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Current trends in Bt crops and their fate on associated microbial community dynamics: a review

Amit Kishore Singh[1] · **Suresh Kumar Dubey**[1]

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Abstract Cry protein expressing insect-resistant trait is mostly deployed to control major devastating pests and minimize reliance on the conventional pesticides. However, the ethical and environmental issues are the major constraints in their acceptance, and consequently, the cultivation of genetically modified (GM) crops has invited intense debate. Since root exudates of *Bacillus thuringiensis* (Bt) crops harbor the insecticidal protein, there is a growing concern about the release and accumulation of soil-adsorbed Cry proteins and their impact on non-target microorganisms and soil microbial processes. This review pertains to reports from the laboratory studies and field trials to assess the Bt toxin proteins in soil microbes and the processes determining the soil quality in conjunction with the existing hypothesis and molecular approaches to elucidate the risk posed by the GM crops. Ecological perturbations hinder the risk aspect of soil microbiota in response to GM crops. Therefore, extensive research based on in vivo and interpretation of results using high-throughput techniques such as NGS on risk assessment are imperative to evaluate the impact of Bt crops to resolve the controversy related to their commercialization. But more studies are needed on the risk associated with stacked traits. Such studies would strengthen our knowledge about the plant-microbe interactions.

Keywords Bt crops · Cry toxic proteins · Rhizosphere · Endophytes · Stacked traits · Soil microorganism · Insect resistant · Genetically engineered crops · Root exudates

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✉ Suresh Kumar Dubey
sureshkd1@hotmail.com

[1] Department of Botany, Banaras Hindu University, Varanasi 221005, India

Introduction

Globally, the major concern is food security for the current century (Fitter 2012). Agriculture sector has been revolutionized through the application of genetically engineered crops that offer immense benefits in terms of improved yield, nutritional security, and resistance to environmental stresses (Pontiroli et al. 2007). The plantation involving genetically modified (GM) crops could be raised to 181 mha by 2014; >100-fold rise over 1.7 mha since 1996 James 2014). Among the widely used GM traits, herbicide tolerant (HT) and insect resistant (IR) are ones. These traits provide growers with benefits of increased yield, lesser insecticide application, and simplified weed control management with fewer and more flexible herbicide applications. Herbicide (glyphosate) tolerance, the most common trait, covers maximum acreage for corn, cotton, soybean, and sugarbeet, and the insect resistance traits in corn and cotton. Despite unabated adoption of GM crops, the global debate concern their impact on the environment with regard to the potential gene flow, weediness or invasiveness of GM plants, and the possible impact on the non-target organisms (Johnson et al. 2006).

Notably, *Bacillus thuringiensis* (Bt) and its insecticidal toxins have been globally used in pest control in maize (*Ostrinia nobilalis*, *Ostinia furnacalis*, *Spodoptera frugiperda*, *Diatraea* spp., *Helicoverpa zea*, and *Diabrotica* sp.) and in cotton (*Heliothis* sp. and *Helicoverpa* sp.). However, their adoption is stringent in majority of EU countries where only Bt maize designated to produce Cry1Ab is commercially cultivated as the crops contain insecticidal toxic proteins and their interactions with non-target organisms warrant risk assessments. Most countries have regulatory bodies (EPA, CFIA, CONABIA, ANZFA, BRAI, APHIS, FDA, etc.) and other specific multidisciplinary inter-institutional advisory groups to assess and resolve the scientific and technical issues through

interactions with the GMOs advisory committee (Craig et al. 2008). Field and laboratory trials involved risk assessment of GM crops related to the non-target organisms (Icoz and Stotzky 2008a). However, the conclusions derived from such studies remain still controversial. For instances, Icoz et al. (2008) reported no effects on soil microbial enzymes and properties while Chen et al. (2012) advocated negative effect on soil microbial and biochemical processes affected by Bt corn. It is expected that transgenic technology get speedy and the newer transgenic technologies for instance stacked traits are introduced. The environmental risk assessment studies determine the possible extent of stacked gene interaction and also the non-target biota that might emerge following cultivation (De Schrijver et al. 2007).

Soils represent a dynamic ecological system characterized by diverse and interacting microbial populations (Singer and Munns 1999). The complexity of soil system hinders risk assessment related to genetically modified crops. The intimate effects of plants on the soil microbiota are on record by many (Brimecombe et al. 2001; Marschner et al. 2001), and many techniques are available to monitor the impact of environmental or anthropogenic factors on the soil ecosystems. The plant-associated microbial community is diverse and the information on microbial responses to environmental changes and perturbations limited (Knief 2014). Genomic studies using restriction fragment length polymorphism (RFLP) and denaturing gradient gel electrophoresis (DGGE) face financial and technological limitations in achieving in-depth information on plant-microbe interactions under a set of conditions (Lindahl et al. 2013). The Next Generation Sequencing (NGS) overcomes many such time-consuming steps of conventional Sanger sequencing. It offers sequencing of a large number of clones to the extent of 10^6, relying on the DNA extracted (Pauwels et al. 2015). Recent studies deployed NGS-based meta-genomic approach for microbial communities in varied habitats (Mardis 2008; Gloor et al. 2010; Bartram et al. 2011; Delmont et al. 2011; Sangwan et al. 2013; Verbruggen et al. 2013) to strengthen the knowledge about microbiota systemics. The pyrosequencing offers huge coverage of genome and seems more reliable and informative over others (Dinsdale et al. 2008; Amend 2010). For further reduction in sequencing costs, the Illumina platform used recently generates larger data (Gloor et al. 2010; Caporasoa et al. 2011; Soni et al. 2015) that surpass 454 GFLX data sets by over an order of magnitude in terms of number of sequences per sample coast (Shendure and Ji 2008). The risk assessment studies on GM crop involving NGS system are less (Hur et al. 2011; Lee et al. 2011; Verbruggen et al. 2012; Kuramae et al. 2013; Dohrmann et al. 2013; Valverde et al. 2014). The major utility of such a cutting age technology could be in deciphering the fate of Cry toxin and their interactions with the soil microbes. Therefore, the application of NGS technology may significantly contribute to our existing knowledge of possible fluctuations in soil microbiota at the genomic level as determined by CryAc-expressing plants.

The last decades witnessed enormous researches on the interaction of soil bacterial, fungal, and actinomycete community with GM crop (Griffiths et al. 2006, 2007; Oliveira et al. 2008). Icoz and Stotzky (2008a, 2008), and Icoz et al. (2008)) reviewed the impact of Bt crops on soils. The latter covered Cry proteins and belowground organisms, existence of such proteins in soil, and the techniques and the indicators available. It is suggested that the deployment of insect-resistant Bt crops with selected Bt proteins had little or even no impact on targets like woodlice, collembolans, mites, earthworms, nematodes, protozoa, and various soil enzymes, and thus offers an alternative to broad-spectrum insecticides. However, the information on the interactive effects of Bt crops with symbiotically microorganisms is still scanty. Arbuscular mycorrhizal fungi (AMF) and endophytes may enhance the suitability of their hosts through facilitating nutrient acquisition as well as protection from insect, pests, and pathogens (Verbruggen et al. 2013). The possible interactions between Bt crops, AMF, and endophytes are, however, little understood.

The present review summarizes the update on the assessment of environmental risks and the fate of Bt crops in the soils with special reference to insecticidal Bt toxins, produced by engineered plants. It is imperative to understand the possible interactions with factors in the soil ecosystem. The future challenges in the transgenic technology including stacked genes, the associated endophytes, and their potential for the accumulation of various bioactive metabolites have also been highlighted.

