly focused on the use of model systems, where the identified microorganisms, as the recipient of the genetically modified DNA, were naturally competent (Dröge et al., 1998). It was observed that while transformation of the soil bacterium *Acinitobacter sp.* by GM sugar beet DNA occurred under sterile soil conditions, it was not observed under a non-sterile soil conditions (Nielsen et al., 2000). The magnitude of transformation in the non-sterile soil was estimated to be only 10^{-10} to 10^{-11} units, which was well below the level of detection. Other studies also confirmed a low probability for incorporation of transgenes in the bacterial genome if a DNA homology was not already existing in the system

(Nielsen et al., 1997). Therefore, while the possibility of transformation of competent bacteria by GM plant DNA; both in the bulk soil and in the rhizosphere; exists, in practice this would be at extremely low frequencies, if at all.

7.2 Gene transfer through conjugation

Although DNA transfer via conjugation generally takes place only amongst closely related bacterial species, it can also occur amongst various bacterial genera and between Gram-positive and Gram-negative bacterium. In this process, the shift of DNA from one bacterial cell to another occurs through direct contact between the cells. DNA is transferred via specific conjugation structures that are encoded by different self-transmissible plasmids and conjugative transposons. While the rates of plasmid DNA transfer could be very high *in vitro* studies, this would drop considerable under heterogeneous soil conditions, where the rates of conjugation between bacteria in the soil may differ widely. However, there may also be hotspots in the soil, such as the rhizosphere, where higher rates of conjugation might occur than the bulk soil because the former would have a greater abundance of bacteria than the latter. In practice, the movement of plasmids from GM microorganisms to native soil bacteria has been observed by Smit et al. (1991). Similarly, the uptake of plasmids by GM microorganisms from indigenous bacteria was also reported by Lilley and Bailey (1997), demonstrating the potential for HGT in soil.

Soil macro-biota, such as earthworms, could play a significant role in HGT. Gene transfer through conjugation was reported amongst bacteria which were spatially separated in a soil microcosm containing earthworms (Daane et al., 1997). This occurred because contact between microorganisms was enhanced when large bacterial populations were confined within the small space of the alimentary tract of the earthworm. In addition, in some insects, the gut environment

639 also provided conditions suitable for the growth and conjugation of bacteria. In fact, conjugation
640 between bacteria was observed in the gut of Rhabditis nematodes (Adamo and Gealt, 1996) and
641 in Collembola (Hoffmann et al., 1999). However, since conjugation involves direct contact and
642 exchange of DNA between two bacteria, it is unlikely to occur in the soil solely due to the
643 cultivation of GM crops.
644
645 **8. Conclusions**
646 In developing countries, GM crops have huge potential to fulfill the food demand of an ever-
647 growing population and make countries self-sufficient in agricultural production. However,
648 despite their rapid uptake worldwide, thorough studies examining the ecological risks induced by
649 GM crops are relatively few. In most of the studies conducted to date, the impact was extremely
650 low and often was insignificant compared to influences from normal background fluctuations in
651 other soil parameters. While some studies showed that GM plants caused considerable changes
652 in the structure and functions of indigenous soil microbial community, the soil heterogeneity,
653 varying nutritional requirements of GM plants, lack of suitable controls and other ecological set-
654 up imposed major difficulties in interpreting the real impact of GM plants on soil
655 microorganisms. Likewise, the practical impact of GM crops on soil biota and rhizospheric
656 processes was limited by the level of robust studies which hinders a complete risk assessment of
657 specific GM crops. Since the current understanding of GM crops on soil biota and their functions
658 remain unclear, future research initiatives should focus on the risk assessment of GM crops at all
659 trophic levels, considering every components of the ecosystem, and this should include emerging
660 potential GM crops, such as brinjal and rice.
661

9. Future directions

As discussed briefly below, this review gives some new insights into researchable issues and strategies necessary for the large-scale adoption of GM crops in order to achieve food and nutritional security *vis-à-vis* ecological safety.

(1) Current research indicates that there have been limited long-term studies which are now considered essential to practically study the impact of GM crops on soil flora and fauna.

(2) To date most of the laboratory studies which have shown soil accumulation of the Bt toxin have not been duplicated under field conditions due to the significant influence of edaphic factors and the biochemical activities of the native soil microorganisms under natural conditions. Hence, accurate estimation of the factors responsible for the transformation of Bt toxin under field conditions urgently needs to be evaluated. Likewise, the possibility of the movement of the *cry* gene from GM crops to non-target crops including wild relatives and weed flora needs is uncertain and needs to be thoroughly investigated.

(3) Very limited information is currently available on the effects of GM crops on soil invertebrates including ants, centipedes, collembola, earthworms, millipedes, mole crickets and nematodes. This is important because soil invertebrates are mainly responsible for the disintegration and decomposition of organic matter in the soil and thus greatly influence the nutrient recycling process. Therefore, a holistic effort is urgently required to compare both floral and faunal diversity under GM *vis-à-vis* non-GM crops.

