

Impact of Genetically Modified Crops and Their Management on Soil Microbially Mediated Plant Nutrient Transformations

P. P. Motavalli,* R. J. Kremer, M. Fang, and N. E. Means

ABSTRACT

One of the potential environmental effects of the recent rapid increase in the global agricultural area cultivated with transgenic crops is a change in soil microbially mediated processes and functions. Among the many essential functions of soil biota are soil organic matter decomposition, nutrient mineralization and immobilization, oxidation–reduction reactions, biological N fixation, and solubilization. However, relatively little research has examined the direct and indirect effects of transgenic crops and their management on microbially mediated nutrient transformations in soils. The objectives of this paper are to review the available literature related to the environmental effects of transgenic crops and their management on soil microbially mediated nutrient transformations, and to consider soil properties and climatic factors that may affect the impact of transgenic crops on these processes. Targeted genetic traits for improved plant nutrition include greater plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic P, and increased assimilation of soil N. Among the potential direct effects of transgenic crops and their management are changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as pesticide applications, tillage, and application of inorganic and organic fertilizer sources. Possible indirect effects of transgenic crops, including changes in the fate of transgenic crop residues and alterations in land use and rates of soil erosion, deserve further study. Despite widespread public concern, no conclusive evidence has yet been presented that currently released transgenic crops, including both herbicide and pest resistant crops, are causing significant direct effects on stimulating or suppressing soil nutrient transformations in field environments. Further consideration of the effects of a wide range of soil properties, including the amount of clay and its mineralogy, pH, soil structure, and soil organic matter, and variations in climatic conditions, under which transgenic crops may be grown, is needed in evaluating the impact of transgenic crops on soil nutrient transformations. Future environmental evaluation of the impact of the diverse transgenic crops under development could lead to an improved understanding of soil biological functions and processes.

THE RECENT RAPID GROWTH in the global agricultural area cultivated with transgenic crops, genetically engineered to have either or both herbicide and pest resistance, has raised public concerns over the potential impact of these crops and their management on the environment (Hoffman, 1990; Grogan and Long, 2000; Nottingham, 2002). In 2001, 52.5 million ha of biotechnology-derived crops were planted worldwide and dur-

ing that year 46% of the world's total soybean [*Glycine max* (L.) Merr.] area, 7% of the corn (*Zea mays* L.) area, and 20% of the cotton (*Gossypium hirsutum* L.) area were planted to biotechnology-derived seed (Council for Agricultural Science and Technology, 2002). Possible direct environmental hazards associated with the release of transgenic crops include movement of transgenes in the environment, escape of the whole plant, effects on nontarget organisms, and resistance evolution (National Research Council, 2002). Changes in soil and crop management over large land areas due to widespread cultivation of transgenic crops, such as changes in pesticide use and tillage practices, could also have both positive and negative environmental effects (Council for Agricultural Science and Technology, 2002; Nottingham, 2002).

Although some research has examined the environmental impacts of the “aboveground” portion of transgenic crops, relatively less research effort has examined the effects of these crops on soil microbially mediated processes and functions in soils (O’Callaghan and Glare, 2001; Bruinsma et al., 2003). Among the many essential functions of soil biota are soil organic matter decomposition and nutrient mineralization and immobilization. Other microbially mediated processes in soil related to nutrient cycling include oxidation–reduction reactions (e.g., nitrification and denitrification), biological N fixation, and solubilization (e.g., solubilization of soil P).

Among the many challenges facing researchers examining the environmental effects of transgenic crops on biogeochemical cycles is the potential dependence of expression of those effects on factors such as climate, soil chemical and physical properties, crop variety, and time (Trevors et al., 1994). A major problem in evaluating the environmental effects of transgenic crops is the lack of sufficient long-term baseline information on the variation in soil microbially mediated nutrient cycling in diverse agroecosystems to compare with agroecosystems in which transgenic crops have been introduced (Dale et al., 2002; Bruinsma et al., 2003). In addition, knowledge of the complex diversity of soil microorganisms is limited since only a small portion of soil microbial populations can be cultured and identified using standard analytical methods, although some new methodological approaches, including use of molecular biological techniques, show some promise in helping to understand the impact of transgenic crops on soil microbial ecology (Angle, 1994; Bruinsma et al., 2003).

The introduction of transgenic crops expressing multiple traits (“stacked” genes) may further complicate determination of environmental impacts due to potential

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interactions among the inserted genes (National Research Council, 2002). An additional challenge will be to evaluate the potential environmental impacts of the wide range of transgenic crops under development. These new transgenic crops include plants containing modifications for improved resistance to stress conditions, plants with better nutritional qualities and vitamins, plants with enhanced N fixation and photosynthesis, and plants that produce industrial products or precursors, such as bioplastics, pharmaceutical proteins, and vaccines (Nottingham, 2002).

The objectives of this paper are to (i) review the available literature related to the environmental effects of transgenic crops and their management on soil microbially mediated nutrient transformations and (ii) consider soil properties and climatic factors that may affect the impact of transgenic crops on nutrient transformations. Although the current literature on the issue of the environmental impact of transgenic crops on soil nutrient transformations is not extensive, a review at this time may facilitate the determination of future priorities for research.

TARGETED TRANSGENIC TRAITS AFFECTING PLANT NUTRIENT ACQUISITION

The recognition that abiotic stress, such as nutrient deficiency, is a major factor limiting crop performance under different environmental conditions and agricultural management practices has led to efforts to develop transgenic crops with improved traits to acquire nutrients from soil (Hirsch and Sussman, 1999). Examples of targeted traits that have been researched include improved plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic P, and increased assimilation of soil N (Table 1).

Improvements in plant nutrient acquisition have also been observed in transgenic crops, which have traits targeted for overcoming other abiotic stresses, such as soil salinity and drought tolerance. Removal of plant growth limitations may be expected to increase plant utilization of soil nutrients since nutrient demand would be higher in healthier plants, but additional changes in plant physiological characteristics due to the introduction of novel traits may also affect nutrient acquisition. For example, Garg et al. (2002) observed higher Fe content in drought- and salt-tolerant transgenic rice developed to accumulate higher levels of trehalose, a non-reducing disaccharide of glucose.

