

AN APPROACH FOR IMPACT ASSESSMENT OF TRANSGENIC PLANTS ON SOIL ECOSYSTEM

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Abstract. Transgenic crops are new products of agriculture biotechnology. The environmental risks and benefits of transgenic crops are topic of hot debate. Current agriculture management practices and ecosystems have their own impacts on the environment and further any additional negative effect of transgenic crops may mitigate their positive impacts as well as increase the background value of negative impacts due to new agriculture practices. Most of the risk assessment studies on transgenic plants have done observations on changes in their respective aboveground environment and its biota. Very few reports are available on the impacts of transgenic plants or their products (that they release in soil) on soil biota (both invertebrates and microorganisms) and soil processes mediated by them. However, observations of these studies were not delivering anything conclusively and creating state of confusion also regarding impact of transgenic plants on soil ecosystem. As some of the studies suggested that If production and release of the transgene products from transgenic plants through different routes in soil exceed to its consumption/ biodegradation, may lead to their accumulation beyond threshold levels, which may have acute as well as chronic effect on soil ecosystem. Impacts of transgenic plants are also dependent upon spatial and temporal environmental variables. Whereas some of the studies observation suggests that transgenic plants don't have any negative impact on soil ecosystem. Keeping this status in background we prepared this manuscript. Our manuscript is divided in two parts, first part comprises review of the available literature on impacts of commercialized transgenic plants on soil ecosystem and its diversity, and in second part keeping above information as background, a framework is proposed for future comparative impact assessment of transgenic plants and its non transgenic isolate on soil ecosystem. In this approach each transgenic crop along with its non-transgenic isolate should be dealt separately according to its construct. The proposed approach is precautionary at each step, if there is any doubt at any stage they should be clarified by repetition of experiments. This approach will be helpful in filling of information gaps, which still exists in impact assessment studies of transgenic plants on soil ecosystem. This approach suggested monitoring should be carried out prior as well as post release of transgenic plants. Impact assessment of transgenic plants with respect to soil ecosystem should be made mandatory in current regulatory framework of transgenic crops throughout the world, to assure the use of transgenic technology without affecting the diversity and functioning of soil ecosystem.

Keywords: *Transgenic, soil, impact assessment, ecosystem*

Introduction

One key challenge for the twenty-first century is to provide food security to growing population of the world. This challenge has led agriculture sector towards gene revolution after green revolution with the help of advanced biotechnology. It has been proclaimed as third technological revolution following the industrial and computer revolution [1]. The gene revolution of agriculture involve understanding and modifying the organization of traits within the chromosomes of the species and conversion of traits of an organism by transferring individual genes from one species to another i.e. creation of transgenic.

First developed transgenic plants, in early 1980s were tobacco [30] and petunia [33] having trait for resistance to the antibiotic kanamycin. Since then, numbers of transgenic crops were developed to achieve novel and desired traits. The purpose of creation of transgenic plants are profound such as pest resistance, herbicide tolerance, long shelf life, phytoremediation, vaccine production, nutrient supplement and tolerance to biotic and abiotic stresses.

Number of countries cultivating transgenic crops commercially have increased from one in 1992 to twenty one in 2005. The estimated global area under transgenic crop cultivation till 2005 was approximately equivalent to 222 million acres [41]. The major traits harbouring transgenic crops were herbicide resistance (HT) with 72% of the total transgenic area followed by insect resistance (IR) with 19.5% and both HT and IR stacked in one crop occupied only 8.5%. Whilst, virus resistance and other traits engaged very little area <1%. The principal commercialised transgenic crops are soybean (48.4m ha), corn (19.3mha), cotton (9.0 mha), canola (4.3 mha) alfalfa and potato (<0.1mha), (James, 2005). Other non-commercialized but ready for trial transgenic crops are papaya, squash, rice, bringal, aubergine, sugarbeet and tomato. More crops under development in labs for being transgenic are apple, mango, banana, pineapple, barley, sweetpotato and coconut.

Transgenic plants contain foreign genes responsible for release of compounds, which help them in growth and establishment outside their natural habitat, with enhanced survival, persistence and competitive capabilities. Now a days several ecological concerns are arising with respect to the cultivation of the transgenic crops such as - invasiveness, gene flow to indigenous organisms, development of resistance in target pests, and direct or indirect effects on nontarget organisms and ecosystems [72, 73]. Among direct or indirect effects of transgenic plants, concerns about their impacts on soil ecosystem have been raised due to the chemical and biological properties of soil [54]. Soil materials have large sorptive capacities for biological molecules, including DNA and insecticidal bacterial proteins. There is increasing scientific data to show that soil can, in some cases, protect these molecules from biological degradation, enabling their toxic properties and genetic information to be retained in soil for unexpectedly long periods.

The impact of transgenic plants on non-target soil microorganisms will depend on the nature of the recombinant protein (i.e. its spectrum of activity) and the extent of exposure. Some transgenic plants (insect resistant) change its rhizosphere environment through root exudates, which consequently promote or retard the growth of microorganisms in the rhizosphere [54, 68]. Transgenic Bt cotton and Bt corn plants release Bt endotoxin into the soil from their different parts (roots, leaves etc), which persists in soil and retained its immunological and biological activity [64, 66]. A major problem in evaluating the impacts of transgenic crops on soil microbial diversity is the lack of baseline information on- diverse agroecosystems to compare with agroecosystems in which transgenic crops have been introduced [10, 17], and lack of universally approved approach for carrying out impact assessment of the transgenic plants on soil ecosystem. Consequently, it is important to review literature for the systematic understanding of risks of transgenic plants and their products observed till now on soil microbial diversity as well as to propose a framework based on approach which should be followed worldwide for impact assessment of transgenic crops on soil ecosystem at various regulatory stages prior to their release as well as after their commercialization.