Routes of Cry proteins exposure to soil microorganisms

The potential adverse impact of Bt crops on soil microbial community may arise through different routes. Some soil microorganisms thrive in close association with the plants or plant debris in the field, and may thereby be exposed to Cry protein in Bt plants (Fig. 1). Transgenic Bt plants may also release their engineered products (Cry proteins) into the soil via root exudates that may actively persist therein (Saxena et al. 2002). Several studies suggested that the potential fraction of carbon fixed during photosynthesis is released into the rhizosphere by roots, whose composition and quantity is plant species specific (Berg and Smalla 2009). Plants and soils interact via roots exudates, along with the plant residues collectively that act as the main C source for microbes. Therefore, GM crops, in line with other crop, are likely to regulate the soil microbial structure and functions. The foremost mechanism through which GM crops affect the soil microbiota could be via intentional or unintentional changes in root-exudate quantity and quality (Hannula et al. 2012). The latter does not only affect root-exudates composition (sugars, organic acids, and amino acids) but also the

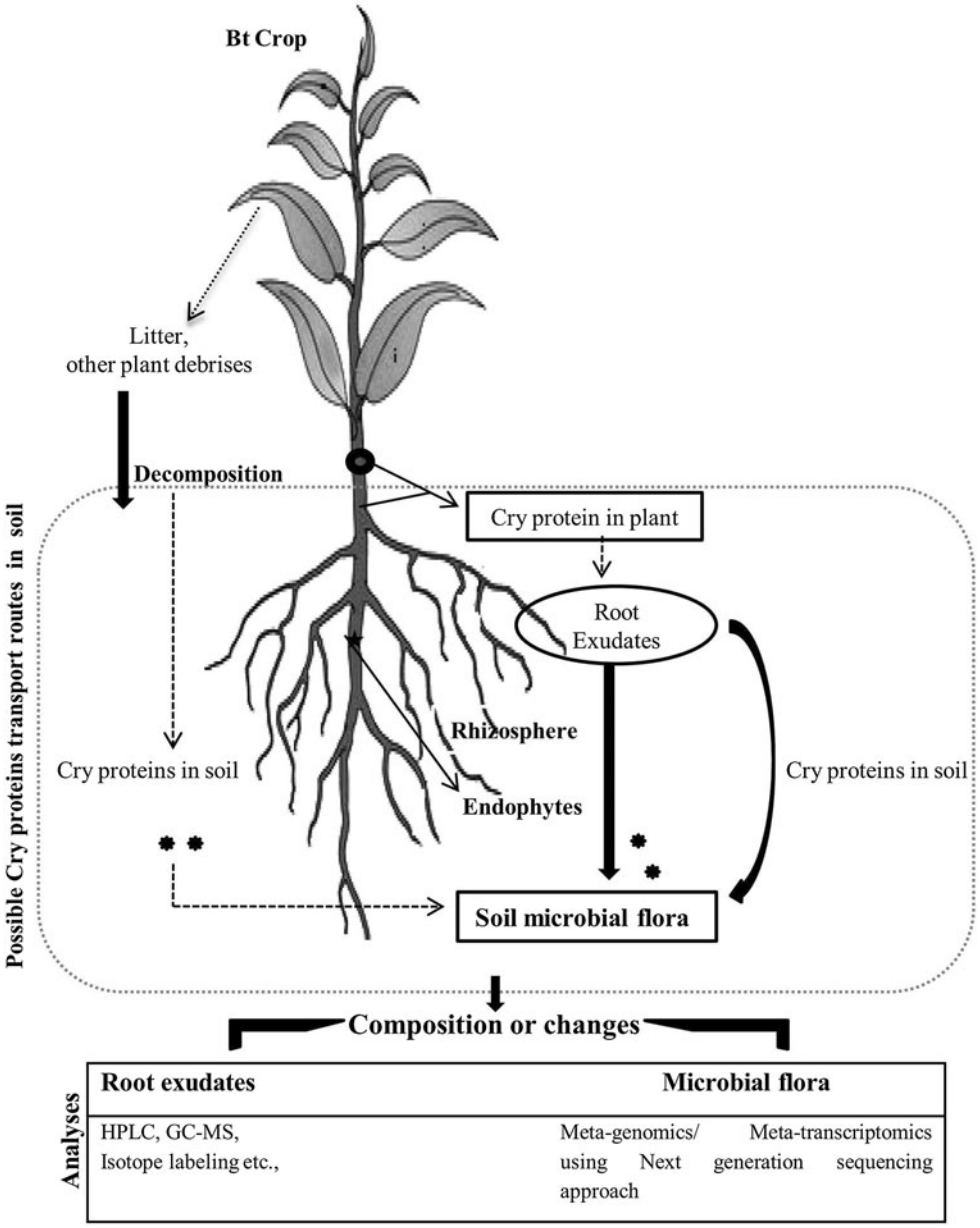

Fig. 1 Possible Cry protein transport routes from the plant to soil environment and proposed approach that could be used to study the plant-microbe interaction in response to GM crops

toxins as introduced via GM. The novel compound(s) in root exudates of the transgenic plants also offered selective advantage to the specific domain of soil bacteria that feed over these (Savka and Farrand 1997). Such substances may affect soil microorganisms even after the plants were removed, and may possibly alter populations of plant-beneficial- (PGPR) or plant-pathogenic microorganisms.

The different routes of Cry proteins exposure may have varied effects on soil microorganisms that may be (1) direct one through exposure to released transgene products that may still persist in the soil and (2) close contact with the plant litter or the post-harvest crop residues. These have the potential to significantly change the plant-microbe interacting zone (rhizosphere/endosphere), microbial dynamics, soil biodiversity, and nutrient mineralization. While the restricted pesticide use in case of Bt cotton varieties is beneficial, little is known about the potential non-target effects of Bt cotton plants on the soil microbiota and associated biological processes critical for sustained crop productivity and ecosystem health. Several investigations on structural and functional compositions of microbial communities as affected by the associated Bt crops are also covered in the present compilation.

Persistence of Cry proteins in soil

The exposure of soil microbes to Cry proteins varies and the persistence of the latter is regulated by soil type, pH,

temperature, and other physicochemical and biological characteristics as advocated in the review by Icoz et al. (2008)). Similar conclusions were also drawn by Feng et al. (2011) and Singh et al. (2013b) while working on Bt maize (Cy1Ab) and Bt brinjal (Cry1Ac), respectively. Apart from the single trait, a study (Chen et al. 2011) used stacked traits (insect resistance conferring toxin-CpTi, a small polypeptide Bowman-Birk type of double-headed serine protease inhibitor) along with Cry1Ac protein and revealed that the concentrations of Cry1Ac and CpTI proteins in soils of transgenic cotton are relatively higher over its non-transgenic counterpart. The outcome of such studies supports that crop variety, soil physicochemical and biological attributes may affect the soil degradation of Cry proteins. The observations on the fate of soil Cry proteins based on laboratory and field trials are listed (Table 1). It is clear from the observations presented in Table 1 that the possible reason for the presence of soil Cry proteins is still debated. Although researchers covered the specific components of soil Cry toxin including the entry, persistence, degradation, and genetic modifications, for the interdisciplinary and systematic study, it is still awaited.

Various salts and hydroxides in soil may alter Cry proteins levels in the ecosystem. Studies revealed that Bt toxins in the soils and soil lead the former to get promptly adsorbed on to the clay component (Zhou et al. 2007) and humic acids Muchaonyerwa et al. 2006). The soil accumulation of Bt toxins depends on their adsorption onto soil components and the bioavailability. Adsorption is very crucial in deciding the persistence of Cry proteins into the complex soil matrix, and little understood. Pagel-Wieder et al. (2007) indicated that the surface assimilation of Cry1Ab protein decreased with the increasing concentrations of Na-montmorillonite. The increase in specific surface area and charge density of soil particles improved Bt toxin adsorbtion (Helassa et al. 2009).

Some recent attempts developed the molecular biology-based approaches to understand the interactions of Cry proteins with the soil particles (Table 1). Some dealt with insight into the adsorption mechanism using Cry1Ab protein as the model (Madliger et al. 2010; Sander et al. 2010; Madliger et al. 2011; Tomaszewski et al. 2012) and suggested that the non-uniform surface charge distribution of Cry 1Ab gave rise to patch-controlled electrostatic attraction of Cry1Ab towards the surfaces that carried the same net charges as applicable to protein. Cry1Ab adsorption on to humic substances also had a strong contribution from the hydrophobic effect. These findings indicate that soil pH, ionic strength, and the polarity of soil organics strongly affect the Cry1Ab fate in soils. A higher Cry1Ab mobility and bioavailability is expected with increasing pH and ionic strength. The bioassay revealed comparable growth inhibition of *Ostrinia nubilis* by Cry1Ab absorbed on to soils and to major mineral, and the organic soil constituents along with the solubilized Cry1Ab (Tomaszewski et al. 2012). Therefore, the adsorbed Cry1Ab is to be considered in every aspect while assessing the fate and impact of Cry1Ab in the soil environment.

