Chapter 8
Transgenic Cotton and Its Impact on Microbial Diversity

Kulandaivelu Velmourougane and D. Blaise

Abstract Introduction of GM crops into agricultural production systems increased public concern and renewed interest in research on the possible environmental consequences of growing GM crops including human health and ecosystem functioning. Globally, *Bacillus thuringiensis* (*Bt*) cotton occupies 15 million ha which comprised 43 % of the total cotton area of 35 million ha. *Bt* cotton was developed by incorporating the *cry* gene of the soil bacterium *Bacillus thuringiensis*. This gene expresses the protein endotoxin (*Cry*) that has insecticidal activity against the common cotton lepidopteran insect pests. While the benefits of *Bt* cotton are well known, there is a wide spread concern about growing transgenic cotton. This stems from the fact that the *Bt* toxin produced in leaves, stems and roots of *Bt* cotton is introduced in soil which might affect general soil health. Several workers have studied the effects of transgene products and transgenic cotton on the soil biological properties. Quite a few studies assessed the risk of growing *Bt* cotton on flora and fauna in diverse agro-ecosystems. This chapter attempts to review the work done so far related to growing transgenic *Bt* cotton on the soil microbial diversity and other related soil functions.

8.1 Introduction

Genetically modified (GM), genetically engineered, or transgenic crops refer to plants produced by the insertion of specific pieces of nucleic acids into the plant's DNA using recombinant DNA technology (i.e., *Agrobacterium*-mediated transformation or direct gene transfer methods) (Griffiths et al. 2005). This biotechnological approach allows genes to be introduced into a plant genome from any source (i.e., plant, animal, bacterial and fungal) resulting in potential transfer of a wide range of genetic resources between unrelated species, a major difference compared to traditional plant breeding that is limited to exchange of genetic material only between sexually compatible close relatives of a given plant (Mirkov 2003). Transgenic plants that show herbicide tolerance, resistance to viral, bacterial and fungal

K. Velmourougane (✉) · D. Blaise
Division of Crop Production, Central Institute for Cotton Research, ICAR, Nagpur, Maharashtra, 440010, India
e-mail: velicar@gmail.com

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Table 8.1 Commercial *Bt* crops and genes expressed by them

Crops	Gene	Target pest
Cotton	*cry1Ac*	Bollworm
Cotton	*cry2Ab*	Bollworm
Corn	*cry1Ab*	European corn borer
Corn	*cry9C* (discontinued)	European corn borer
Corn	*cry1Ac*	European corn borer
Corn	*cry3Bb*	Corn rootworm
Corn	*cry1F*	European corn borer, southwestern corn borer, fall armyworm and black cutworm
Potato	*cry3Aa* (discontinued)	Colorado potato beetle
Crops under development		
Cotton	*cry1Ac+cry2Ab*	Bollworm
Cotton	*cry1Ac+cry1F*	Bollworm and fall armyworm
Cotton	*Vip3A*	Bollworm and fall armyworm
Corn	*cry34Ab/35Ab*	Corn rootworm

diseases, insect resistance, improved product quality and superior agronomic properties were introduced in the mid-1990s. Plant species that have been genetically engineered include: maize, tomato, cotton, soybean, oilseed rape and to a lesser extent potato, squash, beet, rice, flax, papaya and cichorium.

Worldwide 12 major crops such as soybean, maize, cotton, canola, potato, sugar beet, alfalfa, papaya, squash, tomato, poplar and sweet pepper have been genetically modified, commercially cultivated (James 2010). Soybean is the leading GM crop occupying 75.4 m ha, followed by maize (51.00 m ha), cotton (24.7 m ha) and canola (8.2 m ha). Initially, six countries namely USA, China, Canada, Mexico, Australia and Argentina took up cultivation of GM crops, this number increased to 29 in 2011. Growing awareness of GM crops and acceptance by farmers resulted in an increase in global area under GM crops from 1.7 m ha in 1996 to 160 m ha in 2011 (James 2010). Presently, two principal transgenic technologies dominate the market: (i) herbicide-tolerant (HT) crops and (ii) insect resistant crops (*Bt* crops). *Bt* crops increased productivity and reduced insecticide usage, providing additional benefits to human health and the environment (Table 8.1).