Interaction between transgenic plants and soil microorganisms

Soils are among the most species rich habitats on the planet Life in soils includes vertebrates, macrofauna, micro- and mesofauna as well as algae, lichens, protozoa, fungi, bacteria and viruses (*Figure 1.*). Soil diversity is often several orders of magnitude greater than that of which occur aboveground [40]. Microbial diversity of soil play important role in maintaining its resiliency [55].

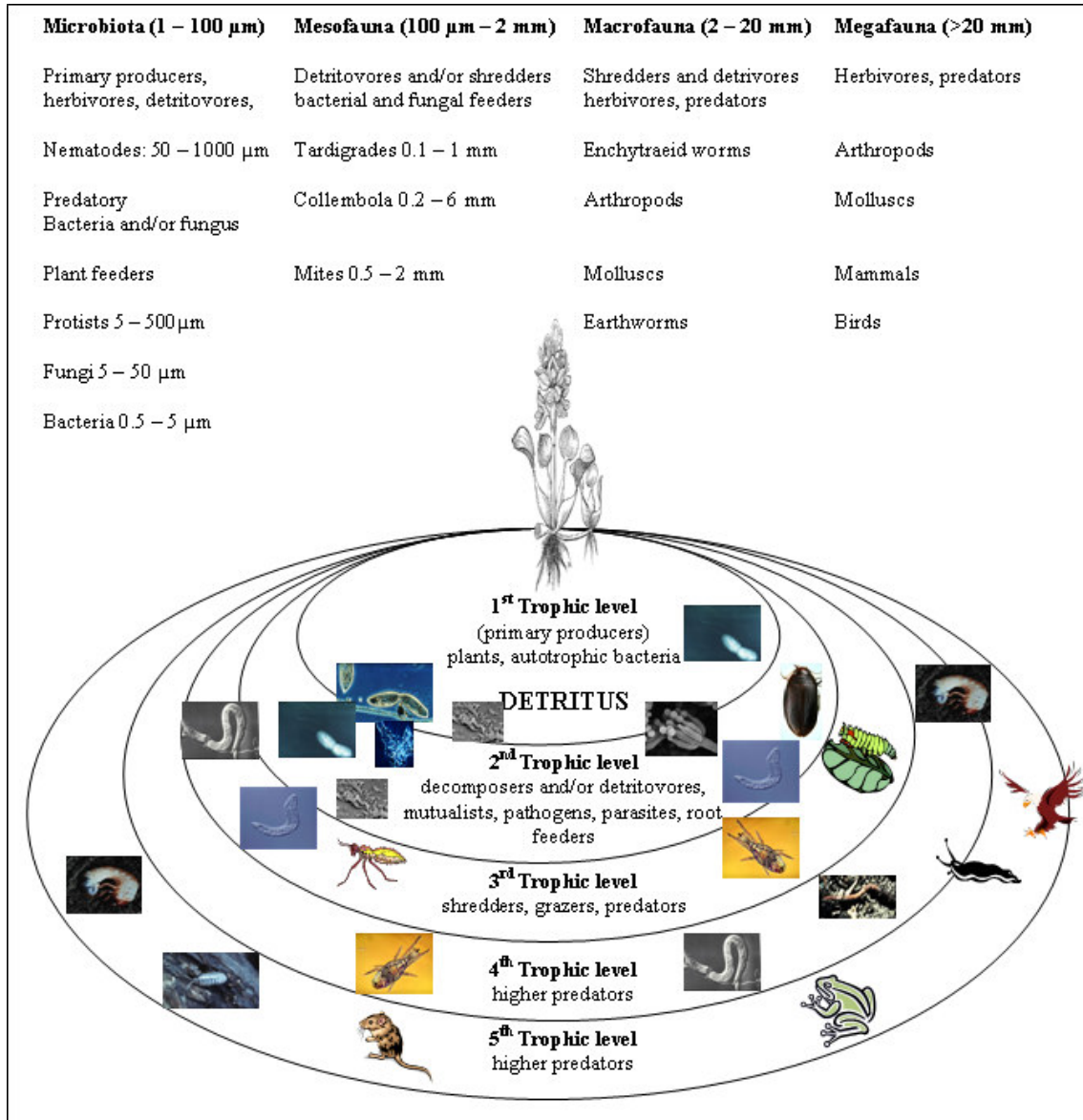


Figure 1. Soil food web showing an outline classification of the soil biota based on type, body size and trophic level. These components have variety of interaction ranging from the competitive or predatorial to the cooperative and symbiotic. Size class is based on the width of the organism according to Swift [78].

Biodiversity of soil provides it an insurance against environmental perturbations, because different species or communities respond differently to these fluctuations, leading to more stability of ecosystem properties [47].

Addressing the impact of transgenic plants on a soil ecosystem is an important issue but distinctions must be made between an impact on soil microbial diversity (the range of organisms the soil contains) and one on soil function (what the soil does?). Reduction in soil microbial diversity might not necessarily cause a decrease in soil function. Studies investigating the links between diversity and function have reported both- a strong role for diversity in soil function [80], and also that function can be maintained with just a relatively small number of species present [47]. The general consensus among researchers is that there is 'no predictable relationship between diversity and function' and that species richness is not a dominant factor in the overall functioning of soils [9].