Interaction of Bt crops with soil microbiota

It is expected that Cry protein expressing Bt crops may exert some effects, may be positive or negative on soils, both in term of biomass and activity. Studies related to population density and the effects ranging from "transient" to "no" are compiled in Table 2. Xue et al. (2005) observed gram-positive to gram-negative bacteria ratio lowered for soils with Bt maize compared to near-isogenic non-Bt maize, contrary to the higher ration for soils with Bt potato. However, no differences were found in the fungal/bacterial population ratio for soils having Bt and non-Bt maize or those with Bt and non-Bt potato. Rui et al. (2005) reported increased number of culturable bacteria (potassium-dissolving, inorganic phosphate-dissolving, nitrogen-fixing) in rhizosphere soils of non-Bt cotton over soils with Bt cotton during the initial and middle plant growth stages. However, the differences were quite small in the following growing season. WeiXiang et al. (2004)) reported some occasional, prominent variations in the colony-forming units (CFU) of aerobic bacteria, actinomycetes, fungi, anaerobic fermentative, denitrifying, hydrogen-producing acetogenic, and methanogens in paddy soils with Bt-transgenic rice (Cry 1Ab protein) straw and the non-Bt rice straw during early incubations. Such variations could be attributed to alterations in the nutritional makeup of transgenic rice straw due to the transgene. There was prominent lowering in the bacterial and actinobacterial population in Bt cotton soils over the non-Bt cotton counterpart (Tarafdar and Rathore 2012). However, the fungal population remained unaffected by the Bt cotton. By contrast, several studies indicated no effect of Bt crop on the microbial population (Kapur et al. 2010; Pangrikar et al. 2014; Zhang et al. 2014). Saxena and Stotzky (2001)) reported insignificant differences in CFUs of culturable bacteria (including actinomycete), fungi, protozoa, and nematodes in rhizosphere soils of Bt and non-Bt corn or among those added with the Bt and non-Bt corn biomass. Singh et al. (2012) observed that the inclusion of peanut and farm yard manure for Bt cotton crops enhanced the microbial population and could even mask the essence of Bt toxin. Singh et al. (2013a, b, 2014) observed paramount decline in actinomycete, bacterial, and fungal population size in the Bt brinjal-planted soils relative to non-Bt brinjal soils. Their population size estimate restricted to the flowering stage revealed the major but transient effect of developmental stages of the genetically modified brinjal crop.

The obligate biotrophic AMF may be at the high risk covering non-target impacts of transgenic Bt crops owing of their close association with the plant roots. Although Bt proteins get expressed in roots of most Bt maize lines Saxena et al.

Table 1 Summary of the Cry proteins persistency in soil

Bt crop/protein	Experimental condition/soil condition	Inferences	References
Bt maize, Bt cotton, and Bt potato; Cry1 Ab, Cry1Ac and Cy3Aa	Laboratory condition; biomass of Bt cotton, maize, and potato amended in soil	No accumulation of protein in soil; proteins degraded in soil with ½ life of 20 days	Ream et al. (1994)
Bt cotton; Cry1Ab and Cry1Ac	Laboratory condition; purified protein or biomass of Bt cotton amended in soil	Purified protein and the Bt cotton protein were encountered up to 28 and 56 days, respectively	Donegan et al. (1995)
Bt maize; Cry 1Ab	Laboratory condition; soil amended with biomass of Bt maize	50 % Cry1Ab protein activity decrease in 1.6 days and 90 % decrease in 15 days	Sims and Holden (1996)
Bt cotton; Cry 2A	Laboratory condition; biomass of Bt cotton amended in soil	½ life of Cry 2A activity was estimated at 15.5 days	Sims and Ream (1997)
Bt cotton; Cry 2A	Field condition; Bt cultivation	½ life of Cry 2A activity was estimated at 31.7d	Sims and Ream (1997)
Cry 1Ab	Laboratory condition; purified protein amended in soil	After 234 days, protein still detectable in soil	Tapp and Stotzky (1998)
Bt cotton, Cry 1Ac	Field condition; Bt cotton cultivation	Not detected	Head et al. (2002)
Bt maize; Cry 1Ab	Laboratory condition; soil with Bt maize or biomass of Bt maize amended in soil	Cry1Ab protein released from root exudates and in plant biomass of Bt maize persisted up to 180 and 350 days, respectively	Saxena and Stotzky (2002)
Bt maize; Cry 1Ab	Laboratory and field condition; biomass of Bt maize amended in soil or Bt maize cultivation for 4 years	Cry 1Ab protein of Bt maize did notcontinue in soil	Hopkins and Gregorich (2003)
Bt maize; Cry 1Ab	Laboratory condition; biomass of Bt maize amended in soil	Protein persisted in the soil due to the clay particles and not accessible for microbial digestion	Muchaonyerwa et al. (2004)
Bt maize; Cry 1Ab	Field condition; Bt maize cultivation	Protein detected from Bt maize litter persisted up to least 8 months	Zwahlen et al. (2003a, b)
Bt maize; Cry 1Ab	Field condition; Bt maize cultivation	No recovery of protein	Baumgarte and Tebbe (2005)
Bt maize; Cry 1Ab	Field condition; Bt maize cultivation	No persistence for 3 years	Dubelman et al. (2005)
Bt cotton; Cry proteins	Laboratory condition; Bt cotton cultivation	Altering levels of Bt toxin persist in the Bt cotton rhizospheric soils	Rui et al. (2005)
Bt maize; Cry 3Bb1	Field condition; Bt maize cultivation	No observable level of protein in soil during 3 consecutive yrs	Ahmad et al. (2005)
Bt rice; Cry 1Ab	Laboratory and field condition; biomass of Bt rice amended and Bt rice cultivation	The ½ life of protein in Bt rice straw (4 % ww^{-1}) amended alkaline soils soil was 11.5 days and for acidic soils it was 34.3 days	Wang et al. (2006)
Bt maize; Cry1Ab and Cry 1Ac	Field condition; Bt cultivation	Degradation rate of Cry1Ac toxin varied in the soil types (sandy loam and clay)	Marchetti et al. (2007)
Bt maize; Cry 1Ab	Field condition; Bt maize cultivation	Protein recovered in soils even after 4 years of successive cultivation	Sun et al. (2007)
Bt maize; Cry 3Bb1	Laboratory condition; soils amended with biomass of Bt maize	Protein was recovered up to 21 days in soils amended with monmorillonite and 40 days in soils amended with kaolinite (K); after adjustment of pH of the K soils to *ca*.7, protein was detected for only 21 days	Icoz and Stotzky (2008a)
Bt cotton; Cry1Ac	Field and lab incubation condition; Bt cotton soils amended with leaves of Bt cotton	Bt cotton toxin decomposes fewer in soil (0.003 μg/g); Bt leaves are more recalcitrant due to low mineralization rate	Das et al. (2009)
Bt maize; Cry 1Ab	Laboratory condition; molecular study for understanding the forces governing the adsorption of Cry 1Ac protein	Uneven surface charge dispersion of Cry1Ab led patch-controlled electrostatic attraction with sorbents that carried the same net charge as Cry1Ab	Sander et al. (2010)
Bt maize; Cry 3Bb1	Field condition; Bt maize cultivation	Cry3Bb1 protein does not accumulate in soil	Miethling-Graff et al. (2010)
Bt maize; Cry 1Ab	Field condition; Bt maize cultivation	Cry 1Ab protein concentration increase initially (6–9 weeks) after incorporation of plant biomass into the soil and degrade slowly after 12–15 weeks; Cry1Ab protein does not accumulate in soil after addition the soil from Bt maize planted soil	Badea et al. (2010)

Table 1 (continued)