There has been a strong debate on the safety of genetically modified plants ever since the introduction of transgenic plant products into the market. This debate is still very much alive and several issues were raised, including the safety of transgenic food and the environmental impact of transgenic plants (Schubert 2002; Dale et al. 2002; Liu et al. 2005). However, the potential development of resistance to the *Bt* toxin by insect pests and the indirect damage of *Bt* toxins to non-target species are major concerns related to their use. Introduction of GM crops took place in 1996, when biotechnology-derived herbicide tolerant (HT) and insect-resistant traits were launched into the market in soybean, cotton, corn, and canola. These 'input traits' were designed to benefit the farmer directly and aimed to increase productivity per hectare, reduce agrochemical use, decrease production costs, have greater flexibility and efficiencies in production regimes, and improve grower health (Hossain et al. 2004; Huang et al. 2005).

8.2 Mechanisms of Transgenic Plants Affect Soil Microorganisms and Functions

With the introduction of GM crops into agricultural production systems public concern increased. This also renewed interest in research on possible environmental consequences of growing GM crops including human health and ecosystem functioning (Sessitsch et al. 2004; Brookes and Barfoot 2005; Marvier et al. 2007). Soil microorganisms are responsible for different key functions in ecosystems as they are involved in many decomposing processes as well as in all major biogeochemical cycles, in the recycling of essential elements. Studies of the impact of genetically modified organisms should therefore, also focus on microbial community functions as they are key elements in a healthy ecosystem (Lamarche and Hamelin 2007). Cultivation of transgenic crops are reported to affect soil functions by direct (transgene proteins) and indirect effects (changes in plant protein, root exudates composition, modification in metabolic pathways). GM crops have the potential to influence soil microbiology through (i) the exudation of transgenic proteins from the root system, (ii) the release of transgenic proteins from broken and dying roots, (iii) the incorporation of above ground plant material into the soil, and (iv) differences in exudation chemistry (Gupta and Watson 2004; Knox et al. 2006; Saxena and Stotzky 2001).

8.3 Indicators of Soil Biological Quality: Why to Measure?

Soil quality is defined as, "the capacity of a soil to function within its ecosystem boundaries to sustain biological productivity and diversity, maintain environmental quality, and promote plant and animal health" (Brady and Weil 1999). Soil quality cannot be measured directly, so we evaluates through indicators. Indicators are measurable properties of soil or plants that provide clues about how well the soil can function. Indicators can be physical, chemical, and biological properties, processes, or characteristics of soils. Soil quality indicators are useful to policy makers to monitor the long-term effects of farm management practices on soil quality; assess the economic impact of alternative management practices designed to improve soil quality by including not only environmental values but also taking into account economic and social factors. Some of the key indicators of soil biological quality are presented in Table 8.2.

8.4 What is *Bt*?

"*Bt*" is short for *Bacillus thuringiensis* it is a soil bacterium occurring naturally. *Bt* was first discovered in 1901 by Shigetane Ishiwatari. In 1911, *B. thuringiensis* was re-discovered in Germany by Ernst Berliner. He isolated the cause of a disease

Table 8.2 Key indicators of soil biological quality

Indicators	Functions
Soil respiration	Soil respiration is a useful index of the overall biological activity in soil and is a critical determinant of ecosystem carbon storage. It reflects the intensity of the soil organic matter decomposition and mineralization and the incidence of the microorganisms in soil, and it is often used for the biomass determination
Fluorescein diacetate hydrolysis (FDA)	FDA hydrolysis assay measures the enzyme activity of microbial populations and can provide an estimate of overall microbial activity in an environmental sample. The assay is considered non-specific because it is sensitive to the activity of several enzyme classes including lipases, esterases, and proteases
Soil microbial biomass (SMB)	SMB is a living pool containing 1–5 % of the soil organic matter. Microbial biomass determinations indicate changes in the soil organic matter before they can be detected by measuring total soil carbon making possible its use as an indicator of early changes in soil organic matter content. Microbial biomass consists of both dormant and metabolically active organisms and has been considered as an integrative indicator of microbial significance of soils
Soil urease	Urease plays an important role in the efficient use of urea fertilizer in soils and the changes in urease activity can be used as an indirect indicator of the variation in the pool of potentially available N in a soil
Soil dehydrogenase	The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil
Phosphatases	Phosphatases are a broad group of enzymes that are capable of catalysing hydrolysis of esters and anhydrides of phosphoric acid. In soil ecosystems, these enzymes are believed to play critical roles in P cycles
ß glucosidase	This enzyme plays an important role in soils because it is involved in catalysing the hydrolysis and biodegradation of various ß-glucosides present in plant debris decomposing in the ecosystem. Its final product is glucose, an important C energy source of life to microbes in the soil
Arylsuphatases	They are responsible for the hydrolysis of sulphate esters in the soil and are secreted by bacteria into the external environment as a response to sulphur limitation
Soil microorganisms	Microorganisms have double role in relation to soil fertility. On one hand, the microbes are the agents that mineralise and liberate plant nutrients from the organic material. On the other hand, the microorganisms can also be viewed as a collective observer of the soil environment. Since the microbes are in close contact with all three soil phases (Solid, water and air), they can sensitively and rapidly probe responses to soil perturbations
Glomalin content	Indicator of mycorrhizal activity in soil. Reported to involved in soil aggregation