The mechanisms by which these transgenic crops may overcome nutrient deficiencies, such as increased root exudation of organic acids, may directly or indirectly affect soil microbially mediated nutrient transformations. For example, the decrease in rhizosphere soil pH due to increased root exudation of organic acids may reduce the rate of microbially mediated processes sensitive to acidic pH (e.g., nitrification). However, the presence of organic anions in soil can also stimulate microbial activity and increase nutrient availability (Ryan et al., 2001). More subtle and complex environmental effects may occur to soil microbially mediated processes when previously marginal lands are converted to cultivation of stress-tolerant transgenic crops with concomitant changes in soil management (National Research Council, 2002).

POTENTIAL NONTARGETED DIRECT AND INDIRECT EFFECTS

The impact of the introduction of transgenic crops and accompanying changes in management practices on soil microbially mediated nutrient transformations may occur through several unintended (nontargeted) direct and indirect effects that may be mediated by variation in climate and soil properties (Fig. 1). Among the potential direct effects are changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as pesticide applications, tillage, and application of inorganic and organic fertilizer sources.

The primary indirect effect of transgenic crops on soil microbially mediated processes that has been studied has been the effects of changes in the amount and composition of crop residues from transgenic crops. A simplified example of the possible indirect effects of transgenic Bt corn (transgenic corn engineered to express *Bacillus thuringiensis* toxin) residues on nutrient transformations in soil is illustrated in Fig. 2. Reductions in corn borer damage or differences in the composition of Bt corn residues may increase the amount of undamaged, low N-containing residues remaining on or in soil after harvest, thereby possibly reducing the rate of decomposition and nutrient mineralization. Determination of the overall impact of increased crop residues from transgenic Bt corn on microbially mediated nutrient processes is complicated by the possible reduction

Table 1. Examples of targeted transgenic traits for improved plant nutrient acquisition from soil.

Trait	Mode of action	Crop	Reference
Tolerance to low Fe availability in alkaline soils	root exudation of mugineic acid phytosiderophores	rice (<i>Oryza sativa</i> L.), sorghum [<i>Sorghum bicolor</i> (L.) Moench], corn (<i>Zea mays</i> L.)	Takahashi et al. (2001)
Tolerance to low P availability in acidic and alkaline soils	root exudation of organic acids (e.g., citric and malic acids) to solubilize soil P root exudation of phytase extracellular enzyme to utilize organic P in soil	corn and other monocots -	López-Bucio et al. (2000) Richardson et al. (2001)
Improved assimilation of soil N	increased activity of NADH-glutamase synthase in plant	tobacco (<i>Nicotiana tabacum</i> L.)	Chichkova et al. (2001)

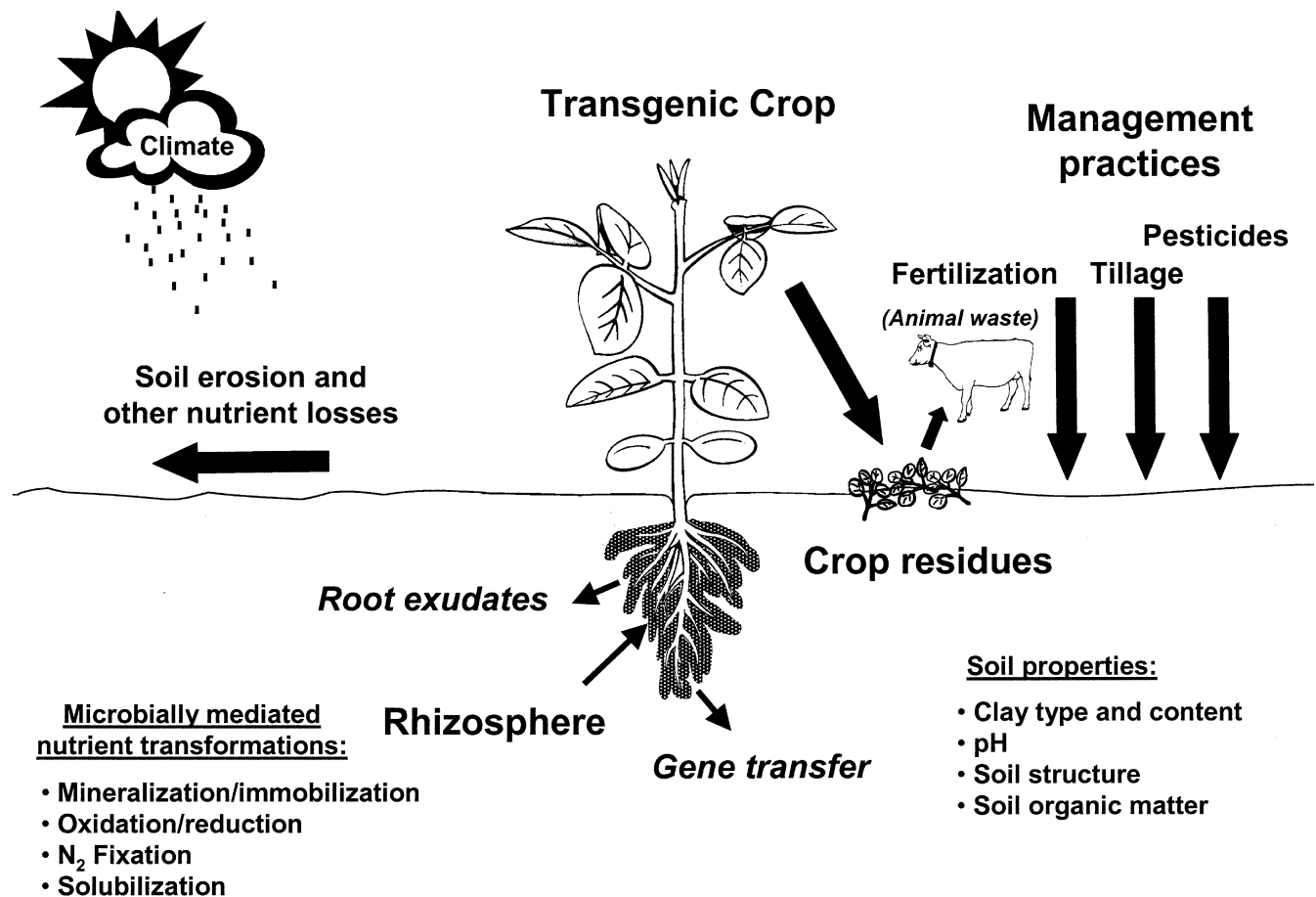


Fig. 1. Potential nontargeted direct and indirect effects of transgenic crops on microbially mediated soil nutrient transformations.