(4) The current understanding of the effects of GM crops on soil biota and their functions are unclear; therefore, detail studies are required which assess the risks of GM crops with a special emphasis on edible GM crops.

(5) While many previous studies concerning the impact of GM crops on soil processes involved traditional microbial enumeration methods, <1% of the natural soil microorganisms can be cultured in the laboratory. Future studies evaluating the effects of GM crops on soil microorganisms therefore should include state of the art molecular techniques such as soil metagenomics and metabolomics to understand the community structure and function level processes.

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Figure caption

Fig 1. Schematic diagram representing impact of genetically modified crops on soil microbial communities and microbe-mediated processes. (PGP: Plant Growth Promoters; HGT: Horizontal Gene Transfer).

Fig. 2: Effects of Bt cotton on selected soil biochemical and biological indicators (adapted from Sarkar et al., 2009); MBC: Microbial biomass C, MBN: Microbial biomass N, MBP: Microbial biomass P, MiQ: Microbial quotient; PNM: Potential N mineralization, NF: Nitrification; NR: Nitrate reductase, Alk-P: Alkaline phosphatase, Acid-P: Acid phosphatase.

Figures

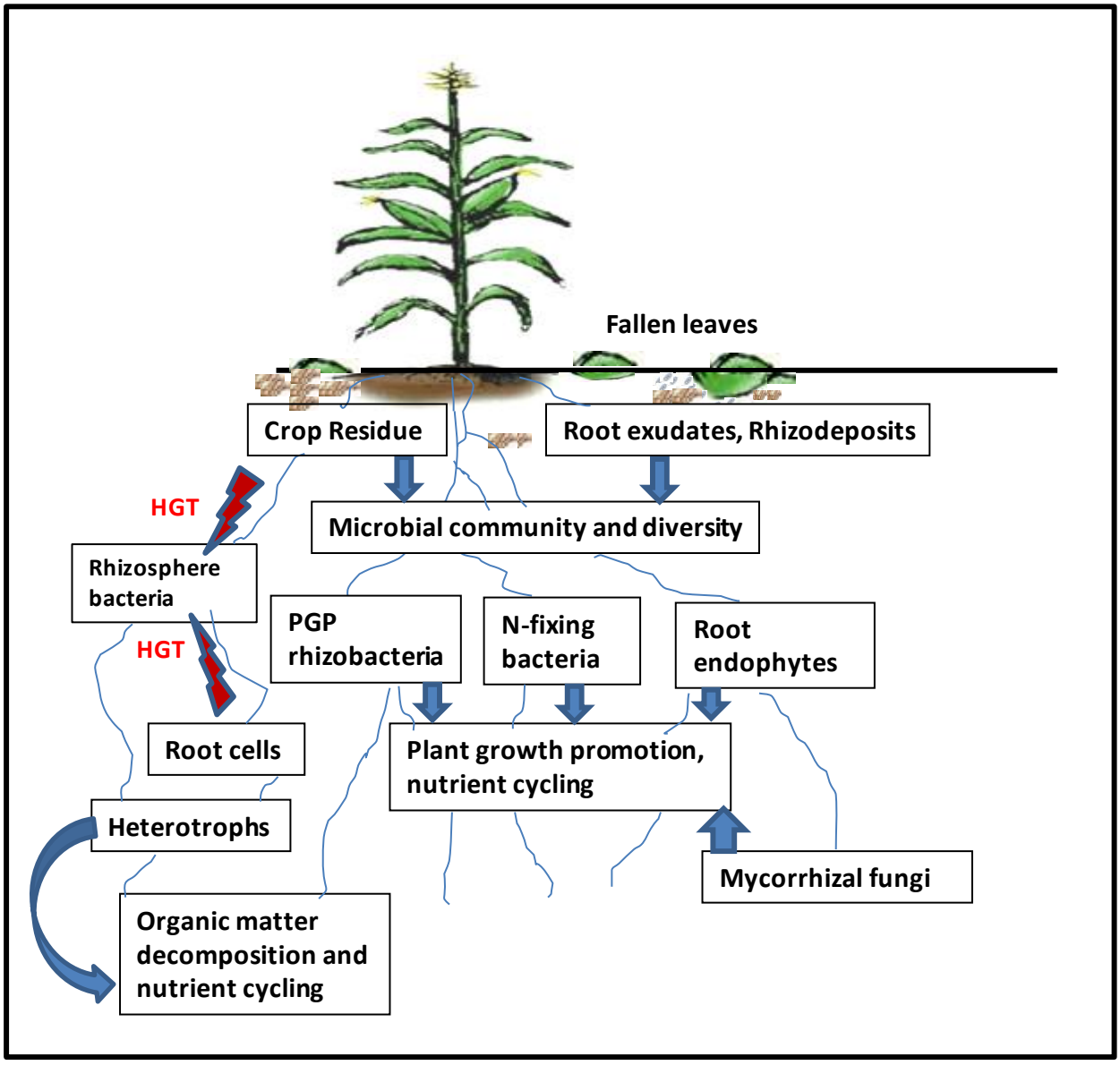

Fig. 1

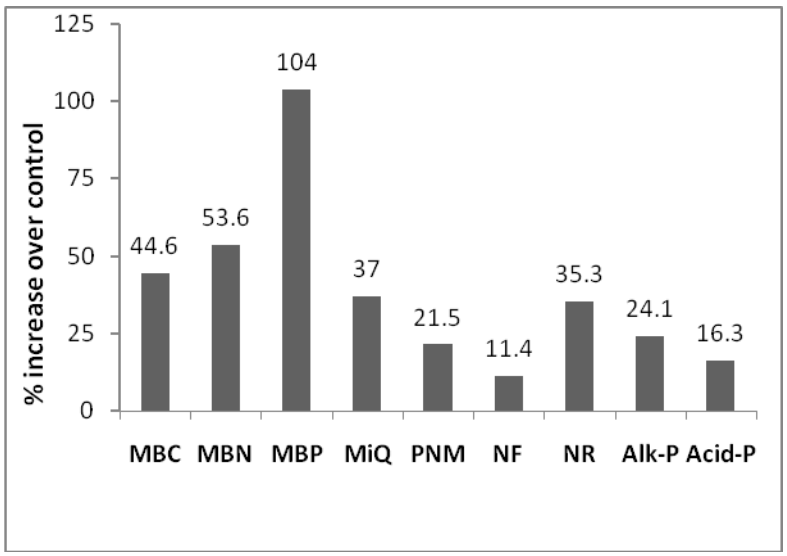
% increase over control
125
100
75
50
25
0
44.6
53.6
104
37
21.5
11.4
35.3
24.1
16.3
MBC
MBN
MBP
MiQ
PNM
NF
NR
Alk-P
Acid-P