called *Schlaffsucht* in flour moth caterpillars. A unique feature is its produce *cry*stal proteins called as "*CRY* proteins" or "Insecticidal *Cry*stal Protein" (ICP) that selectively kills specific groups of insects for example Lepidopteran caterpillars (moth and butterflies), Diptera (mosquitoes and black flies), Coleoptera (beetles), and nematodes.

8.5 Mode of Action by ICP

The target organ for *Bt* toxins is the insect larvae's mid-gut. The mid-gut of the larvae is a simple, tubular epithelium that dominates the internal architecture of the insects. After ingestion by insect's larvae, the *Bt* δ-endotoxin disrupt of the epithelium in the insect mid-gut. The alkalinity of insect mid-gut (pH 12) dissolves the *Cry*stals, releasing the *Cry* pro-toxin where it is cleaved by insect proteases to generate the trypsin resistant core of the active δ-endotoxin. The active toxin traverses the peritrophic membrane to bind receptor of brush border cells of the insect mid-gut. Integration of the toxin into the epithelial membrane, resulted in osmolysis of the cells, and paralysis occurred and dies within 2 days. Different *Bt* strains produce different *CRY* proteins, and there are hundreds of known strains which have identified more than 60 types of *Cry*-proteins that affect a wide variety of insects.

8.6 Persistence of cry1 Toxins from *Bt* Cotton

During plant litter decomposition, most transgene protein(s) appear to be rapidly degraded. However, some proteins can bind to surface-active particles and reduce their availability to microbes. Sims and Ream (1997) estimated that a potential maximum of 1.6 mg Cry2A protein kg^{-1} soil would result from the incorporation of *Bt* cotton residues into the top soil (Table 8.3). Recently, Sun et al. (2007) reported that the CryIAb protein persisted in soil for at least 56 days after incubating a slit loam soil with *Bt* cotton tissues.

8.7 Impact of Transgenic *Bt* Cotton Cultivation

Cotton (*Gossypium* spp.) belonging to the genus *Gossypium* in the family Malvaceae is an important fiber crop of global importance. Cotton is grown in tropical and subtropical regions of more than 80 countries. It is an important source of oil and high quality protein and plays a significant role in the national economy. Besides being the backbone of the textile industry, cotton and its byproducts are also part of the livestock feed, seed-oil, fertilizers, papers and other consumer products. Handling, processing and production of various consumer based products of cotton

Table 8.3 Studies on persistence of Cry proteins in soil

Protein	Experiment	Findings	References
Cry1Ab Cry1Ac Cry3Aa	Soil amended with biomass of *Bt* cotton	No persistence of proteins in soil; proteins degraded in soil with a half-life of 20 days	Ream et al. (1994)
Cry1Ab Cry1Ac	Soil amended with purified protein or biomass of *Bt* cotton	Purified protein was detected up to 28 days and the protein from *Bt* cotton was detected up to 56 days	Donegan et al. (1995)
Cry1Ab Cry1Ac	Soil amended with purified protein or biomass of *Bt* cotton	Purified proteins and Cry proteins from cotton tissue decreased rapidly, with a half-life of approximately 4 and 7 days, respectively, by ELISA	Palm et al. (1996)
Cry2A	Soil amended with biomass of *Bt* cotton *Bt* cotton cultivation	Half-life of bioactivity was estimated at 15.5 days by insect assay Half-life of bioactivity was estimated at 31.7 days by insect assay	Sims and Ream (1997)
Cry1Ac	*Bt* cotton cultivation	No detectable level of protein in soil for 3–6 consecutive years	Head et al. (2002)

also play an important role in the social and industrial structure. Cotton is long duration crop and it is reported to be attacked by more than 162 species of insect pests including sucking pests, tissue borers and defoliators at various stages of growth, causing losses up to 60 %. Cotton is vulnerable to a number of insect species, especially to the larvae of lepidopteran pests. The cotton bollworm complex is a major and serious threat to the cotton, causing potential yield losses across the world and reported that the annual loss of at least US $ 300 million. High level of insecticide resistance in bollworms necessitates repeated application of insecticides, thereby aggravating the problem of resistance and also leading to heavy expenditure on cultivation and crop failures. Therefore it was important to initiate the development of alternative technologies such as genetic modification to enable plants to resist against the insect attack.