in soil erosion that occurs with greater Bt corn residues left on the soil surface. Decreased loss of topsoil relatively higher in soil organic C compared with subsoil would maintain or enhance soil microbial activity. Boyle et al. (2001) also provide an example of the possible indirect ecological effects of the introduction of transgenic disease-resistant trees on mycorrhizae and other soil biota important in decomposition and nutrient cycling in forest soils.

Root Exudates

The main interaction zone between the plant and soil biota is at or near the root surface in the rhizosphere (Kennedy, 1998). Both plant roots and soil microorganisms alter and are affected by soil chemical and physical properties in the rhizosphere (see Fig. 3 for an illustration of the effects of this relationship on soil P availability). In the example of factors affecting soil P availability, root exudates, such as organic acids, H⁺ ions, sugars, and phosphatases, facilitate the solubilization and desorption of mineral P as well as affect the activity of rhizosphere microorganisms (Ryan et al., 2001) (Fig. 3). Alterations in the composition and quantity of root exudates through the introduction of new genetic traits may, therefore, directly affect processes, such as mineral P solubilization, or indirectly affect P availability through changes in the activity of rhizosphere microorganisms.

Plant roots excrete several substances into the rhizosphere soil, including exudates, secretions, mucilages, mucigel, and lysates (Kennedy, 1998). For the purposes of this paper, these substances will be collectively termed as root exudates.

Evidence that root exudates can promote or retard the growth of rhizosphere microorganisms can be observed in the study of transgenic plants that change the rhizosphere environment for the possible development of biopesticides or plant growth enhancers (Salmeron and Vernooij, 1998; Mansouri et al., 2002). One example of this type of research is the development of transgenic bird's-foot trefoil (*Lotus corniculatus* L.) and black nightshade (*Solanum nigrum* L.) plants that produce opines, a root exudate that favors opine-degrading rhizobacteria (Mansouri et al., 2002). This transgenic trait to produce a bacterial growth substrate to select for specific rhizosphere microbes utilizing the substrate was not affected by the limited number of soil types and plant species tested and remained constant over the 18-wk observation period (Mansouri et al., 2002).

A well-documented nontarget effect of root exudates has been with transgenic crops that incorporate the *CryIAb* gene from the bacterium *Bacillus thuringiensis* that codes for the production of an insecticidal protein that kills Lepidopteran pests (Schnepf et al., 1998; Donegan and Seidler, 1999; Saxena et al., 1999). The insecti-

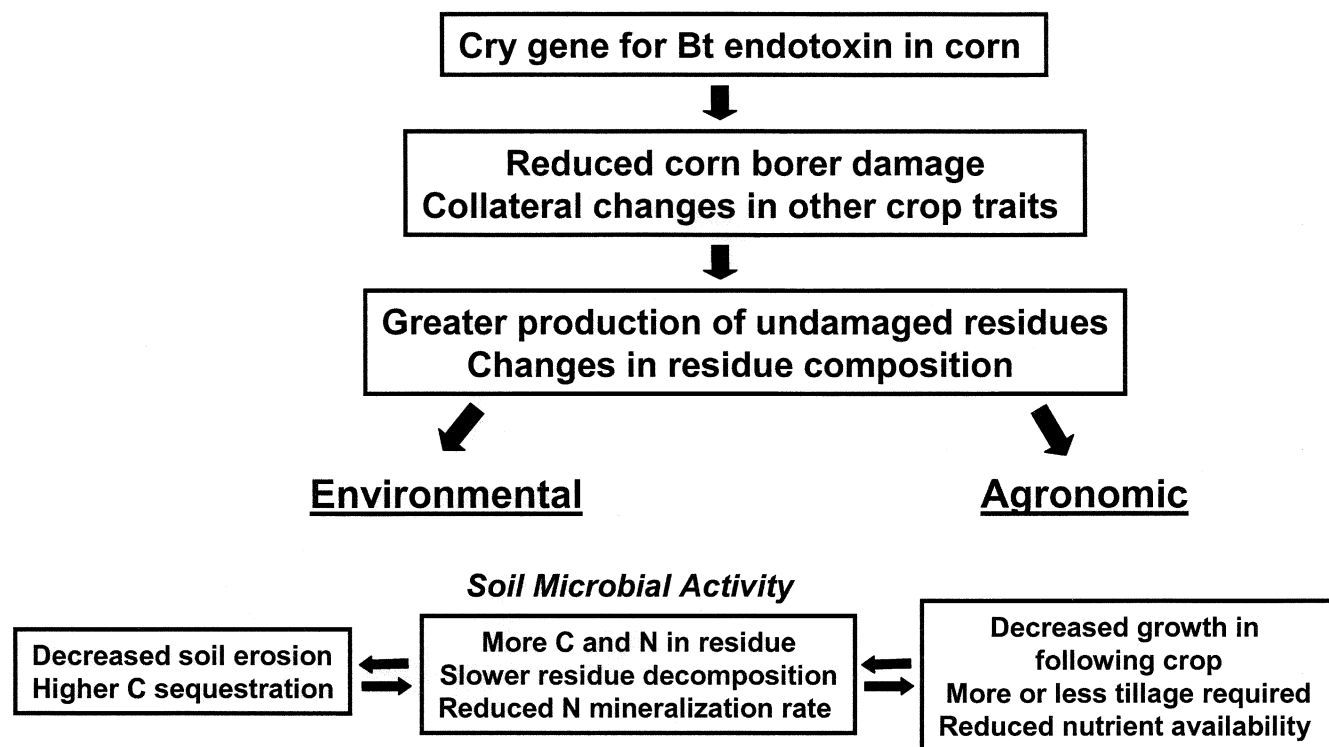


Fig. 2. A flow diagram illustrating the possible indirect effects of transgenic Bt corn (i.e., corn engineered to express *Bacillus thuringiensis* toxin) residues on soil nutrient transformations.