Bt crop/protein	Experimental condition/soil condition	Inferences	References
Bt cotton, Cry 1Ac and CpTi proteins	Field condition; Bt cotton cultivation	Cry 1Ac and CpTi proteins persisted in the soil and their content differ in the transgenic cotton-planted soil	Chen et al. (2011)
Bt maize (Cry1Ab)	Field condition; Bt maize cultivation and harvested and straw were dried	Cry1Ab released from straw were decline at early stages but a slow decline at middle and late stages of Bt corn; in the late stage (180 days after the experiment commenced) 0.03–1.51 % and 0.02–0.91 % of initial Cry1Ab protein released from 34B24 and 1246 1482 straw was detected in soil	Feng et al. (2011)
Cry 1Ab	Laboratory condition; insect bioassay, adsorption of Cry1Ab to humic acid and fulvic acid	Cry1Ab retains insecticidal activity over short-term sorption-desorption cycles to humic acids highlights the need to include SOM-adsorbed Cry proteins in the assessment of the environmental fate and potential risks of Cry proteins	Tomaszewski et al. (2012)
Bt brinjal, Cry1Ac	Field condition; Bt brinjal cultivation	Cry1Ac protein content detected up to 0.6 ng g^{-1} during flowering stage of consecutive two year Bt brinjal cropping	Singh et al. (2013b)

2004; Icoz and Stotzky 2008b), the direct role of Cry proteins in AMF is still ambiguous. Some studies reported reduced AMF colonization of Bt maize line (Bt 11) (Castaldini et al. 2005; Cheeke et al. 2011) and Bt 176 (Turrini et al. 2004) expressing Cry1Ab, while others observed no difference for Bt maize encoded with same protein (MON810, Cry1Ab) (de Vaufleury et al. 2007) or even Bt cotton with other Bt proteins (Cry1Ac and Cry2Ab) (Knox et al. 2008). Noticeably, these studies were based on varied experimental conditions and AMF inocula, Bt cultivar, Cry protein, fertilizer level, and harvest time, and the observations were inconclusive. However, under similar environmental condition, low level of AMF colonization is reported in different Bt maize roots compared to non-Bt ones (Cheeke et al. 2011; 2013). The outcome stated the possibility of pleiotropic and certain type of genetic changes that influence crop physiology (i.e., sugar allocation, enzyme activity in roots, lignin content, etc.) may affect the ability of selected lines of Bt maize to form associations with AMF. However, another study reported that Cry34/35Ab1 proteins expressing Bt (DAS-59122-7 event) maize may negatively affect the initial development of AMF under field conditions (Cheeke et al. 2012, 2013), but the effect was not observed during the last two sampling dates (82 and 135 days). The probable reason for this inconsistency is still unknown (Seres et al. 2014).

The evaluation by de Souza Vieira et al. (2011) of the impact on endophytic fungi associated with leaves, stem, and roots of Cy1Ac expressing Bt cotton revealed Bt modifications to have no impact on the endophytes, while the tissue and plant stage significantly affected the fungal community composition. Such observations were corroborated by others wherein low levels of endophyte infection in Bt tissues was not due to the direct effect of Cry protein on the fungi, but the indirect one following Bt gene incorporation (Suryanarayanan et al. 2011).

Changes in population density/diversity indices might not always elucidate the changes in ecosystem function as soil-microbe interaction and soil function are complex and far from clear (Nielsen et al. 2011). Therefore, still more functional aspects of the taxonomic groups have to be monitored in parallel with the diversity estimations. Moreover, it is necessary to combine such parameters as the single index for meaningful information on diversity and functional attributes.

Impact of Bt crops on soil microbial community structure

Many laboratory and field trials on the impact of transgenic crops on soil biota considering the different variables and techniques for evaluating the risk on community structure are given in Table 3. Most such studies used culture-dependent approach, such as substrate utilization pattern (i.e., BIOLOG) and culture-independent ones, such as DGGE, T-RFLP, and SSCP (Liu et al. 2008; Tan et al. 2010). However, the results were invariably inconsistent as the effects ranged from no to minor transient changes (Blackwood and Buyer 2004; Brusetti et al. 2004; Devare et al. 2004; Fang et al. 2005, 2007). Studies based on above techniques revealed minor or no Bt gene expressing specific effects on soil microbial community, and the age and plant type and other environmental factors (soil texture, soil pH, moisture, redox potential, N concentration, temperature, precipitation, etc.) dominantly determined the microbial

Table 2 Summary of the effect of Bt crops on soil microbial population

Site/Crop	Organism	Findings	References
USA; Bt (Cry1Ac) and non-Bt maize	Culturable bacteria and fungi	Substantial but temporal increase in numbers in soil with Bt cotton; no response on bacteria and fungi compared to control	Donegan et al. (1995)
Hermiston Agricultural Research and Extension Center, Hermiston, Oregon. USA; Bt (Cry3A) and non-Bt potato	Culturable aerobic bacteria and fungi	Minimum alteration in population load were encountered	Donegan et al. (1996)
Agricultural field of East Marion, Long Island, New York; Bt (Cry1Ab) and non-Bt maize	Culturable bacteria, fungi, protozoa, nematodes, and earthworms	Insignificant variation in microbial population size between Bt and non-Bt maize biomass amended soils or in rhizospheric soils of respective soils	Saxena and Stotzky (2001)
Bt (Cry1Ab, Cry3A and Cry4) and non-Bt	Bacteria, fungi, and algae	No reaction on the microbial development were observed	Koskella and Stotzky (2002)
Experimental site of Londrina, PR, Brazil; Bt (Cry1Ab) inocula infesting soybean crop	Heterotrophic bacterial and saprophytic fungal populations and carbon-cycling microorganisms (cellulolytic, amylolytic, proteolytic) and arbuscular mycorrhizae	No reaction on the populations when compared to non-inoculated soil; temporal variation in population size compared to non-inoculated soil. No response on arbuscular mycorrhizae population size when inoculated with ICP protein but inhibition of fungal colonization was observed when inoculated with spores of Bt*k*	Ferreira et al. (2003)
Experimental rice field at Zhejiang University, Hua-jia-ci Campus, Hangzhou, China; Bt (Cry1Ab) and non-Bt rice	Culturable bacteria including actinomycetes and fungi	No detrimental reactions on population load	WeiXiang et al. (2004)
Field of the Inner Mongolia Autonomous Region north China and Chinese Academy of Agricultural Sciences, Beijing, China, Bt (Cry1Ab) and non-Bt maize	Culturable bacteria and fungi	Low proportion of gram positive to gram negative bacteria in Bt maize-planted soil; no variation in bacterial and fungi population load	Xue et al. (2005)
Bt (Cry 3A) and non-Bt maize and soil with Bt and non-Bt potato	Culturable bacteria and fungi	Large proportion of gram-positive to gram-negative in Bt potato-planted soil compared to non-Bt potato-planted soil, and no alteration in the ratio of fungi to bacteria was encountered	Xue et al. (2005)
Experiment Station of China Agricultural University, Beijing, China; Bt (Cry1Ac) and non-Bt cotton	Culturable functional bacteria (potassium-dissolving bacteria, inorganic phosphate-dissolving bacteria, and nitrogen-fixing bacteria)	Elevated population load of functional bacteria in non-Bt cotton soil compared to Bt cotton in early and middle cotton growth stages; insignificant differences in population size followed by growing season	Rui et al. (2005)
Farm, Long Island, New York, USA; Transgenic (*Cry* gene)) plant of corn, rice, canola, tobacco, cotton and tomato and their non-Bt counterparts	Field trial; population density of bacterial including actinomycetes and fungi using CFU method on soil extract agar and Rose-Bengal-streptomycin agar	Insignificant variation	Flores et al. (2005)
Experimental field of Department of Crop Plant Biology, University of Pisa, Pisa, Italy; Bt (Cry1Ab) and non-Bt maize;	Microcosm study; culturable heterotrophic bacteria and mycorrhizae	Low intensity of mycorrhizal infection by *Glomus mosseae* in transgenic maize	Castaldini et al. (2005)
Rosemount Experiment Station of the University of Minnesota; Bt (Cry1Ab and Cry 3Bb1) and non-Bt maize	Field trial; microbial populations	No persistent significant reaction on population size of culturable bacteria, gram-negative bacteria, chitin- and cellulose-utilizing bacteria, nitrifiers, denitrifiers, protozoa, and fungi	Icoz et al. (2008)

Table 2 (continued)