8.8 Transgenic *Bt* Cotton on Pest Control

The era of transgenic cotton began when Perlak et al. (1990) introduced *cry* 1A(b) and *cry* 1A(c) genes into cotton (*G. hirsutum*) plants and transformed plants showed a high level of resistance to *Helicoverpa*. During the field and laboratory tests, it was demonstrated that transgenic cotton is highly effective against neonate larvae of *H. armigera* (cotton bollworm), *H. virescens* (Tobacco budworm), and *Pectinophora*

Table 8.4 Event released for commercial cultivation of *Bt* cotton

Gene	Events	Company/institute
cry I Ac	MON 531	Monsanto company
cry I Ac and *cry* II Ab	MON 15985	Monsanto company
cry I F	2581-24-236	Dow Agrosciences
cry I Ac	3006-210-23	Dow Agrosciences
cry I Ac	31807/31808	Calgene
Vip 3A(a)	COT 102	Sangenta Seeds
cry I Ac and *cry* I F	Das-21023-5 * Das 24236-5	Dow Agrosciences
cry I Ab/*cry* I Ac		CAAS/Nath Seeds
cry I Ac		NRCPB/UAS Dharwad
cry I E/C, *cry* I Ac		NBRI/Swarna Bharat Biotechnics Pvt. Ltd
cry I Ac	Event I	IIT Kharagpur/JK Agri-genetics
cry I Ac, CPTi		Mahyco
cry I Ac, CPTi		Nath Seeds, CICR

gossypiella (pink bollworm). The *Bt* gene from the original genetically engineered mother plant was Coker-312. Transferred to advanced cotton cultivars through backcrossing. Later Gene stacking, involving the introduction of more than one gene of similar effects is becoming an attractive alternative for developing durable resistance and for simultaneous and effective control of more than one insect together. For instance, in cotton, Monsanto transgenic event 'Bollgard-II' carries two genes viz, *cry* 1Ac (against American Bollworm and *cry* 2Ab (Against tobacco bud worm) (Table 8.4).

Bt cotton occupies globally 15 million ha which comprised 43% of the total cotton area of 35 million ha in nine countries namely USA, Mexico, China, Argentina, South Africa, Colombia, India, and Brazil (ISAAA 2006). GM cotton were developed by incorporating and expressing crystal protein endotoxin (*Cry*) encoded by the *cry* gene of the soil bacterium *B. thuringiensis* having insecticidal activity against the common cotton infecting insects belonging to orders Lepidoptera, Diptera, and Coleoptera (Wallimann 2000). Although there is large-scale adoption of *Bt* cotton by the farmers because of immediate financial gain, there is concern that transgenic *Bt* crops release *Bt* toxins into the environment which affect associated and succeeding crops due to a reduction in soil chemical and biological activities (O'Callaghan et al. 2005; Sarkar et al. 2008).

8.9 Transgenic *Bt* Cotton on Microbial Diversity and Soil Functions

In experiments to evaluate the persistence of Cry1 toxins from *Bt* cotton leaves incorporated into soil microcosms, Palm et al. (1996) found that degradation of the toxin was microbially mediated as suggested by various reports from the Stotzky group (Koskella and Stotzky 1997; Crecchio and Stotzky 1998). Bacterial

Table 8.5 Cry proteins from *Bacillus thuringiensis* on the microbial population and diversity