cidal toxin in root exudates (as well as pollen and crop residues) produced by Bt corn binds rapidly to montmorillonite and kaolinite clay minerals, humic acids, and organomineral complexes, which protect the toxin from microbial degradation (Tapp et al., 1994; Crecchio and Stotzky, 1998, 2001; Saxena et al., 2002). The bound toxin retains its insecticidal activity and has been observed to persist in soil for up to 234 d (Saxena et al., 1999). Similar results on the persistence of the insecticidal toxin of Bt cotton but for shorter periods of time (up to 140 d) have also been reported by Palm et al. (1996). That study observed a general pattern of rapid degradation of the toxin during the first 14 d of incubation followed by a slower rate of degradation over the total 140-d study period. Zwahlen et al. (2003) observed some variation in long-term persistence of the Bt toxin derived from degradation of corn residues in field trials depending on tillage practice.

The presence of the insecticidal toxin from Bt crops either through root exudates or by incorporation of residues of Bt crops has not generally been shown to have a significant effect on soil biological populations, which may affect soil nutrient transformations. Saxena and Stotzky (2001a) observed no apparent effects of Bt toxin from corn added to soil on earthworms, nematodes, protozoa, bacteria, and fungi. However, they suggested that more detailed research needs to be conducted to determine the effects of the toxin on soil biodiversity (Saxena and Stotzky, 2001a). Another study reported no direct effects of purified Bt toxin and Bt toxin contained in transgenic Bt cotton on soil microorganisms, but observed a transient but significant in-

crease in culturable aerobic bacteria and fungi with two of three transgenic cotton lines, which was attributed to unexpected changes in plant characteristics as a result of genetic manipulation or tissue culture (Donegan et al., 1995). A survey of levels of *CryIAC* protein levels contained in soil samples of six fields grown to continuous Bt cotton for 3 to 6 yr indicated that no detectable *CryIAC* protein or biological insecticidal activity was present in any of the fields despite incorporation of the transgenic Bt cotton plant residues into the soil by postharvest tillage (Head et al., 2002). Similarly, aerobic bacterial and fungal populations, fungal species diversity, and abundance in soil planted to transgenic Bt potato (*Solanum tuberosum* L.) did not differ significantly from soil planted with non-transgenic potato plants (Donegan et al., 1996). The specificity of the Cry proteins for target pests, the small amount of the protein contained in plants, and its rapid degradation in soil reduces the risk of environmental impacts from cultivation of Bt crops (Betz et al., 2000).

Some evidence exists that transgenic plants currently under development may affect soil nutrient transformations, but whether root exudates or other nontargeted physiological changes in the plant are the mechanisms for these effects is unclear. Soil from field plots in which lignin peroxidase-producing transgenic alfalfa (*Medicago sativa* L.) was grown over two years had significantly higher population levels of culturable, aerobic spore-forming and cellulose-utilizing bacteria, lower activity of the soil enzymes dehydrogenase and alkaline phosphatase, and higher soil pH levels compared with that of the parental non-transgenic lines (Donegan and