Plants are the main drivers of soil ecosystem and soil provides spatially and temporally heterogeneous environment to plants and soil microbes. Plants influence soil through rhizodeposition (root exudates, sloughing of root cells and root turnover), plant litter, water, gas and nutrient exchange. Interaction between soil microorganisms and plant roots satisfy important nutrient requirements for both the plants and the associated microorganisms [12]. Interactions between transgenic plants and soil biota occur naturally as well as through anthropogenic activities.

Natural interactions

Naturally soil microorganisms interact with plant roots on the rhizoplane and within the rhizosphere[43]. In rhizosphere, plant roots have direct influence on the composition and density of soil microorganisms through rhizospheric effect. Normally microbial population is large and more active in rhizospheric zone as compared to bulk soil. Not only physical and chemical conditions are different in the rhizosphere, but plant roots also provide the majority of substrate for energy and biosynthesis of microorganisms in soil. Plant roots release carbohydrates, aminoacids, organic acids, lipids, hormones, vitamins, enzymes, mucilages, mucigel, and lysates into the rhizosphere as root exudates [43]. Plants can therefore through its rhizosphere have a profound effect on soil-borne microbial communities and processes; vice versa soil microorganisms affect plant growth and performance through their activities.

Naturally, transgenic plants according to introduced transgenic trait releases different transgene products (Bt toxin and T4 lysozyme) at different growth stages into the soil ecosystem actively via different routes such as- from the roots as root exudates, via leachates following plant injuries, from sloughing off root / root cap cells and through the decomposition of senescent leaves and left over transgenic plant biomass in field after final harvest [22]. It has been reported that Bt endotoxin released from root exudates (as well as from pollen and crop residues) binds rapidly to montmorillonite and kaolinite clay minerals, humic acids, and organomineral complexes [14, 15, 69, 79]. The soil bound toxin retains its activity and has been observed to persist in soil for up to 140 days [65] to 234 days [68]. Palm[65] observed a general pattern of rapid degradation of the toxin during the first 14 days of incubation followed by a slower rate of degradation over the total 140-days study period.

Anthropogenic intervention

Interaction between transgenic plants and soil biota is also influenced due to anthropogenic interventions. These anthropogenic interventions are man managed agriculture practices and includes- tillage activities, number of pesticide or herbicide and irrigation applications etc. Transgenic crops residue incorporated in soil through tillage practice, which during its biodegradation interact with soil biodiversity and influence it either positively or negatively. In zero-tillage practice, crop residues are left concentrated on the soil surface, which limits the soil microorganisms to come in contact with the protein at the soil surface. Whereas in conventional tillage, the plant litter incorporated into the soil, diluting the concentration of the transgene products but increasing the number of organisms exposed [6]. Zwahlen [86] also observed variation in long-term persistence of the Bt toxin from transgenic corn residues in field trials with respect to tillage practices. The presence of transgene products in soil may influence the structure and function of the microbes and their community by selectively stimulating or arresting the growth of organisms that can use them.

Effects of transgenic plants on structure and functions of soil microorganisms

The introduction of transgenic crops and their novel products in soil ecosystem may alter structure of soil microorganisms directly or indirectly. Most of the risk assessment studies on transgenic plants have tried to address the impact of commercialized transgenic plants on the structural alteration of soil microbial community as compared to its non-transgenic isolate. The observed and reported impacts of transgenic plants on structural pattern of its soil microbial community are listed in (*Table 1*). Donegan [20] examined the effects of decomposing transgenic cotton litter on structure of soil microbial communities and observed transient and significant increase in culturable aerobic bacteria and fungi in two out of three transgenic lines, which was attributed to unexpected changes in plant root exudates. Similarly, aerobic bacterial, fungal population and fungal species diversity in soil samples of Bt potato (*Solanum tuberosum* L.) differ significantly from non-Bt potato plants [21]. Soil from fields of transgenic alfalfa (herbicide resistant) has shown significantly higher population levels of culturable, aerobic spore-forming and cellulose-utilizing bacteria compared with that of the parental non-transgenic lines [23]. Lottmann and Berg [50] in Germany analysed rhizobacterial population of transgenic potato crop (expressing T4 lysozyme against the bacterial pathogen *Erwinia carotovora*) and nontransgenic potato and found that many bacterial and fungal species are sensitive to T4 lysozyme in vitro, but significantly high colony counts of T4-lysozyme tolerant *Pseudomonas putida* (potential biocontrol bacterial strains) were recovered from the transgenic plants than from control plants [50]. Donegan et al. [22] also found differences in population size of nematode and Collembola and earthworm in the soil surrounding the transgenic tobacco plant (expressing Protease Inhibitor I) litterbags. Whereas Saxena and Stotzky [69] observed no apparent effects of Bt toxin from corn added to soil on earthworms, nematodes, protozoa, bacterial and fungal population. However, they suggested that more detail research should be conducted to determine the effects of the toxin on soil biodiversity.