Protein	Microorganisms	Experiment	Findings	References
Cry1Ac	Culturable bacteria and fungi	Soil with *Bt* and non-*Bt* cotton	A significant, increase in numbers in soil with *Bt* cotton	Donegan et al. (1995)
Cry1Ac	Microbial population	*Bt* and non-*Bt* cotton	No adverse effect	Valasubramanian (2001)
Cry1Ac	Composition of soil microbiota	Rhizosphere soils of *Bt* and non-*Bt* cotton	More extensive fungal colonization, higher ratios of fungi to bacteria, and different types of fungal spores in soil with *Bt* cotton	Gupta and Watson (2004) Gupta et al. (2002)
Cry1Ac	Culturable functional bacteria	Rhizosphere soils of *Bt* and non-*Bt* cotton	No significant differences in numbers after the growing season	Rui et al. (2005)
Cry1Ac	Functional diversity of microbial communities	Soil with *Bt* and non-*Bt* cotton	No adverse effects	Shen et al. (2006)
Cry1Ac	Methylobacteria	*Bt* and non-*Bt* cotton	No adverse effects	Balachandar et al. (2008)
Cry1A	Culturable functional bacteria	Multiple-year (0–5 years) cultivation of *Bt* cotton	No adverse effects	Hu et al. (2009)
Cry1Ac	Microbial diversities	Soil with *Bt* and non-*Bt* cotton	No adverse effects	Kapur et al. (2010)
Cry1Ac	Microbial population and diversity	Soil with *Bt* and non-*Bt* cotton	Higher microbial population and diversity in *Bt* cotton	Velmourougane and Sahu (2013)

community structure was less affected by the c*ry1Ab* protein than by other environmental factors, such as plant age or field heterogeneity (Baumgarte and Tebbe 2005). However, undue decrease in microbial community richness with the use of genetically modified cotton is also reported (Dunfield and Germida 2004). Previous studies have shown that the qualitative and quantitative differences in root exudation of *Bt* cotton could strongly influence the structure of microbial communities in the rhizosphere (Oger et al. 2000; Yan et al. 2007). A significant but transient increase in the populations of culturable bacteria and fungi was observed in soil amended with leaves of *Bt* cotton (*Gossypium hirsutum* L.) expressing the *Cry1Ac* protein in comparison to the wild type plant measured by BIOLOG analysis and DNA fingerprinting (Donegan et al. 1995). Higher microbial counts in transgenic cotton grown soil have also been reported by several workers (Shen et al. 2006) (Table 8.5). Head et al. (2002) demonstrated that the amount of Cry1Ac protein accumulated as a result of continuous use of transgenic *Bt* cotton, and subsequent

incorporation of plant residues into the soil by postharvest tillage for 3 to 6 consecutive years is extremely low and does not result in detectable biological activity. Rui et al. (2005) reported that the fortification of pure *Bt* toxin into rhizospheric soil did not result in significant changes in the numbers of culturable functional bacteria, except the nitrogen-fixing bacteria when the concentration of *Bt* toxin was higher than 500 ng/g. The results indicated that *Bt* toxin was not the direct factor causing decrease of the numbers of bacteria in the rhizosphere, and other factors may be involved. Balachandar et al. (2008) studied the diversity richness of Pink-pigmented facultative methylotrophs (PPFMs) present in the phyllosphere, rhizoplane and internal tissues did not differ between *Bt* and non-*Bt*-cotton and reported that there was no evidence to indicate any adverse effects of *Bt* cotton on the diversity of plant-associated methylobacteria.

Hu et al. (2009) reported that there were no consistent differences in the numbers of different groups of functional bacteria between rhizosphere soil of *Bt* and non-*Bt* cotton in the same field. Further, no obvious trends were observed with regard to the numbers of the various groups of functional bacteria with an increasing duration of *Bt* cotton cultivation. Sarkar et al. (2009) concluded from their study that there were some positive or no negative effects of *Bt*-cotton on the soil quality indicators (microbial biomass carbon, microbial biomass nitrogen, microbial biomass phosphorus, total organic carbon, microbial quotient, potential nitrogen mineralization, nitrification, nitrate reductase, acid and alkaline phosphatase activities, Root dry weights, and root volume). Therefore cultivation of *Bt* cotton appears to pose no risk to soil ecosystem functions (Table 8.6). The microbial community structure in soil was not affected by the cropping of *Bt* cotton and the total microbial population and diversity of experimental fields remain quite similar during the cropping of both *Bt* cotton and non-*Bt* cotton (Kapur et al. 2010). Based on the studies conducted at Central Institute for Cotton Research, Nagpur, it was found that growing *Bt* cotton does not affect the soil biological properties (soil respiration, urease activity, dehydrogenase activity, and microbial biomass carbon). The results obtained with culturable microbial population and microbial diversity index analysis further proved that the microbial activity in soil was not affected by the cropping of *Bt* cotton (Velmourougane and Sahu 2013). Cry proteins from *B.thuringiensis* was also not reported to affect the soil invertebrates (Table 8.7). These results suggest that cultivation of *Bt* cotton expressing *cry1Ac* gene may not poses ecological or environmental risk.