Site/Crop	Organism	Findings	References
Australian Cotton Research Institute (ACRI), Narrabri, NSW, Australia; Bt cotton (Cry1Ac and Cry2Ab) and conventional varieties	Field trial; mycorrhizal colonization assessment	Genetic modification did not pose negative effect on AMF colonization	Knox et al. (2008)
Bt maize (Cry 1Ab protein) and their isogenic non-Bt maize lines	Field trail; culturable aerobic bacteria, fungi and actinomycetes	No significant variation was encountered on the microbial populations	Oliveira et al. (2008)
Agricultural field Boading, Hebei Province, China; Bt cotton (Cry 1A and CpTI) and non-Bt cotton	Field trial; Quantification of N_2 fixing, inorganic-PO_4, organic-PO_4, and K dissolving bacteria using respective media for CFUs count	Numerous year of Bt cotton cropping may not affect the bacterial load	Hu et al. (2009)
Agricultural field Hotala, Maharashtra, India; Bt cotton (Cry) and non-Bt cotton	Field trial; total bacterial, fungal, and actinomycetes population using SCDA and TSA culture media for CFUs	No variation were encountered the population load of microbes	Kapur et al. (2010)
Agricultural farm of Baibi town, Henan Province, China; Bt cotton (Cry1Ac) and non-Bt cotton	Field trial; culturable bacteria, fungi, and azotobacter	Prominent effect of natural factors compared to genetic transformation	Li et al. (2011)
Portland State University, Portland, USA; Bt 11 maize (Cry1Ac) and parental iso-lines	Greenhouse microcosm; Mycorrhizal fungal colonization assessment	No changes in AMF colonization was observed between the Bt 11 and the following maize cultivars	Cheeke et al. (2011)
Experimental farm of Central Institute of Cotton Research, Nagpur, India; Bt (Cry) and its isogenic non-Bt cotton	Field study; infection frequency of endophytes were calculated from different plant healthy tissue	No variation in endophytes numbers obscured from the respective plants; Bt cotton receive low infection frequency	Suryanarayanan et al. (2011)
Federal Rural University of Pernambuco (UFRPE), Recife-PE, Brazil; Bt (Cry1Ac) and non-Bt cotton	Isolation of endophytes using PDA media and microscopicobservation of fungal structure	The most periodic fungal endophyte were *Phomopsis archeri* from leaves (22.9 %) and stems (16.8 %) and *Phoma destructive* from roots (11 %) from both the cotton genotypes; the cotton tissue and the plant developmental stage significantly affected the diversity and composition of the fungal community compared to Bt modification	de Souza Vieira et al. (2011)
Portland State University, Portland, USA; Multiple Bt maize (Cry1Ab; Cry34/35Ab1; Cry3Bb1; Cry1F) and parental iso-lines	Greenhouse microcosm; mycorrhizal fungal colonization assessment	Bt maize receive minor intensity of AMF colonization in their roots compared to counterpart parental lines; reductions in colonization were not related to the Bt protein	Cheeke et al. (2012)
Indian Agricultural Research Institute, New Delhi, India; Two cropping systems sole Bt cotton (Cry), cotton+peanut)	Enumeration of total bacterial, fungal and actinomycetes population using soil extract agar, Martin's Rose agar and Kuster's agar media, respectively	No negative response of transgenic cotton on soil microbial population	Singh et al. (2012)
Agricultural land of Vidarbha, Maharashtra, India; Bt cotton (Cry 1Ac) and non-Bt cotton	Field trial; total bacterial, fungal, and actinomycetes population	Bacterial (85.9×10^6 CFU g^{-1} in non-Bt cotton and 73.7×10^6 CFU g^{-1} in Bt cotton) and actinomycetes (52.5×10^5 CFU g^{-1} in isogenic counterpart and 43.6×10^5 CFU g^{-1} in Bt cotton) population significantly decreased under Bt cotton compared to non-Bt cotton	Tarafdar and Rathore (2012)
Experimental field, Indian Agricultural Research Institute, New Delhi, India; Bt cabbage (Cry) and non-Bt cabbage	Pot experiment; Total bacterial, actinomycetes, fungi, and phosphate solubilizing using CFU method	No significant changes on bacterial, actinomycetes, fungal, and phosphate solubilizing bacterial population between the Bt and its counterpart	Dutta et al. (2012)

Table 2 (continued)

Site/Crop	Organism	Findings	References
Agricultural Field of Indian Institute of Vegetable Research, Varanasi, India; Bt Brinjal (Cry1Ac) and non-Bt brinjal	Field trail; total actinomycetes bacterial (16S rRNA) and fungal (ITS rRNA) population load	Actinomycetes and bacterial population load were significantly reduced under the soil planted with Bt brinjal compared to non-Bt brinjal; effect of Cry1Ac gene was masked by crop growth stages	Singh et al. (2013a, b, 2014)
Experimental field of Central Institute of Cotton Research, Nagpur, India; Bt (Cry1Ac) and non-Bt Cotton	Field trial; bacterial, actinomycetes, fungal, and functional microflora population enumeration	Bacterial and fungal population were significantly greater in Bt cotton owing to the crop type; no effect of of genetic transformation was observed	Velmourougane and Sahu (2013)
Experiment field near Corvallis, OR, USA; Multiple Bt maize (Cry1Ab; Cry34/35Ab1; Cry3Bb1; Cry1F) and parental iso-lines	Field trial; mycorrhizal fungal colonization assessment and spore density	No effect of genetic modification on the colonization of AMF in *G. max* in field condition	Cheeke et al. (2013)
Experiment field of Julianna-major, Nagykovácsi, Hungary; Bt maize (Cry34/35Ab1) and near isogenic non-Bt maize	Field trial; AMF colonization assessment	Negative effect on the development of AMF	Seres et al. (2014)

community (Blackwood and Buyer 2004; Baumgarte and Tebbe 2005; Fang et al. 2005; Icoz et al. 2008; Chen et al. 2011). Liu et al. (2008) reported that KMD1(Bt) rice expressing Cry1Ab did not have pronounced adverse effect on bacterial and fungal community or their vital processes, and also, the variations in the rhizosphere associated-soil microbial community outweigh the application of triazophos and Cry1Ab modifications over 2 years of rice cropping. Miethling-Graff et al. (2010) observed no significant differences between the rhizosphere bacterial community structure of Bt maize and other cultivars over three consecutive years of study. Also, the bacterial and fungal community composition did not differ between *Myxococcus xanthus protoporphyrin oxidase* (Mx PPO) transgenic and non-transgenic parental rice at the seedling, tillering, heading, or maturing stage over two successive years of cultivation (Chun et al. 2012). However, a few studies reported significant differences in microbial community structure between soils with Bt and non-Bt planted crops (Lu et al. 2010; Tan et al. 2010). Castaldini et al. (2005) observed consistent and significant differences in the composition of soil microbial community with regard to Bt or non-Bt maize. Lu et al. (2010) observed minor effects of Cry1 Ab modification in the Xiushui 11 rice genome on the residue decomposition-associated bacteria or fungi during a 2-year study. Similarly, Wei et al. (2012) reported minor impact on the rhizosphere-associated bacterial, fungal, and actinomycete community. Singh et al. (2013a, b, 2014) observed actinomycete, bacterial, and fungal groups exclusively restricted to plant flowering and maturation stages, suggesting the transient effect of Cry1Ac compared to crop growth stages during 2 years of trial. The contrasting observations on the impact of Bt crops on the associated soil microbes may possibly reflect differences in the type of Cry protein, plant variety, and the experimental methods applied along with the soil type and the environmental factors (Ciccazzo et al. 2014). The species and functional variations in soil microbial community is influenced by many direct and indirect environmental factors. The direct effects depend on both: the range of activity of proteins encoded by the transgenes (Oger et al. 1997) and their amount accumulating in the environment. In comparison, the indirect effects are possibly mediated by fluctuations in the chemical composition of plant biomass and root exudates resulting from modifications in the normal metabolic pathways of the plant.