Fig. 2

Table 1. Area and type of GM crops grown in different countries in 2017 (adapted from James, 2017).

Country	Area (M ha)	Type of GM crop
USA	75.0	Soybean, maize, cotton, rapeseed, sugarbeet, squash, papaya
Brazil	50.2	Soybean, maize, cotton
Argentina	23.6	Soybean, maize, cotton
Canada	13.1	Rapeseed, maize, soybean, sugarbeet
India	11.4	Cotton
Paraguay	3.0	Soybean
Pakistan	3.0	Cotton
China	2.8	Cotton, poplar, papaya, tomato, sweet pepper, petunia
South Africa	2.7	Maize, soybean, cotton
Bolivia	1.3	Soybean
Uruguay	1.1	Soybean, maize
Australia	0.9	Cotton, rapeseed, carnation
Philippines	0.6	Maize
Other countries	≈1.4	Maize, cotton, soybean, canola, eggplant

Table 2. Presence of toxin in corn root exudates with and without the *cry 1 AB* gene (adapted from Saxena and Stotzky, 2000).

Growth condition	Days after germination of seed					
	7		15		25	
	(Bt^-)	(Bt^+)	(Bt^-)	(Bt^+)	(Bt^-)	(Bt^+)
Hoagland's solution (SHPC)	-	+	-	+	-	-
Soil	-	+	-	+	-	+

(Bt^-): Non–Bt corn; (Bt^+): Transgenic Bt-corn; SHPC: Sterile hydro phonic culture

Table 3. Growth of microorganisms upon utilization of Bt toxin (adapted from Koskella and Stotzky, 1997).

Organism	Toxin	Binding clay fraction	Growth on toxin	
			Free	Bound
P. vulgaris	*B.t.* subsp. *kurstaki*	Ca-montmorillonite	+	-
E. aerogenes	*B.t.* subsp. *kurstaki*	Ca-montmorillonite	+	-
Mixed microbial culture	*B. t.* subsp. *tenebrionis*	Na-montmorillonite	+	-

Table 4. Effects of Bt-cotton on available nutrient contents in soil (adapted from Sarkar et al., 2008).