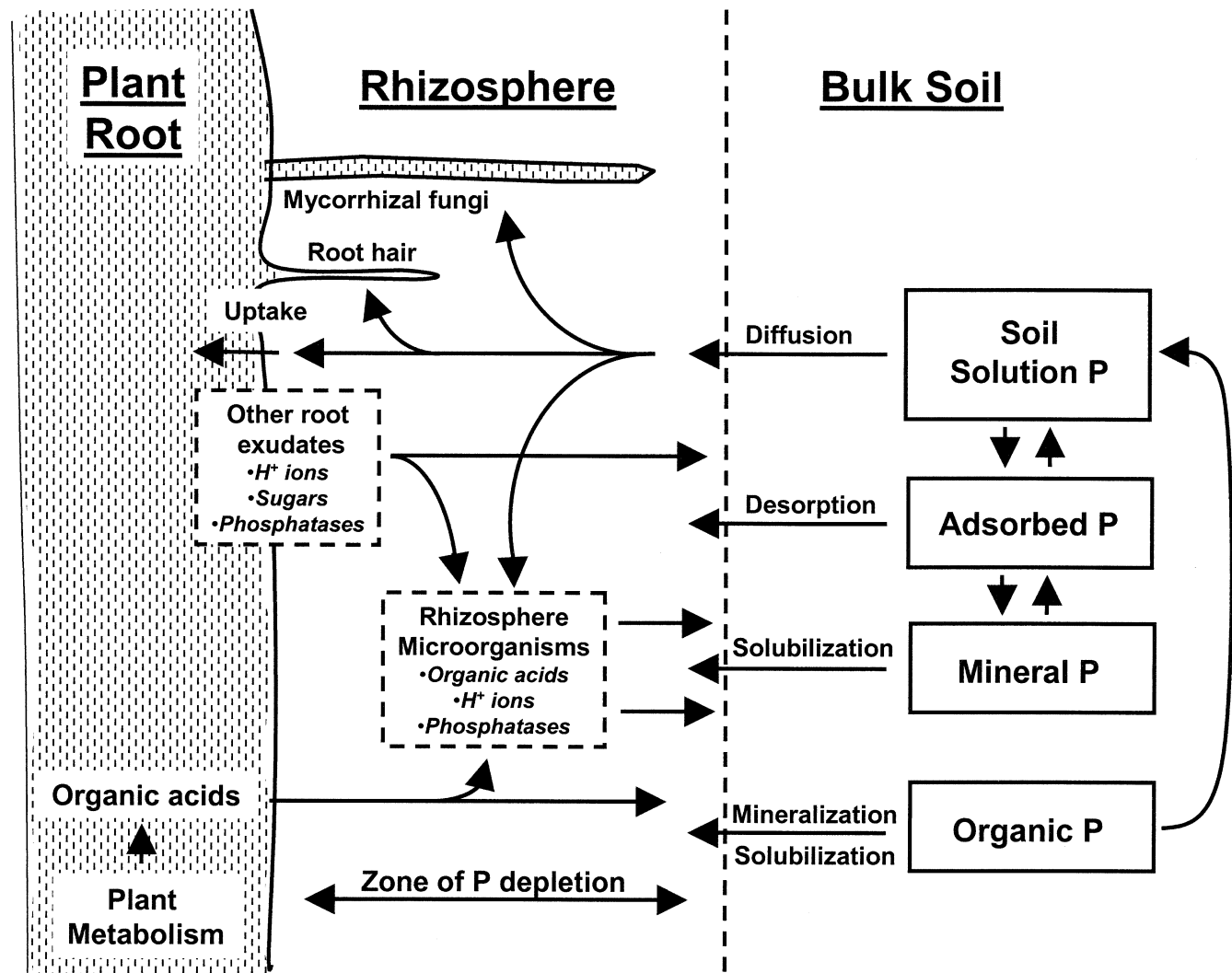


Fig. 3. Processes influencing soil P availability in the soil rhizosphere (adapted from Ryan et al., 2001).

Seidler, 1999). However, population levels of protozoa, nematodes, and microarthropods, DNA fingerprints of indigenous bacteria, and rates of microbial substrate-induced respiration were not significantly affected by the growth of the transgenic alfalfa (Donegan and Seidler, 1999). The study also observed that the transgenic alfalfa had significantly lower shoot weight and higher tissue N and P contents compared with the parental non-transgenic plants. Further research examining nontargeted traits in transgenic crops under development and the mechanisms by which these traits may affect soil biologically mediated processes, including changes in root exudates, is needed to better understand the potential impact of these introduced crops on soil biological functions.

Herbicide Applications

One of the major changes in crop management practices that has occurred with the introduction of transgenic crops with herbicide resistance has been the widespread application of glyphosate [commonly applied as the isopropylamine salt of *N*-(phosphonomethyl) gly-

cine]. In 2001, glyphosate-resistant soybean accounted for 46% (33.3 million ha) of the total area in the world planted to soybeans (Council for Agricultural Science and Technology, 2002). In the United States, glyphosate-treated land constituted 62% of the total area planted to soybeans in 2000 (Council for Agricultural Science and Technology, 2002).

An extensive review of the ecotoxicological risks associated with use of glyphosate and its formulations, including the herbicide Roundup (Monsanto, St. Louis, MO), is presented in Giesy et al. (2000). Among the factors affecting the toxicity of glyphosate and Roundup to soil microorganisms and invertebrates are the rate of glyphosate applied, soil properties such as soil texture and organic matter content, and whether the testing was done in artificial substrates or natural soil ecosystems (Giesy et al., 2000).

One potential effect of the application of glyphosate-based herbicides to soil is the stimulation or inhibition of soil microbially mediated nutrient transformations and related processes (Table 2). Toxic effects of glyphosate on soil microorganisms may occur because the

Table 2. Effects of glyphosate on soil nutrient transformations cited in research literature.

Effect of glyphosate	Mode of action	Soil or medium characterization	Reference
Increased C and N mineralization and soil microbial biomass C and N	glyphosate contains organic C and N	Weswood silt loam (fine-silty, mixed, superactive, thermic Udifluventic Haplusteps); nine soils from Georgia and Texas	Haney et al. (2000, 2002)
Decreased growth of ectomycorrhizal fungi	direct antagonism	growth medium	Chakravarty and Chatarpaul (1990), Estok et al. (1989)
Delayed N ₂ fixation and lower N accumulation in soybean, especially when soil water content low; effect is rate-dependent and may not affect plant growth in the field	direct antagonism of glyphosate on symbiont, <i>Bradyrhizobium japonicum</i>	potting media, Bosket sandy loam (fine-loamy, mixed, active, thermic Mollic Hapludalfs)	King et al. (2001), Reddy et al. (2000)
Inhibited nodule development in field-grown glyphosate-resistant soybean	glyphosate accumulates in nodules	Dundee silt loam (fine-silty, mixed, active, thermic Typic Endoaqualfs)	Reddy and Zablotowicz (2003)
Toxic to bacteria and fungi in growth medium but no detectable effects when grown in soil	direct or indirect antagonism; inactivation of glyphosate in soil; variability due to effects of climate, substrate availability, and management	growth medium; three soils from pine plantations in northern California	Busse et al. (2001)

enzyme 5-enolpyruvylshikamate-3-phosphate synthase (EPSPS), which glyphosate inhibits, is in both plants and soil microorganisms (Busse et al., 2001). However, Haney et al. (2000, 2002) observed significantly higher C and N mineralization when Roundup Ultra was added to nine soils from Georgia and Texas with a range in soil pH, soil organic C, and clay content. The difference in C and N mineralization with added herbicide was directly attributed to the low C to N ratio of Roundup Ultra. They also observed higher rates of C mineralization of added Roundup Ultra in soils with higher organic C, which they attributed to the larger initial microbial biomass in these soils. However, differences in soil organic C, pH, and clay content among the soils in the study did not relate to the potential rate of C mineralization of the added Roundup Ultra.

In contrast, Busse et al. (2001) found no differences in soil respiration when glyphosate was added at expected field application rates (5–50 $\mu\text{g g}^{-1}$) to three pine plantation soils with a range in soil clay and Fe and Al oxide contents. However, a 100-fold higher rate of glyphosate applied to soil did result in significantly higher soil respiration compared with the unamended soils. In the same study, 9 to 13 yr of repeated glyphosate application among the plantation sites had no significant effects on soil microbial characteristics such as basal respiration, metabolic quotient, metabolic diversity, and total bacteria, but these characteristics were more strongly affected by seasonal variations and differences in vegetation composition and growth.