Apart from the external soil biota, endophytes are well known for their plant beneficial potential (Sessitsch et al. 2004; Berg et al. 2005). The expression of Cry protein might lead to modifications in the plant metabolite composition that induces alterations in the associated endophytic community compared to the nearly isogenic wild-type. Nevertheless, studies on GM crops associated endophytes are relatively rare (Heuer et al. 2002; Rasche et al. 2006). Recent studies under the containment on two different soils compared the endophytic bacteria in three transgenic Bt maize lines MON89034 (cry3Bb1), MON88017 (cry1A105 and cry2Ab2), and the stacked event MON88017×MON89034 (cry1A105 and cry2Ab2, cry3Bb1) with the respective near-isogenic line, and plants of three additional, conventional maize lines. The endophyte community associated with the Bt lines was closely related with isogenic lines suggesting that both the soil environment and plant cultivar were the major determinants of endophytic bacteria (Prischl et al. 2012). Recently, comparative study using modern high-throughput techniques (454 GFLX sequencing and T-RFLP) revealed

Table 3 Summary of the effect of Bt crops on soil microbial community structure

Site/ Crop	Experimental design	Findings	References
Experimental field of Salisbury, Marlboro, Australia; Bt corn (Cry) and non-Bt gene	Growth chamber experiment:, PLFA and CLPA were used to asses community structure	Response of Bt corn was small and temporal	Blackwood and Buyer (2004)
Vegetable farm of Cornell University, New York; Transgenic Bt corn (Cry 3Bb)	T-RFLP for bacterial community analysis	No impact on soil bacterial community structure	Devare et al. (2004)
Agricultural field sites, Sachsen-Anhalt and Nordrhein-Westfalen, Germany; Bt–maize hybrid MON810 (Cry1Ab)	Field trial, bacterial community structure analysis using 16S rDNA-SSCP	Cry1Ab protein recovered in MON810 soil; minor effect on bacterial community structure compared to natural factors	Baumgarte and Tebbe (2005)
Bradford Agronomy Research Center' USA; Bt maize and non-Bt maize	Field trial and green house study; biolog, bacterial community structure analysis using 16S rDNA-DGGE	Bacterial communities affiliated with rhizospheric soil affected by soil texture compared to crop varieties	Fang et al. (2005)
Greenley Agricultural Experiment Station USA; Bt maize and non-Bt maize	Field trial and microcosm study; biolog, bacterial community structure analysis using 16S rDNA-DGGE	Addition of Bt residue containing prominent lignin and lignin/N ratio in soil incomparably influenced the microbial composition compared to the residue of its counterpart	Fang et al. (2007)
Department of Crop Plant Biology, University of Pisa, Italy and field of The Centro Interdipartimentale di Ricerche Agro Ambientali, Pisa, Italy; Bt corn (Cry1Ab) and non-Bt corn	Microcosm and greenhouse experiment; 16S rDNA–DGGE for eubacterial community	Model study revealed variation in rhizospheric eubacterial communities; greenhouse experiment showed differences between Bt and non-Bt corn plants in rhizospheric heterotrophic bacterial communities and mycorrhizal colonization	Castaldini et al. (2005)
Agricultural field Zhejiang province, China; Bt rice (Cry 1Ab*)* (Bt,), non-Bt (Ck) and non-Bt with triazophos (Ckp)	Field trial, DGGE and T-RFLP for compare bacterial and fungal compositions	Seasonal variations affects bacterial composition compared to genetic modification	Liu et al. (2008)
Agriculture field of Suwon, Kyonngi Province and Yesan, Chungnam Province, Korea	Field trial; 16S rDNA-DGGE profile for bacterial communities	Genetic transformation did not pose negative impact on bacterial composition	Jung et al. (2008)
Agricultural field Hotala, Maharashtra, India ; Bt cotton (Cry) and non-Bt cotton	T-RFLP for bacterial community analysis	Bt cotton cultivation did not pose negative impact of bacterial diversity	Kapur et al. (2010)
Agricultural field Wurzburg, Bravia, Germany; Bt maize (event MON88017 Cry 3Bb1 and CP4 EPSPS) and three non-Bt cultivar	Bacterial community analysis by SSCP of 16S rRNA	No significant differences in bacterial communities between Bt maize and other cultivar	Miethling-Graff et al. (2010)
Experimental field of South China Agricultural University, China; Two transgenic Bt corn hybrids (Cry1Ac and Cry1A, respectively) and their near-isolines	Field trial; 16SrRNA and 18S rRNA PCR-DGGE profile for bacterial and fungal community, respectively	No variation in the microbial community structure between the Bt corn hybrids and its counterpart	Tan et al. (2010)
Zhejiang University's Research farm, Zhejiang Province, China; Bt rice (Cry 1Ab) and non-Bt rice	Field trial, T-RFLP analysis of bacterial and fungal community analysis	No significant differences in the bacterial and fungal composition of Bt rice and non-Bt rice	Lu et al. (2010)
Experimental farm, Zhejiang, China; Two transgenic lines (Cry1Ab) (HC and TT) and non-transgenic parental varieties Jiazao 935 (JZ) and Minghui 63(MH), and non-transgenic distal parental rice varieties Zhongjiu B (ZJ) and 9311	16S rRNA–DGGE for bacterial community analysis	Vegetation and straw amendment of transgenic lines did not have negative effect on the bacterial communities	Fang et al. (2012)

Table 3 (continued)

Site/ Crop	Experimental design	Findings	References
Experimental field, Shanghai Academy of Agricultural Sciences, Shanghai, China; Bt rice (Cry1Ac) and non-Bt rice	DGGE for bacterial, fungal and actinobacterial community Composition	Bt rice pose little effect on the dominant rhizospheric microbial community structure	Wei et al. (2012)
Agricultural Field of lower Austria, Austria; Bt maize Cry1A105, (Cry2Ab2, and stacked genes carrying proteins Cry3Bb1, cry1A105 and Cry2Ab2) and their isolines	Culture-dependent technique; culture-independent technique via T-RFLP of 16S rRNA of endophytes	Study revealed the influence of both the soil type and plant cultivar on endophytes irrespective of genetic transformation	Prischl et al. (2012)
Agricultural field of Netherland; Bt maize (Cry1Ab) and non-Bt maize	Field trial; 454 pyrosequencing and T-RFLP of AM fungi	Non-uniform differences were encountered between the AMF associated with Bt and non-Bt maize; transient changes in AM community was more compared to the genetic modification of the maize crop	Verbruggen et al. (2012)
Agricultural Field of Indian Institute of Vegetable Research, Varanasi, India; Bt Brinjal (Cry1Ac) and non-Bt brinjal	Field trial; 16S, ITS rRNA- PCR cloning for actinomycetal, bacterial and fungal community	Exclusive actinomycetal and bacterial community detected during flowering and maturation stages; genetic modification effect was minor and transient compared to crop developmental stages	Singh et al. (2013a, b, 2014)
Agricultural field of Netherland; Bt maize (Cry1Ab) and non-Bt maize	Field trial; 454 pyrosequencing of fungal community	Detected large groups of AM fungal and basidiomycota; however, no significant differences in soil fungal diversity and community structure associated with different plant cultivars were observed	Kuramae et al. (2013)

no pronounced effect of Cry1Ab toxin expressing Bt corn on the AMF (Verbruggen et al. 2012).