8.10 Conclusion

A major problem in assessing the impacts of transgenic crops on soil microbial attributes is the lack of baseline information on diverse agro-ecosystems to compare with ecosystems in which transgenic crops were introduced and lack of universally approved approach for carrying out impact assessment of the transgenic plants on soil ecosystem. Genetic modifications have been performed with several

Table 8.6 Effects of *Bt* cotton on microbe-mediated process and functions in soil

Protein	Process/function	Experiment	Findings	References
Cry1Ac	Soil enzymes (Urease, Alkaline Phosphatases, dehydrogenase, phenol oxidase, proteases	Soil with *Bt* and non-*Bt* cotton	No differences in the activities of enzymes	Shen et al. (2006)
Cry1Ac	Selected enzymes	Soil amended with *Bt* and non-*Bt* cotton biomass	Biomass of *Bt* cotton stimulated the activities of all enzymes	Sun et al. (2007)
Cry1Ac	N mineralization and Olsen-P	Soil with *Bt* and non-*Bt* cotton	Total mineral-N was reduced in *Bt* cotton, whereas Olsen-P was increased	Sarkar et al. (2008)
Cry1Ac	Root biomass	Soil with *Bt* and non-*Bt* cotton	Root biomass were not different but root volume was significantly higher in *Bt* than non-*Bt* isoline	Sarkar et al. (2008)
Cry1Ac	Microbial biomass C, N and P, organic carbon, microbial quotient, potential nitrogen mineralisation, nitrification, nitrate reductase, AcP, AlP, root volume	Soil with *Bt* and non-*Bt* cotton	No negative effects of *Bt*-cotton on the indicators.	Sarkar et al. (2009)
Cry1Ac	Urease activity, nitrate reductase, Acid and alkaline phosphatases	Soil with *Bt* and non-*Bt* cotton	No significant difference enzyme activities	Mina et al. (2011)
Cry1Ac	Dehydrogenase and $KMnO_4$-N content	Soil with *Bt* and non-*Bt* cotton	Positive correlations between *Bt* cotton cultivation and $KMnO_4$-N content and dehydrogenase in soil	Singh et al. (2013)
Cry1Ac	Soil respiration, fluorescein diacetate hydrolysis, urease, dehydrogenase, microbial biomass carbon	Soil with *Bt* and non-*Bt* cotton	Higher biological activities in soil grown with *Bt* cotton than the non-*Bt* cotton	Velmourougane and Sahu (2013)

Table 8.7 Effects of Cry proteins from *Bacillus thuringiensis* on soil invertebrates

Protein	Organism	Experiment	Findings	References
Cry1Ab Cry1Ac	Collembola Mites	Fed leaves of *Bt* and non-*Bt* cotton	No significant effects on oviposition, nos. of eggs, and body length	Yu et al. (1997)
Cry1Ac	Earthworm	Transgenic cotton	No adverse effect on earthworms	Valasubramanian (2001)
Cry1Ab Cry1Ac	Collembola	Cultivation of *Bt* and non-*Bt* cotton	No effects on numbers	USEPA (2001)
Cry1Ac	Micro, meso and macro fauna	Cultivation of *Bt* and non-*Bt* cotton	micro, meso and macro fauna were more in *Bt* cotton rhizosphere	Mina et al. (2011)

plant species (maize, cotton, wheat, and rice), with targeted goals such as resistance to insect pests or herbicides, increased growth, and increased nutritional quality. Enormous studies have been conducted to assess the potential beneficial and/or detrimental effects of genetically engineered plants on soil microbes. Special attention was paid to study the impact of *Bt* plants, which express the cry toxin of *B. thuringiensis*, on microbial communities. Though few studies demonstrated a negative impact of *Bt* crops on soil microbes (Castaldini et al. 2005; Wu et al. 2004), most of the studies showed no adverse effects (Baumgarte and Tebbe 2005, Blackwood and Buyer 2004; Brusetti et al. 2004; Devare et al. 2004, Griffiths et al. 2005; Liu et al. 2005, Muchaonyerwa et al. 2004; Saxena and Stotzky 2001).

From the already published work, following points emanate:

1. *Bt* cotton provided effective control against lepidopteran pests and reduced insecticide spray applications.
2. Cry proteins released in root exudates and from *Bt* cotton residues appear to have no consistent, significant, and long-term effects.
3. Differences in numbers and community structure of microorganisms in soil between *Bt* and non-*Bt* crops were not statistically significant and transient.
4. Although Cry proteins bind rapidly on clays and humic substances, there is little evidence for the accumulation of the proteins in soils in the field, even after years of continuous cultivation of *Bt* cotton.

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