Table 1. Effect of transgenic plant on structure and functions of soil microorganisms and their communities

Plant	Transgenic trait	Effect on soil biota	Reference
Cotton	Insect resistance	Significant stimulation in growth of culturable bacteria and fungi with change in substrate utilization	[20]
Rape	Herbicide resistance	Community fatty acid, community level physiological profile altered, taxonomic diversity of root associated community altered, altered diversity of <i>Rhizobium leguminosarum</i> and variation in <i>Pseudomonas</i> population	[37, 74, 75]
Soybean	Herbicide resistance	Incidence of <i>Fusarium</i> (soilborne pathogen) on transgenic soybean roots was greater within 1 wk after the application of glyphosate as compared to non-transgenic isolate.	[45]
Tobacco	Insect resistance	Alteration in community size of nematodes and Collembola	[22]
Potato	Insect resistance	Altered CLPP pattern of microbial community in transgenic rhizosphere	[36]
Wheat	Pathogen resistance	Variation in cultivable rhizospheric community	[58, 59]
Alfalfa	Organic acid expression	Qualitative changes in the abundance of bacterial phylogenetic groups between rhizosphere soils of transgenic and untransformed alfalfa. rhizosphere of transgenic alfalfa had significantly greater microbial functional diversity compared with untransformed alfalfa.	[80]
Lotus	Opine production	Increased population of opines utilizing bacteria as compared to other bacterial species in the rhizosphere of transgenic lotus.	[61]
Potato	Expressing the phage T4 lysozyme gene	Transgenic potato plants root are showing high bactericidal activity against <i>Bacillus subtilis</i> adsorbed artificially on potato roots as compared to nontransgenic plants	[2]
Potato cv. Desiree and harbor	T4 lysozyme-producing plant lines DL4 and DL5	No difference in growth of bacterial communities was observed between the rhizosphere of transgenic potato and non-transgenic potato varieties.	[39]
Rice	Insect resistance	Differences in protease, neutral phosphatase and cellulase activities between soil amended with Bt-transgenic rice straw and non-transgenic rice straw were not persistent. However, differences in dehydrogenase activity, methanogenesis, hydrogen production and anaerobic respiration were persistent.	[85]
Maize	Insect resistance	No significant differences in earthworm, microarthropods, nematodes and protozoan population due to exudation of Bt toxin in rhizosphere of Bt corn as compared to non Bt corn.	[4, 69]
Tobacco	Expression of proteinase inhibitor I	Number of collembella colonies associated with transgenic tobacco litter are less as compared with non transgenic litter. Whereas nematode population is high in transgenic litter as compared to non-transgenic litter.	[22]

Plant	Transgenic trait	Effect on soil biota	Reference
Rape var. Quest	Herbicide resistance	rhizosphere and root interior microbial populations associated with a transgenic canola have altered CLPP and fatty acid methyl ester (FAME) profiles compared to the profiles of a nontransgenic counterpart.	[25]
Oilseed rape	Herbicide resistance	From root interior and rhizosphere fewer <i>Arthrobacter</i> , <i>Bacillus</i> , <i>Micrococcus</i> and <i>Variovorax</i> isolates, and more <i>Flavobacterium</i> and <i>Pseudomonas</i> isolates were found in the root interior of Quest compared to Excel or Parkland. The bacterial root-endophytic community of the transgenic cultivar, Quest exhibited a lower diversity compared to Excel or Parkland.	[75]
Maize	Herbicide resistance	No effect of transgenic maize was observed on genetic diversity of bacterial communities in rhizospheric samples.	[71]

Variations in response of soil diversity with respect to varieties and environment conditions of transgenic plant have been observed in few studies. Heuer [39] reported that the rhizosphere community structure associated with DL 4 line of transgenic potato was different than the community structure associated with a DL5 transgenic line and the control (DES). But contradictory to Heuer, Christopher and Jeffrey [13] observed rhizospheric and bulk soil samples of Bt corn for PLFA and bacterial and fungal CLPP profiles and found 73% and 6.3 -3.8% differences respectively of rhizospheric and bulk soil microbial community. But they found that expression of Cry endotoxin and varietal differences of transgenic corn don't have a significant effect on microbial profiles, except in the high-clay soil where both factors significantly affected bacterial CLPP profiles (accounting for 6.6 and 6.1% of the variability). The persistence as well as intensity of the impacts of transgenic plants on the structure of microbial community is dependent on the environment conditions of cultivation site [25, 26, 36]. Terminal restriction fragment length polymorphisms (T-RFLP) patterns of microbial communities associated with the rhizosphere of transgenic plants varies with spatial and temporal effects and spatial by temporal interactions [51]. Reports on retention time of transgenic products in soil are very limited. Head [38] reported that Cry1Ac protein was undetectable from soil samples of six fields that are under cultivation of Bt cotton from last 3-6 yrs and have incorporated Bt cotton plant residues by postharvest tillage. Whereas Oger et al. [62] observed that changes induced by transgenic plants in soil microbial community structure are persistent for a long time.

Above studies observations has shown variation in response of soil microorganisms to various transgenic plants due to different reasons. Spatial and temporal factors of cultivation site play an important role in determining the impact of transgenic plants on soil ecosystem. Any changes in soil due to introduction of transgenic plants should be crosschecked by replication of the experiment over the long duration. Very few reports are available on the effect of transgenic plants on invertebrate or protozoan population of soil. Experimental studies are urgently needed on the structural alterations of invertebrate or protozoan population of soils due to transgenic plants. Further research for probing non targeted traits of new transgenic crops and the mechanisms by which these traits may affect soil biological structure and processes, including changes in

composition of root exudates, is needed for better understanding of the potential impact of transgenic crops on soil ecosystem.

The major functions of soil microbes in soil ecosystem are to carry out energy flow and nutrient cycling. Energy flow always takes place from primary producers to primary, secondary and tertiary consumers. In soil ecosystem this flow will take place from plants to microorganisms (decomposers and saprophytes). The energy flow in soil ecosystem may be affected if transgenic plants will negatively or positively alter structure and composition of soil microbial community. Energy flow in any ecosystem is mediated through food chains or on larger scale through food webs. Positive or negative impact of transgenic plants on a single link of the soil food web will affect the energy flow. Reports on the energy dynamics in soil ecosystem with respect to transgenic plants are lacking. So this information gap should be filled by future research on transgenic plants and soil ecosystem.