Inhibition of the growth of mycorrhizal fungi by glyphosate applications may affect soil nutrient transformations because of the often significant role of these fungi in plant nutrient acquisition among several plant species. Chakravarty and Chatarpaul (1990) observed reduction in the growth of five species of ectomycorrhizal fungi when exposed to glyphosate concentrations higher than 50 $\mu\text{L L}^{-1}$ in culture media. This study also observed significantly lower fungal and bacterial populations in a sandy loam soil 2 mo after glyphosate treatment with a rate of 0.54 kg a.i. ha^{-1} . Similarly, Estok et al. (1989) observed reductions in radial growth of three species of ectomycorrhizal fungi at glyphosate concentrations of $\geq 1000 \mu\text{L L}^{-1}$ in culture media and

complete inhibition of growth at concentrations of $\geq 5000 \mu\text{L L}^{-1}$. Further field research is required on glyphosate effects on soil mycorrhizae since research findings from artificial media assays may have limited relevance for predicting glyphosate effects on soil microorganisms under field conditions due to the greater complexity and variability of agricultural and forest ecosystems (Estok et al., 1989; Busse et al., 2001).

Another potential toxic effect of glyphosate applied to soil is inhibition of symbiotic N₂ fixation. King et al. (2001) observed in greenhouse and growth chamber experiments using potting media that early applications of glyphosate at a rate of 1.68 kg a.i. ha^{-1} delayed N₂ fixation and decreased biomass and N accumulation in glyphosate-tolerant soybean, especially when soil water content was low. However, affected soybean plants recovered 40 d after emergence. The observed delay in N₂ fixation was attributed to the lack of resistant EPSPS in the N-fixing symbiotic bacteria, *Bradyrhizobium japonicum*, in glyphosate-resistant soybeans (King et al., 2001). In field experiments conducted on a Pembroke silt loam (fine-silty, mixed, active, mesic Mollic Paleudalfs) and a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) in Arkansas by the same researchers, glyphosate-tolerant soybeans had decreased biomass and seed yields at one of two locations, possibly due to conditions of limited soil water at the affected site (King et al., 2001). Similarly, Reddy et al. (2000) determined that glyphosate applications of 2.24 kg a.i. ha^{-1} reduced shoot and root weights in glyphosate-resistant soybeans, and glyphosate applications of both 1.12 and 2.24 kg a.i. ha^{-1} reduced leghemoglobin content of nodules by 6 to 18%. Application of 2.24 kg a.i. ha^{-1} 3 wk after planting resulted in a 30% reduction in nodule numbers and a 39% decrease in nodule mass.

Subsequent field studies revealed that glyphosate, regardless of formulation, reduced both leghemoglobin content and nodule mass of glyphosate-resistant soybean, indicating that nodule development was inhibited (Reddy and Zablotowicz, 2003). In addition, glyphosate accumulated in nodules of glyphosate-treated plants, with concentrations ranging from 39 to 147 ng g^{-1} nodule dry weight. The interference of nodule development combined with the sensitivity of *B. japonicum* to glypho-

sate concentrations at 1 to 5 mM (Moorman et al., 1992) suggests potential adverse effects on nitrogen fixation in soybean. However, the researchers for both studies noted that even though glyphosate application at recommended application rates may reduce nodule development, effects were minimal and soybean varieties studied so far have the potential to compensate after short durations of stress that may occur due to glyphosate application.

Crop Residues and Organic Wastes

Changes in the fate of crop residues from transgenic crops compared with non-transgenic crops may occur through several mechanisms including: (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; (iii) changes in management practices (e.g., tillage) for transgenic crops affecting biotic and abiotic factors involved in decomposition and nutrient turnover of crop residues; and (iv) the effects of any possible physical or chemical changes in transgenic residues on feeding and digestion of the residues in animals that may affect animal waste characteristics and its decomposition in soil (Fig. 1).

Differences in the composition of crop residues due to the introduction of a transgenic trait have been primarily observed in transgenic Bt crops. Masoero et al. (1999) determined that two transgenic Bt corn hybrids had higher starch and lignin and lower protein and soluble N contents compared with non-transgenic corn, depending on the Bt hybrid. Similarly, Saxena and Stotzky (2001b) observed 33 to 97% higher lignin content in 10 Bt corn hybrids, either grown in a plant growth chamber or in the field, compared with their respective non-Bt isolines. In contrast, Escher et al. (2000) found a lower C to N ratio and lignin content and a higher content of soluble carbohydrates in the leaves of one Bt corn variety compared with the corresponding non-transgenic corn variety. These differences in tissue composition resulted in a lower mortality of woodlice (*Porcellio scaber*), but bacterial growth on leaves and fungal growth on feces of *P. scaber* were equal for both corn varieties. However, this study also observed a faster rate of lignin decomposition in transgenic corn.

The effects of crop residue composition and the importance of several indices of residue quality, including the C to N ratio, lignin content, lignin to N ratio, initial N content, polyphenol content, polyphenol to 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 (Franzluebbers et al., 1996; Fernandes et al., 1997; Trinsoutrot et al., 2000). In general, as the lignin or polyphenolic content to N ratio of the plant material increases or the initial N content decreases, the decomposition rate of the material is lower and short-term N availability may be reduced (Fernandes et al., 1997). Therefore, the lower or higher lignin content observed

in some Bt crops may act to increase or reduce rates of residue decomposition and N mineralization of organic N contained in the residues. However, any reductions in the rate of N mineralization due to transgenic residues could be offset by the larger amount of total N added in crop residues under Bt crops, which may have higher yields than non-transgenic crops, especially after exposure to Bt-sensitive insect pests.

Differences in decomposition and N transformations due to changes in the physical form of transgenic versus non-transgenic crop residues have not been extensively studied. For example, Bt tolerant corn residues may have less corn borer damage in stem tissue and therefore, these residues would possibly have less physical accessibility for biological decomposition.