Parameters	Available nutrient contents (mg kg^{-1})			
	No crop	non Bt-cotton	Bt-cotton	LSD ($P < 0.05$)
Ammonium-N	19.7	19.3	18.0	Not significant
Nitrate-N	17.2	17.6	13.6	3.0
Total mineral N	36.8	36.9	31.6	3.6
Olsen-P	9.6	7.7	8.3	0.4

1166 **Table 5.** Effect of Bt–cotton expressing *cry* toxin on soil microorganisms and microbial
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Methods used for risk assessment	Impacts on microorganism/biota	References
Microbial counts (CFUs)	Significant negative differences in the numbers of the three functional bacteria	Rui et al., 2005
Catabolic diversity (CLPP)	No effects on the functional diversity of microbial communities	Shen et al., 2006
ARDRA; RISA; BOX-PCR; ERIC-PCR	No effects on diversity richness of PPFMs	Balachandar et al., 2008
Microbial counts (CFUs)	No significant effects on the numbers of different functional bacteria groups	Hu et al., 2009
Microbial counts (CFUs), T-RFLP.	No adverse effects on the diversity of the microbial communities	Kapur et al., 2010
Biochemical properties, faunal counts (nematode, collembolan, ants)	No differences in the soil biochemical properties, however faunal counts was found higher under Bt-cotton rhizosphere soil	Mina et al., 2011
Microbial counts (CFUs, MPN)	No significant effects on the number of bacteria, fungi, azotobacter, and the diversity indices of microorganisms	Li et al., 2011
Microbial counts (CFUs), biochemical properties	Decline in actinobacteria, bacterial counts and biochemical properties	Tarafdar et al., 2012

Microbial counts (CFUs)	No effects on microbial population and microbial diversity indices	Velmourougane and Sahu, 2013
Microbial counts (CFUs)	Bt-transgenic cotton tissues have no apparent impact on soil bacteria, actinomycetes and fungi	Hu et al., 2013
Microbial counts (CFUs), 16S rRNA and 18S rRNA gene sequencing	rhizosphere soil sample of non-Bt cotton has shown increased number of bacterial and fungal populations indicating adverse effects on soil micro flora.	Pindi and Sultana, 2013
Microbial counts (CFUs)	No apparent impact on microorganism populations	Zhang et al., 2014
DGGE techniques, Microbial properties	No significant influence of cultivar or GM status on the total biomass and rhizosphere bacterial or fungal communities	Knox et al., 2014
DGGE	No effects on microbial communities	Zhang et al., 2015
CFUs, Enzymatic activity	apparently no negative effect on metabolic, microbiological activities	Yasin et al., 2016
qPCR) and denaturing gradient gel electrophoresis (DGGE	no indication of any significant changes of fungal community diversity and population in rhizosphere of Bt-cotton	Xie et al., 2016
Molecular analyses such as immune Dot blot, SDS-	No lethal effects of transgenic Bt protein on the survival of earthworm	Shahid et al., 2016

PAGE, ELISA and PCR		
qPCR and denaturing gradient gel electrophoresis (DGGE)	No significant differences were found in actinobacterial communities in the rhizosphere of transgenic cotton.	Qiao et al., 2017
qPCR, 16S rRNA gene sequencing	No significant differences were detected between the same root zones from Bt and the conventional cotton varieties.	Li et al., 2018
Microbial community analysis via rDNA gene sequencing	Transgenic cotton may not significantly affect soil microorganisms compared with conventional cotton	Qi et al., 2018
Microbial counts (CFUs), Biochemical properties	No adverse effect on soil beneficial microorganism and soil enzyme activities	Mandal et al., 2019
Quantitative and metagenomic analyses (marker gene 16S rRNA)	Cultivation of transgenic cotton does not seem to affect the quantity and diversity of natural soil bacteria	Fernandes et al., 2019
Microbial counts (CFUs), Biochemical characterization	No significant differences were observed in relation to parameters like bacterial population, colony morphologies, biochemical activities.	Yaqoob et al., 2019
Microbial counts (CFUs)	No adverse effects on community structures and total number of culturable bacteria and fungi in the rhizosphere.	Shahmoradi et al., 2019
Catabolic diversity (Biolog)	The original functional diversity of soil	Zhang et al.,

	microbial communities was affected by planting transgenic Bt cotton in one year and immediately returning residues.	2019

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Table 6. Pros and cons of genetically modified crops (Adapted from Van Acker et al., 2017)

Prospects	Limitations
Resistance to insects and pests	Allergic reactions to people
Potentially withstand adverse climatic conditions	Not fully proven for eco-friendliness
Increased promise on the productivity of GM plants	May be toxic to non-target organisms
Environmental benefits with less emission of greenhouse gases, soil erosion and soil pollution	Possibility of decreased sensitivity towards existing agrochemicals/drugs
Extended protection of the crops	Not totally safe at different trophic levels
More nutritional quality and biofortified foods	Cross pollination and genome contamination
Less depend on pesticide use	Risk of gene transfer to wild relatives and resurgence of minor pests
Less exposure of pesticide chemicals and residues to food crops	Uncertainty of sustainable productivity and erosion of biodiversity due to rapid increase in cultivated area of GM crops
Pesticide reduction has positive influence on the diversity of beneficial insects	Buildup of resistance in target pests will necessitate the novel strategy to combat with the pests