The two major pools of soil nutrients are- the mineral (the clay fraction) and the organic matter. Organic matter part of soil nutrients is composed of decomposed plant and animal residue. Nutrient turnover from residues of transgenic crops as compared to non-transgenic isolines may vary because of the following reasons (i) alterations in the composition, quantity, and physical form of residues from transgenic crops; (ii) inhibition or stimulation of soil microbial communities involved in nutrient transformations, possibly through chemical compounds contained in the transgenic residues; and (iii) changes in management practices (e.g., tillage) for transgenic crops, which affect biotic and abiotic factors involved in decomposition and nutrient turnover of crop residues.

Differences in the composition of crop residues due to the introduction of a transgenic trait have been primarily observed in transgenic Bt crops. Masoero [52] reported that two transgenic Bt corn hybrids had higher starch, lignin and lower protein and soluble nitrogen contents compared with non-transgenic corn. Saxena and Stotzky [70] also observed 33 to 97% higher lignin content in 10 Bt corn hybrids, compared with their respective non-Bt isolines. In contrast, Escher [28] found low carbon:nitrogen ratio (C / N), lignin content and higher content of soluble carbohydrates in the leaves of one Bt corn variety compared with the corresponding non-transgenic corn variety. The effects of crop residue composition and the importance of several indices of residue quality, including the C/N ratio, lignin content, lignin / N ratio, initial N content, polyphenol content, polyphenol /N ratio, and initial soluble C concentrations of the residue, on decomposition and N mineralization have been extensively examined under both temperate and tropical conditions [29, 32]. In general, as the lignin or polyphenolic content / N ratio of the plant material increases it will slow down the decomposition rate of the crop residue and reduce short-term N availability [29]. Therefore, the lower or higher lignin content observed in some Bt crops may affect rate of residue decomposition and N mineralization of organic N contained in the residues. However, any reduction in the rate of N mineralization due to transgenic residues could offset normal nitrogen cycle of soil ecosystem.

Inhibition or stimulation of growth of soil microbial communities through chemical compounds released from the transgenic residues may affect their normal functioning as evidenced by changes in enzymatic activity. Wu and Min [85] observed protease, dehydrogenase, neutral phosphatase and cellulase soil enzymes activities in soil amended with Bt-transgenic rice straw and non-transgenic rice straw and reported high dehydrogenase activity in the transgenic straw amended soil compared to the control,

with the differences persisting over 80 days. Whereas the activity of dehydrogenase and alkaline phosphatase soil enzymes in transgenic alfalfa (herbicide tolerant) rhizosphere was low as compared to its nontransgenic isoline [23]. As phosphatase enzymes were involved in soil organic phosphorous mineralization and plant nutrition, and dehydrogenase enzyme play important role in biological oxidation of organic compounds and also reflect the total viable microbial population responsible for decomposition of organic matter [31]. Alterations in soil enzymes activity shows the indirect impact of transgenic plants on cycling of nutrient in soil.

Studies are very limited on the response of soil microbe's to transgenic plants under sustainable and conventional agricultural practices. Only few studies have reported impact of different tillage practice with respect to transgenic plants and their impact on soil ecosystem [6]. Sharon [77] observed the decomposition of the transgenic Bt cotton and glyphosate tolerance (roundup ready) cotton crop residue within agricultural systems under conventional-tillage (CT) and no-tillage (NT) management, and found that the mass loss with subsurface decomposition of transgenic cotton residue in the CT reached 55% but surface decomposition in the NT reached only 25%. Under CT more transgenic biomass will come in contact of soil microbial diversity, which may affect its structure and function. Whereas NT causes deposition of transgenic crop biomass on the soil surface prevents soil erosion. In addition, improved weed control with herbicide-tolerant crops has also stimulated a larger proportion of producers to adopt conservation tillage leaving more crop residues on the surface and potentially reducing soil erosion [3, 56]. Transgenic crops (herbicide tolerant) could support a reduction in tillage through direct drilling into a weedy field, which may be beneficial to soil organisms. But more detailed experiments are needed in future to understand impact of various agriculture practices of transgenic plants on functions of soil microbes.

Timms-Wilson [82] refer plants rhizosphere as an 'environmental hotspot' for gene transfer. The horizontal transfer of genes takes place in nature between organisms of different species (*Figure 2.*). It has been suggested that exchange of gene between transgenic plants and soil microorganisms in belowground ecosystem may take place, which can cause structural and functional alterations in soil microorganisms [7, 27]. The plant roots exudate DNA, so transgenic plant DNA is available to soil microorganisms. The persistence of plant DNA in the soil is dependent on abiotic and biotic factors, the content and type of clay minerals and the presence of DNAase enzyme in the soil [8, 83]. Through natural transformation method soil organisms may be transformed by free DNA, released from decomposing plant tissue and stabilized on soil particles. But for natural transformation to occur in a soil environment, free DNA and competent bacteria in the soil need to be in close vicinity [76]. Natural transformation is one of the methods that may allow the dispersal of foreign transgenes, such as antibiotic resistance markers, to native soil bacteria [59, 60,63]. Widmer [16, 83] quantified the antibiotic resistance marker gene persistence in the field, and reported that marker genes from tobacco (*Nicotiana tabacum* L.) and potato (*Solanum tuberosum* L.) were detectable in soil for 77 and 137 days, respectively. Gebhard and Smalla [35] in Germany also reported transfer of transgenic DNA from the transgenic sugar beet plant debris to bacteria in the soil. The kanamycin resistance marker gene was transferred to the soil bacterium *Acinetobacter* in an experiment using DNA that was extracted from homogenized plant leaf from a range of transgenic plants, including potato, tobacco, sugar beet, oil-seed rape and tomato [18].