Increased crop residues that are more or less resistant to decomposition due to the growth of transgenic crops may also affect the amount of environmental nutrient loss through soil erosion and runoff since a higher content of crop residues, especially on the soil surface, has been linked with increased soil conservation (Alberts and Neibling, 1994; Lal et al., 1994). 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 (Council for Agricultural Science and Technology, 2002).

The effects of transgenic crop residues on any changes in animal waste characteristics and the subsequent decomposition of that animal waste in soil has also not been extensively examined. One problem in assessing the impact of transgenic crop residues on soil microbial processes and functions is the wide range in feed quality that occurs in non-transgenic residues (Klopfenstein, 1994). Folmer et al. (2002) studied the utilization of Bt corn residues by grazing beef steers and found no significant differences in daily weight gain of the steers and in grazing preferences between Bt and non-Bt corn residues. Transgenic low phytate or high available phosphorus (HAP) crops are being developed that would contain less phytate thereby increasing the efficiency of feed P absorption in monogastric animals, such as pigs and poultry (Huff et al., 1998; Sands et al., 2001). This increased efficiency would result in possible reductions in the P content of animal wastes applied to land, effectively lowering the potential for water quality problems due to excessive P in the environment. Since research related to transgenic HAP crops has been primarily focused on use of HAP grain as the feed source, information related to the fate in soil of other residues of HAP crops and associated transgenic crops that produce phytase, an enzyme that facilitates the breakdown of phytin, is limited.

CONCLUSIONS

Additional research evaluating the potential effects of transgenic crops and their management practices on microbially mediated soil nutrient transformations is required because of the wide range and complexity of possible targeted and nontargeted direct and indirect

effects of novel crop traits on soil processes and because of the increasingly large land area cultivated with transgenic crops. Despite widespread public concern, no conclusive research has yet been presented that currently released transgenic crops are causing significant direct effects on stimulating or suppressing soil nutrient transformations in field environments. The net indirect effects on soil nutrient transformations of the introduction of transgenic crops and their associated management practices, including effects on land use and soil erosion, have not been fully explored. In addition, further consideration of the effects of a wide range of soil properties, including the amount of clay and its mineralogy, pH, soil structure, and soil organic matter, and variations in climatic conditions, under which transgenic crops may be grown, is needed in evaluating the impact of transgenic crops on soil nutrient transformations. Ongoing and future environmental evaluation of the diversity of transgenic crops under development may pose a unique scientific challenge that may provide an opportunity for an improved understanding of soil ecology.