Response of microbial processes to Bt crops in soil

With the emergence of molecular techniques, it is possible to retrieve the specific microbial groups as affected by Bt crops. However, the functional role of microbes in soils for growing Bt crops could be better defined by the processes they impart. Enzymatic activities, microbial biomass, respiration, CO_2 evolution, etc. could be the better parameters to decipher the impact of GM crops on the functional aspects of soil microbial processes (Icoz et al. 2008). Previous studies demonstrated effect, ranging from "no" to "minor" and "significant," of GM crops on the microbial community (Stotzky 2004; Rui et al. 2005; Xue et al. 2005; Shen et al. 2006; Sun et al. 2007; Icoz et al. 2008; Chen et al. 2011; Tarafdar and Rathore 2012). Some studies highlighted the input of transgenic crops on dehydrogenase, invertase, acid phosphomonoesterase, urease, cellulase, etc. (WeiXiang et al. 2004; Flores et al. 2005; Icoz et al. 2008; Hussain et al. 2011; Chen et al. 2011, 2012) in soils under field and laboratory conditions. Some studies, however, indicated insignificant differences in the activity of phosphatases and catalase in soils planted with Bt and non-Bt maize (Flores et al. 2005; Lang et al. 2006; Icoz et al. 2008). WeiXiang et al. (2004) observed no apparent variation in neutral phosphatase in soils supplied with Bt and non-Bt rice straw. While dehydrogenase activity was significantly higher (~1.95-fold) in soils with Bt-transgenic straw from d7 to d14 but not from d21 to d49 over the soils treated with the non-Bt counterpart (~1.5-fold). The possible reason could be the alterations in the nutritional quality/quantity of the transgenic rice straw owing to the expression of the Cry1Ab protein. Similarly, Liu et al. (2008) also observed no significant difference in enzyme activities in the rhizosphere of transgenic Bt rice and non-parental rice under field and laboratory conditions as well indicating that crop growth effect could have masked the effect of genetic modifications. In contrast, a few studies reported significant impact of Bt crops on soil enzymes. Flieβbach et al. (2012) reported reduction (5 %) in the soil dehydrogenase activity for Bt maize varieties compared to non-Bt counterpart under experimental field conditions suggesting that the anticipated changes in the plant composition due to transformation could modify the soil-mediated processes. Similar result was also observed under

Table 4 Summary of soil microbial processes over Bt crops

Site/crops	Experimental variables/microbial parameters	Findings	References
Experimental rice field of Zhejiand University, China; transgenic rice (Cry1Ab) and non-transgenic rice	Field trial; determination of soil dehydrogenase and neutral phosphatase enzyme activity	Substantial difference in the soil dehydrogenase activity between the Bt and its counterpart	WeiXiang et al. (2004)
Vegetable farm of Cornell university, New York; transgenic Bt corn (Cry 3Bb)	Field trial; N mineralization, microbial biomass N and C, soil respiration	No detrimental effects of Bt born on N-mineralization, MBC and soil respiration	Devare et al. (2004)
Experimental field of university of Missouri, USA; Bt (Cry) maize hybrids and their non-transgenic isolines	Field trial and laboratory-scale N-mineralization and lignin content	No effect on N dynamics in laboratory and field condition; non-uniform variation in N-mineralization rate	Mungai et al. (2005)
Experimental field of Chinese Academy of Sciences, China; Bt-transgenic cotton (Cry1A) and non-Bt cotton	Field trail; enzymatic activities in soil	Except dehydrogenase, activities of other enzymes decline. No negative effects of Bt cotton on the soil enzymes of Bt and non-Bt cotton-planted soils	Shen et al. (2006)
Experimental field of Chinese Academy of Science; Bt-transgenic cotton (Cry1A) and non-transgenic Cotton	Field trial; analysis of Bt toxin concentration and enzyme activities in soil	Soil urease, phosphomono-esterase, invertase and cellulase were accelerated by the addition of Bt cotton tissues	Sun et al. (2007)
Vegetable Farm of Freeville, New York; Bt corn and non-transgenic isoline	Field trial; microbial biomass, N-mineralization	No negative reaction of Bt maize on microbial biomass and other soil processes	Devare et al. (2007)
Experimental station of University of Minnesora; Bt corn varieties with either of Cry1Ab and Cry3Bb	Field trial; soil enzymes	No uniform variation in the processes of soil enzymes under Bt maize	Icoz et al. (2008)
Research farm, Indian Agricultural Research Institute, New Delhi, India; Bt cotton (Cry) and isogenic non-Bt cotton	Field trial; soil respiration, soil dehydrogenase activity	Significant reduction in the soil respiration (−3.5 %) and dehydrogenase (−17 %) under Bt cotton rhizosphere soil	Sarkar et al. (2008)
Agricultural field Zhejiang province, China; Bt rice Cry 1Ab (Bt,), non-Bt (Ck) and non-Bt with triazophos (Ckp)	Field trial; soil dehydrogenase, soil neutral phosphatase activity	No variation in the enzymatic activities between the rhizosphere soil of Bt, Ck, and Ckp over cultivation period	Liu et al. (2008)
University of Nebraska-Lincoln West Central Research and Extension Center, NE, USA; Bt corn (Cry1Ab) hybrids and their non-Bt isolines	Field trial; decomposition rate for leaves, cobs, and stalks using litter bag technique	No variation in the rates of decomposition and biomass C left over between the Bt and non-Bt corn residues	Tarkalson et al. (2008)
South Dakota State University's Dakota Lakes Field Station, USA; Four Bt corn hybrids and isogenic-non-Bt corn	Field trial; decomposition rate of residues using litter-bag technique	Decomposition rate is constant (0.25 day^{-1}) for all varieties; no variation in the composition of Bt and non-Bt residues	Lehman et al. (2008)
Kellogg Biological Station long-term ecological research (LTER), Michigan, Bt corn (Cry1Ab) and non-Bt corn	Field trial; C mineralization	Continuous cropping of Bt corn did not affect C mineralization	Kravchenko et al. (2009)
Experimental farm of Indian Agricultural Research Institute, New Delhi, India; Bt (Cry1Ac) and non-Bt cotton	Field trail; decomposition of Bt cotton leaf using incubation method	Cry1Ac protein decomposes into the soil, due to the recalcitrant nature they remain into the soil compared to the non-Bt cotton leaf	Das et al. (2009)
Experimental farm of University of Maryland Research and Education Centre, MD, USA; Bt corn hybrid (Cry3Bb1) its isogenic non-Bt corn, including other untreated negative control lines	Field trial; enzyme assays from detrimental organic matter estimation	Bt corn had no significant impact on extracellular enzymes activities	Lawhorn et al. (2009)
University of Fort Hare (UFH) Research Farm, Province of	Field trial; decomposition rate of maize residues using litter-bag technique	No considerable concentration of Cry 1Ab protein was recovered. The	Daudu et al. (2009)

Table 4 (continued)

Site/crops	Experimental variables/microbial parameters	Findings	References
South Africa; Bt-maize (Cry1Ab) and its near-isogenic line		results revealed that Bt-maize residues degrade at a similar rate as of other maize cultivars, and that the recovery of the free Cry1Ab protein in soil could be minimal	
Experiment field of Swiss plateau, Switzerland; Bt (Cry1Ab and Cry 3Bb1) and their respective maize isolines	Field trial; leaf residue decomposition using litter bag technique	No variation in the trend of decomposition rate of Bt and non-Bt maize; no negative effects of Bt maize on the decomposition rate	Zurbrügg et al. (2010)
Experiment site of Shenyang Agricultural University, China; three pairs of cotton—Bt cotton (Cry1Ac) with its isogenic, CpTI + (transgenic Bt+CpTI cotton; non-transgenic Bt+CpTI cotton with its isoline), CpTI + + (transgenic Bt+CpTI cotton; non-transgenic Bt+CpTI cotton with its isogenic)	Pot experiment; microbial biomass carbon (MBC); various soil enzymes	Soil enzymes activities (besides urease and phosphodiesterase) greatly reduced in toxin producing cotton soils; consecutive cultivation of transgenic cottons might pose adverse impact on soil microbial and biochemical properties	Chen et al. (2011)
Macdonald Research Farm, Ste. Anne de Bellevue, Quebec, Canada, USA; Bt (Cry1Ab) and non-Bt maize	Field trial; aerobic soil incubation, lignin-derived phenol analysis	Genetic modification elevated CO_2 production from stem-amended soils and reduced N mineralization in root-amended soils	Yanni et al. (2011)
Experiment farm of Cornell University's Musgrave New York; Bt corn (Cry3Bb) And non-Bt corn	Field trial; decomposition of maize cob, shoots, and roots using litter bag technique, lignin concentration	Corn residue decomposition was not influenced by Cry3Bb toxin; although, environmental factors led to variation for most variables measured	Xue et al. (2011)
Indian Agricultural Institute, New Delhi, India; two cropping systems, Sole Bt cotton (Cry), cotton+peanut)	Field trial; dehydrogenase activity	No impact	Singh et al. (2012)
Agricultural land of Vidarbha, Maharashtra, India; Bt cotton (Cry 1Ac) and Non-Bt cotton	Field trail; microbial C, N, P; soil dehydrogenase, esterase, acid and alkaline phosphatase, and phytase enzyme activity	Significant reduction in the microbial biomass under Bt cotton; significant reduction in dehydrogenase, acid phosphatise, phytase, and nitrogenase enzymes under Bt cotton compared to its counterpart	Tarafdar and Rathore (2012)
Agricultural field of Shanxi University, China; Bt cotton (Cry1Ac) and conventional variety near isogenic to Bt cotton	Field trial; soil protease, urease, alkali phosphatase, sucrase, and dehydrogenase; nutrient content determination	Negative impact on soil enzymes and soil nutrient	Yang et al. (2012)
Experimental farm, Zhejiang, China; Two transgenic lines (HC and TT) and non-transgenic parental rice varieties Jiazao 935 (JZ) and Minghui 63 (MH), and non-transgenic distal parental rice varieties Zhongjiu B (ZJ) and 9311	Field trial; soil enzymes (catalase, urease, neutral phosphatase, and invertase)	Insufficient detrimental response on soil enzymes due to transgenic rice lines compared to parent rice varieties	Fang et al. (2012)
Experimental field, Shanghai Academy of Agricultural Sciences, Shanghai, China; Bt rice (*Cry1Ac*) and non-Bt rice	Field trial, soil protease, urease, sucrase, dehydrogenase, catalase, and polyphenol oxidase	No variation in dehydrogenase, invertase, phenol oxidase, acid phosphatase, urease, and protease between Bt and its counterpart	Wei et al. (2012)
Agricultural field of Shenyang Agricultural University, Liaoning province, China; Two Transgenic varieties: Bt (Cry)	Field trial; soil microbial biomass (MBC) and soil enzymes	Sequential cropping of transgenic cotton posses adverse impact on microbial activities and enzyme activities in Bt cotton rhizospheric	Chen et al. (2012)