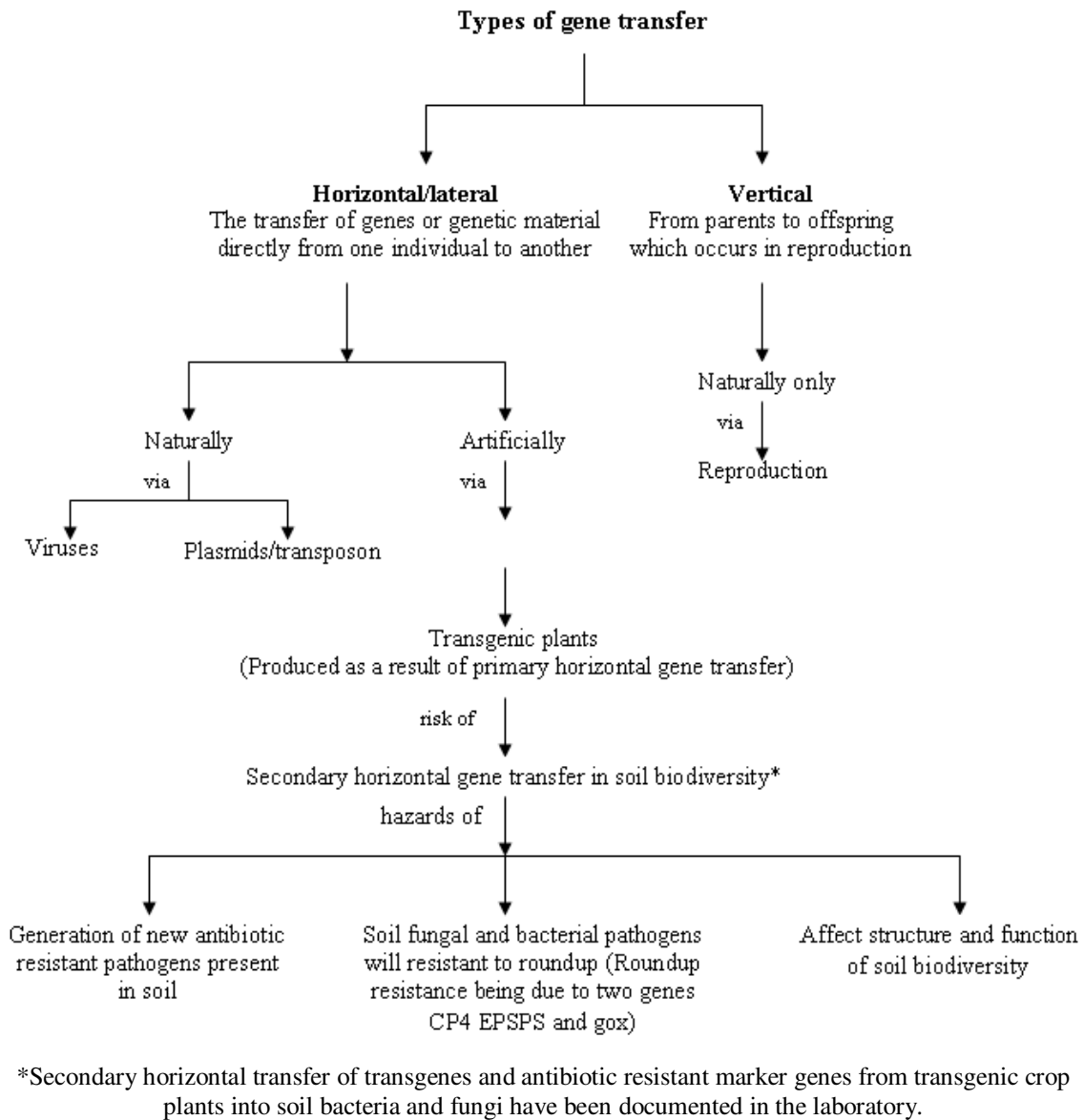


Figure 2. Gene transfer in soil ecosystem

Major issue of concern from gene exchange is transfer of antibiotic resistance genes to pathogenic microbes present in the soil, rendering them resistant to treatment with such antibiotics. Another potential complication might occur when uptake of *Bt* toxin genes (released from *Bt* crops in soil) by soil microbes. If this occurs, this might lead to more *Bt* toxin production in the soil, to an extent that may be harmful to soil invertebrates that contribute to soil fertility. Horizontal transfer of genes between soil microorganisms and transgenic plants may cause changes or disturbances in the functioning of the microorganisms that indirectly affect that soil ecology and fertility.

Future framework to assess impact of transgenic plants on soil ecosystem

After reviewing the reports of the studies carried out to assess impact of transgenic plants on soil ecosystem, we observe - (i) results of different studies contradict each other (ii) major gaps in information and baseline data on interactions of transgenic and living and nonliving components of soil still exist. All this at last leads us towards a state of confusion only, which is due to lack of universal schematic approach based framework for assessing the ecological risks and benefits of transgenic plants on soil. The universal framework is imperative and must be based on holistic approach, which will directly reflect response of soil ecosystems to transgenic plants. Transgenic plants impact-monitoring concepts needs to integrate available impact assessment data, environment monitoring networks baseline data, threshold values for impacts with future impact assessment studies on transgenic plants. Future impact assessment studied must be long term and should be carried out prior as well as post release of transgenic crops. As long term monitoring only truly represents slowly changing variables such as biodiversity or accumulation of transgenic crops product in soil. In the integrated framework changes in different soil ecological variables (physical, chemical and biological) due to transgenic plants should be prioritized. As integrated prioritization of impact assessment approach is not only critical in creating reliable monitoring data, the methodology also helps in fast decision making at advance stages of monitoring. The components of future impact assessment framework should be –

(i) Monitoring of retention time or half life of transgenic crops products in soil, if the retention time of transgenic products in soil is very less than they don't have any affect on soil and its diversity, however if retention time is high its its negative or positive impacts should be monitored in different agro climatic zones soil ecosystem.

(ii) Monitoring of changes in spatially and temporally distributed soil ecological variables due to transgenic crops

(iii) Integrated agro-environmental and transgenic plants monitoring network

(iv) Within transgenic plants monitoring network, Inhibition or stimulation of activity of specific microorganism or its community due to differences in the amount and composition of root exudates released from transgenic plants should be observed in different agro environments

(v) Impact of various agriculture practices of transgenic crops agro ecosystems on soil diversity and processes and,

(vi) Monitoring of horizontal gene transfer between transgenic crop and soil diversity at commercialized transgenic crops cultivation sites field

(vii) Changes in functions of microbial populations of soil community due to rare events i.e.,horizontal gene transfer.

Capacity of soil to carry out all its functions sustainably is defined as soil quality [42]. Quality of soil is assessed through the physical, chemical and biological parameters of soil. The physical parameters are -bulk density, texture, infiltration, water holding capacity, aggregation, top soil depth, chemical parameters includes - pH, Organic matter content, nitrogen content in different forms, salinity, nutrient availability and biological parameters are - microbial biomass, shift in trophic level and diversity, soil communities and soil respiration rate etc. But till now integrated studies on impact of transgenic plants on all soil quality indicators are almost lacking. In Future impact assessment studies for assessing the impact of transgenic plants on soil quality of

diverse agro ecosystems various soil parameters should be monitored to extract real picture.

Any alteration in structure and function of biological representative of soil i.e., either a single species or whole community of a species in transgenic agro ecosystem will represent impact of transgenic crops. Important functions and fertility of good soils are regulated by some dominant microbial species. The focus of the future studied should be on monitoring structure and function of microorganisms or their communities, whose demise is expected to result in the loss of a particular soil function and are therefore indicative of any positive or negative impact of transgenic crops on the soil ecosystem [10, 44]. The impact of transgenic plants on soil can also be observed by monitoring biological baseline components such as keystone species along with abiotic indicators as these exhibit high variability in their functioning within different soils. In addition, the specific ecosystem should be considered and the study should be performed to account for impacts on the key players in that ecosystem. This would mean that if *Pseudomonas* species, for example, were particularly important in a specific ecosystem then their populations and processes mediated by them would be evaluated for that specific ecosystem over the course of the study. Selection of keystone indicators should be done on the basis of their agronomic relevance, ecological significance and responsiveness to perturbations, plus the availability of practical assay methods.

It is recognized that, in addition to the use of keystone indicators, there is a need for broader analyses of the impacts on the soil microbial and faunal communities to improve sensitivity and, importantly, to improve detection of unforeseen effects [46]. Such monitoring requires a combination of measures, each responding to different types of change, for example monitoring the biomass, activity and diversity of the microbial and faunal communities. Monitoring of community size of soil microorganisms such as nematode and collembola, substrate utilization pattern as well as composition of rhizosphere bacterial communities and the metabolic fingerprints of the microbial community in the transgenic crops fields soil will also indicate impacts of transgenic plants on soil ecosystem and will helps us in deciding whether transgenic plants are really an option for sustainable agriculture practices or they itself affecting the sustainability of soil ecosystem

Agriculture practices especially tillage and irrigation plays an important role in enhancing or inhibiting any adverse or positive effect of plants on soil microorganisms. In future studies various agriculture management practices associated with transgenic crops should also be monitored for any influence on the activity of soil microbial communities. As agriculture practices can control the exposure or contact of soil microbial communities with transgenic crop biomass. Horizontal gene transfer between transgenic crop and native soil biodiversity of an agroecosystem is also an important aspect which require regular monitoring through modern techniques

A framework (*Fig. 3*) based on above discussed points will redefine the transgenic plants impact assessment process and ensure more reliable results. This will also help researchers in thorough understanding of impact of transgenic plants on structure and function of soil microbes. Since only a small portion of soil microbial populations can be cultured and identified using standard analytical methods. Combining techniques, especially different types of methods, will in almost all cases lead to more accurate and well-balanced view of the soil system (*Table 2*).