Table 4 (continued)

Site/crops	Experimental variables/microbial parameters	Findings	References
and *CpTi* (gene along with non-Bt near isogenic lines		soils compared to non-Bt cotton rhizospheric soils	
Experimental field, Indian Agricultural Research Institute, New Delhi, India; *Bt* cabbage (Cry) and non-*Bt* cabbage	Pot experiment; soil dehydrogenase activity	Soil dehydrogenase activity varied with respect to sampling date only; no effect of *Bt* cabbage on soil processes	Dutta et al. (2012)
Agricultural Field of Indian Institute of Vegetable Research, Varanasi, India; Bt Brinjal (Cry1Ac) and non-Bt brinjal	Field trail; N-mineralization, soil nutrients; organic C, soil moisture, MBC, soil dehydrogenase, FDA, invertase, urease and acid phosphor-monoesterase	Significant reduction of organic C, MBC, dehydrogenase and FDA enzymes in Bt brinjal-planted soil compared to non-Bt; Soil nutrients and soil pH varied significantly across the crop developmental stages only	Singh et al. (2013a, b, 2014)
Experimental field, Central Institute of Cotton Research, Nagpur, India; Bt (Cry1Ac) and non-B Cotton	Field trail; soil respiration, fluorescein diacetate (FDA) hydrolysis, urease, dehydrogenase, MBC	Soil respiration and FDA activity were highest under Bt cotton soil>non-Bt>control bulk soil; no adverse effects of Bt cotton on microbial processes	Velmourougane and Sahu (2013)
Iowa State University Field Extension Education Laboratory Research, USA0; Bt (Cry1Ab) and non-Bt maize	Field trial and laboratory incubation study; decomposition rate of residues using litter-bag technique	No effect on decomposition rate under no-tillage in Bt and non-Bt crops	Al-Kaisi et al. (2013)

Bt cotton-planted soil (Beura and Rakshit 2013). Sun et al. (2007) reported stimulations in soil urease, acid phosphomonoesterase, invertase, and cellulose activities through additions of Bt cotton straw, attributable to the increased microbial activity. Studies on the effect of microbial activity for elucidation of soil health associated with Bt crops are listed in Table 4. Sarkar et al. (2008) reported significant difference between Bt and its near-isogenic non-Bt cotton. Similarly, Tarafdar and Rathore (2012)) reported reductions in activities of soil dehydrogenase, acid phosphatase, phytase, and nitrogenase under Bt cotton compared to non-Bt cotton indicating the possible inhibition of the microorganisms involved in the soil metabolic activities.

In addition, indirect and pleiotropic effects induced due to genetic modification have been widely addressed (Icoz et al. 2008; Turrini et al. 2015). Most of the studies targeted the microbial-mediated processes such as decomposition and mineralization (Table 4). However, relation between the Cry protein and the crop residues remains to be verified.

Future perspectives and conclusions

The predicted climate models indicate global temperature rise by 2 to 11 °F by 2100, depending on the extent of greenhouse gas emissions (NRC 2010). Crops are therefore likely to encounter such environmental stresses and can lead to severe consequences in terms of food security. For instance, transgenic cotton expressing Bt insecticidal protein (Cry) showed decline in the protein level at high temperatures, elevated CO_2, or drought, thus decreased pest resistance (Dong and Li 2007). Therefore, studies have to focus on plant responses to multiple stresses. Cheeke et al. (2011) looked at the fertilizer levels and Bt-trait interactions related to AMF. The strong effect of soil fertilizer and spore density provided some insight to explaining the diversity of AMF as observed previously, and identified some vital environmental considerations for evaluations in future.

Pest resistance arising through mutations in pests enables them to knock out the resistance as conferred by the single Bt trait (ISAAA Pocket K 42). Now, the combination or stacking of different traits or genes in plants is rapidly getting popular in the biotechnology of crop production. The transgenic technology of stacked traits has been applied by many for the pest management; nevertheless, their soil persistence still remains the major challenge. It will be interesting to look for the interplay between the stacking traits and soil components and to know as to how the predominant adsorption mechanisms were affected by stacked traits. For stacking the IR traits, the single ones have extensively been assessed for evaluation of the adverse side effects on the non-target organisms. Therefore, stacked events need a specific risk assessment other than the evaluation of their single transformation event (De Schrijver et al. 2007; Hendriksma et al. 2012). In context of the risk assessment of stacked traits containing transgenic crops, the information available to date is very little (Chen et al. 2011; 2012; Prischl et al. 2012) and, therefore, warrants more study

for the biosafety assessment of GM crops harboring stacked traits.

Another interesting approach would be to analyze the transgenic crops-associated endophytic microorganisms. Genome analysis of endophytic microbes would enable us understand the host-plant symbiotic relationship that may confer greater fitness to plants to fully acclimatize the adverse conditions (Kusari et al. 2012). There has been interest in the bioprospecting of microbial endophytes (Strobel and Daisy 2003). Therefore, it seems imperative to evaluate the risks related to the endophytes and their response to transgenic crops.

Despite the better assessment of soil microbial diversity through molecular tools and techniques, the correlation between microbial diversity and soil functionality remains largely unclear. In some cases, the secondary (unexpected) effects of genetic modifications are likely to remain undetected without their assessment under experimental conditions simulating natural soils. Root exudates are the important factors affecting soil functions and microbial diversity. Recent study update for monitoring of rhizodeposition using carbon labeling and stable isotope probing (SIP) has proven reliable for linking the microbial activity and structure (Wu et al. 2009; Hannula et al. 2012). The in vivo technologies like FISH, phylogenetic probes, etc., coupled with meta-genomics and meta-proteomics/transcriptomics, would be more advantageous for linking changes at the DNA/ mRNA level with the protein expression. This approach is likely to offer a better understanding of the linkage between microbial diversity and soil functionality. The objectives and parameters taken into consideration should be relevant to the concerned environment so that the consequences of GM crops could be understood. Most studies centered on the "immediate effects" while the "delayed effects" omitted. Investigations on the impact of GM crops on soil biota and the consequent risk assessment should be conducted for longer durations under natural conditions incorporating as many treatments as possible to clearly define the baseline representative of the "natural variations," and should also well incorporate the non-genetically transformed control plants as well as controls transformed using only genetic markers. Therefore, the assessment strategies need some more improvements. In the overall, case-by-case assessments of the potential benefits and ecological and environmental risk of each GM crop will be the most appropriate approach to ensure the agricultural sustainability.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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