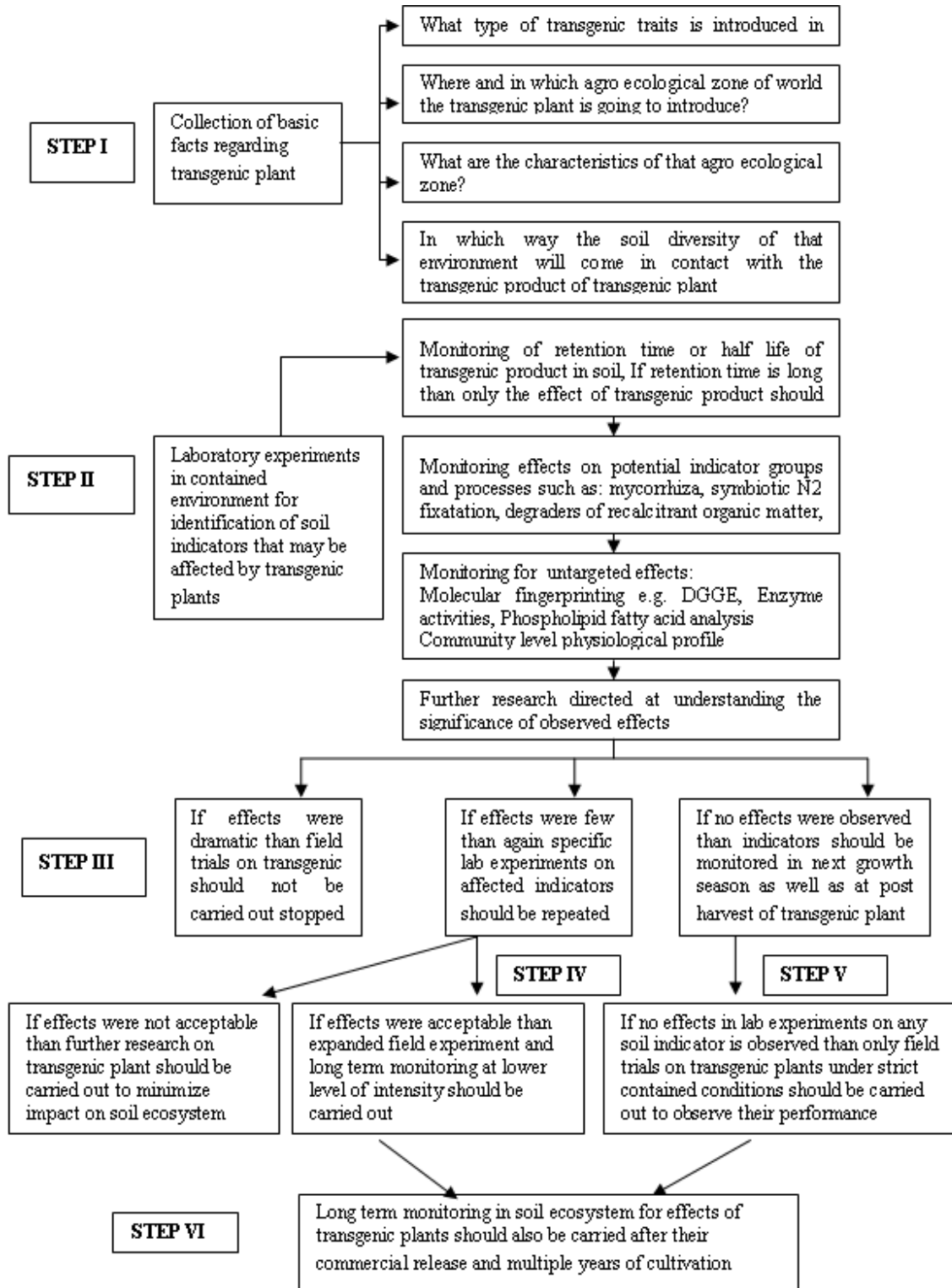


Figure 3. Schematic approach outline for impact assessment of transgenic plants on soil ecosystem and functions

Table 2. Few examples of horizontal gene transfer in soil ecosystem

Type of study	Transgenic plants	Gene for transgenic trait	Transferred to	Reference
Lab experiment	Potato, tobacco, canola and tomato	nptII (kanamycin resistance) genes	<i>Acinetobacter</i> Soil bacteria	[18]
Lab experiment	Sugar beet	(kanamycin resistance) genes	<i>Acinetobacter</i> Soil bacteria	[34]
Lab experiment	Maize	bla gene encoding TEM-1 L-lactamase	<i>E.coli</i> bacteria in the saliva and rumen fluid of adult sheeps	[24]

Conclusion

After reviewing the literature on interactions of transgenic plants with soil components we understood that, though lab and field reports on such interactions are very limited, but they have showed that the transgenic plants and their transgene products directly or indirectly leaving their footprints on soil ecosystem which in long term may affects the structure and functioning of soil ecosystem. However due to transient behaviour of impacts and lack of systematic integrated experimental approach in above discussed studies it is very difficult to establish that those changes in soil ecology are solely due to transgenic plants. Because of the exponentially increasing land area under transgenic crops cultivation in last few years, long-term risk assessment studies based on integrated approach are urgently needed in diverse agro-ecological zones of world for filling information gap as well as to establish the net direct and indirect effects of transgenic crops and their associated management practices on soil ecosystem and biodiversity. Environment plays an important role in creating variability in impacts of transgenic plants on soil ecosystem. So, in integrated approach framework environmental monitoring component should be incorporated to generate baseline data on impacts of transgenic plants on different dimensions of soil ecology from various agro ecological zones of world where commercial cultivation of transgenic crops has been allowed. As some studies have shown that transgenic plants may affect biogeochemical cycling in soil ecosystem in long run, which may affect nutrient cycling in hydrosphere and atmosphere also, as all are interlinked. Transgenic crops have immense potential to provide economic and environmental benefit, but few reports which have shown negative impacts of transgenic plants on soil ecosystem have created a doubt on benefits of transgenic crops. So to clarify those doubts and to say anything conclusively, more long term experimental studies which should monitor each and every aspect of soil ecology are urgently needed in each and every nation which have allowed commercial cultivation of transgenic crops

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