REFERENCES

- Alberts, E.E., and W.H. Neibling. 1994. Influence of crop residues on water erosion. p. 19–39. *In* P.W. Unger (ed.) *Managing agricultural residues*. Lewis Publ., Boca Raton, FL.
- Angle, J.S. 1994. Release of transgenic plants: Biodiversity and population-level considerations. *Mol. Ecol.* 3:45–50.
- Betz, F.S., B.G. Hammond, and R.L. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regul. Toxicol. Pharmacol.* 32:156–173.
- Boyle, J.R., H. Lundkvist, and C.T. Smith. 2001. Ecological considerations for potentially sustainable plantation forests. p. 151–157. *In* S.H. Strauss and H.D. Bradshaw (ed.) *Proc. of the 1st Int. Symp. on Ecological and Societal Aspects of Transgenic Plantations*, Stevenson, WA. 22–24 July 2001. College of Forestry, Oregon State Univ., Corvallis.
- Bruinsma, M., G.A. Kowalchuk, and J.A. van Veen. 2003. Effects of genetically modified plants on microbial communities and processes in soil. *Biol. Fertil. Soils* 37:329–337.
- Busse, M.D., A.W. Ratcliff, C.J. Shestak, and R.F. Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. *Soil Biol. Biochem.* 33:1777–1789.
- Chakravarty, P., and L. Chatarpaul. 1990. Non-target effect of herbicides: I. Effect of glyphosate and hexazinone on soil microbial activity. Microbial population, and in-vitro growth of ectomycorrhizal fungi. *Pestic. Sci.* 28:233–241.
- Chichkova, S., J. Arellano, C.P. Vance, and G. Hernández. 2001. Transgenic tobacco plants that overexpress alfalfa NADH-glutamate synthase have higher carbon and nitrogen content. *J. Exp. Biol.* 52:2079–2087.
- Council for Agricultural Science and Technology. 2002. Comparative environmental impacts of biotechnology-derived and traditional soybean, corn, and cotton crops. CAST, Ames, IA.
- Crecchio, C., and G. Stotzky. 1998. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. *Soil Biol. Biochem.* 30:463–470.
- Crecchio, C., and G. Stotzky. 2001. Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound on complexes of montmorillonite-humic acids-Al hydroxylolymers. *Soil Biol. Biochem.* 33:573–581.
- Dale, P.J., B. Clarke, and E.M.G. Fontes. 2002. Potential for the environmental impact of transgenic crops. *Nat. Biotechnol.* 20:567–574.
- Donegan, K.K., C.J. Palm, V.J. Fieland, L.A. Porteous, L.M. Ganio, D.L. Schaller, L.Q. Bucuo, and R.J. Seidler. 1995. Changes in levels, species, and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Appl. Soil Ecol.* 2:111–124.
- Donegan, K.K., D.L. Schaller, J.K. Stone, L.M. Ganio, G. Reed, P.B. Hamm, and R.J. Seidler. 1996. Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the *Bacillus thuringiensis* var. *tenebrionis* endotoxin. *Transgen. Res.* 5:25–35.
- Donegan, K.K., and R.J. Seidler. 1999. Effects of transgenic plants on soil and plant microorganisms. p. 415–424. *In* S.G. Pandalai (ed.) *Recent research development in microbiology*. Vol. 3. Part II. Research Signpost, Trivandrum, India.
- Escher, N., B. Kach, and W. Nentwig. 2000. Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustacea: Isopoda). *Basic Appl. Ecol.* 1:161–169.
- Estok, D., B. Freedman, and D. Boyle. 1989. Effects of the herbicide 2,4-D, glyphosate, hexazinone, and triclopyr on the growth of three species of ectomycorrhizal fungi. *Bull. Environ. Contam. Toxicol.* 42:835–839.
- Fernandes, E.C.M., P.P. Motavalli, C. Castilla, and L. Mukurumbira. 1997. Management control of soil organic matter dynamics in tropical land-use systems. *Geoderma* 79:49–67.
- Folmer, J.D., R.J. Grant, C.T. Milton, and J. Beck. 2002. Utilization of Bt corn residues by grazing beef steers and Bt corn silage and grain by growing beef cattle and lactating dairy cows. *J. Anim. Sci.* 80:1352–1361.
- Franzluebbers, A., M.A. Arshad, and J.A. Ripmeester. 1996. Alterations in canola residue composition during decomposition. *Soil Biol. Biochem.* 28:1289–1295.
- Garg, A.K., K. Ju-Kon, T.G. Owens, A.P. Ranwala, Y.D. Choi, L.V. Kochian, and R.J. Wu. 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl. Acad. Sci. USA* 99:15898–15903.
- Giesy, J.P., S. Dobson, and K.R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® herbicide. *Rev. Environ. Contam. Toxicol.* 167:35–120.
- Grogan, J., and C. Long. 2000. The problem with genetic engineering. *Org. Gardening* 47:42–47.
- Haney, R.L., S.A. Senseman, and F.M. Hons. 2002. Effect of Roundup Ultra on microbial activity and biomass from selected soils. *J. Environ. Qual.* 31:730–735.
- Haney, R.L., S.A. Senseman, F.M. Hons, and D.A. Zuberer. 2000. Effect of glyphosate on soil microbial activity and biomass. *Weed Sci.* 48:89–93.
- Head, G., J.B. Surber, J.A. Watson, J.W. Martin, and J.J. Duan. 2002. No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollgard) use. *Environ. Entomol.* 31:30–36.
- Hirsch, R.E., and M.R. Sussman. 1999. Improving nutrient capture from soil by the genetic manipulation of crop plants. *Trends Biotechnol.* 17:356–361.
- Hoffman, C.A. 1990. Ecological risks of genetic engineering of crop plants. *Bioscience* 40:434–437.
- Huff, W.E., P.A. Moore, Jr., P.W. Waldroup, A.L. Waldroup, J.M. Balog, G.R. Huff, N.C. Rath, T.C. Daniel, and V. Raboys. 1998. Effect of dietary phytase and high available phosphorus corn on broiler chicken performance. *Poult. Sci.* 77:1899–1904.
- Kennedy, A.C. 1998. The rhizosphere and spermophere. p. 389–407. *In* D.M. Sylvia, J.F. Fuhrmann, P.G. Hartel, and D. Zuberer (ed.) *Principles and applications of soil microbiology*. Prentice Hall, Upper Saddle River, NJ.
- King, C.A., L.C. Purcell, and E.D. Vories. 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. *Agron. J.* 93:179–186.
- Klopfenstein, T. 1994. Crop residue use as animal feed. p. 315–342. *In* P.W. Unger (ed.) *Managing agricultural residues*. Lewis Publ., Boca Raton, FL.
- Lal, R., T.J. Logan, D.J. Eckert, W. Dick, and M.J. Shipitalo. 1994. Conservation tillage in the Corn Belt of the United States. p. 73–114. *In* M.R. Carter (ed.) *Conservation tillage in temperate agroecosystems: Development and adaptation to soil, climatic and biological constraints*. CRC Press, Boca Raton, FL.
- López-Bucio, J., M.F. Nieto-Jacobo, V. Ramírez-Rodríguez, and L. Herrera-Estrella. 2000. Organic acid metabolism in plants: From adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Sci.* 160:1–13.
- Mansouri, H., A. Petit, P. Onger, and Y. Dessaux. 2002. Engineered rhizosphere: The trophic bias generated by opine-producing plants

- is independent of the opine type, the soil origin, and the plant species. *Appl. Environ. Microbiol.* 68:2562–2566.
- Masero, F., M. Moschini, F. Rossi, A. Prandini, and A. Pietri. 1999. Nutritive value, mycotoxin contamination and in vitro rumen fermentation of normal and genetically modified corn (cry1A(b)) grown in northern Italy. *Maydica* 44:205–209.
- Moorman, T.B., J.M. Becerril, J. Lydon, and S.O. Duke. 1992. Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. *J. Agric. Food Chem.* 40:289–293.
- National Research Council. 2002. Environmental effects of transgenic plants: The scope and adequacy of regulation. Natl. Academy Press, Washington, DC.
- Nottingham, S. 2002. Genescapes. Zed Books, New York.
- O'Callaghan, M.O., and T.R. Glare. 2001. Impacts of transgenic plants and microorganisms on soil biota. *N. Z. Plant Protection* 54:105–110.
- Palm, C.P., D.L. Schaller, K.K. Donegan, and R.J. Seidler. 1996. Persistence in soil of transgenic plants produced *Bacillus thuringiensis* var. *kurstaki*-endotoxin. *Can. J. Microbiol.* 42:1258–1262.
- Reddy, K.N., R.E. Hoagland, and R.M. Zablotowicz. 2000. Effect of glyphosate on growth, chlorophyll, and nodulation in glyphosate-resistant and susceptible soybean (*Glycine max*) varieties. *J. New Seeds* 2:37–52.
- Reddy, K.N., and R.M. Zablotowicz. 2003. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. *Weed Sci.* 51:496–502.
- Richardson, A.E., P.A. Hadobas, and J.E. Hayes. 2001. Extracellular secretion of *Aspergillus* phytase from *Arabidopsis* roots enables plants to obtain phosphorus from phytate. *Plant J.* 25:641–649.
- Ryan, P.R., E. Delhaize, and D.L. Jones. 2001. Function and mechanism of organic anion exudation from plant roots. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52:527–560.
- Salmeron, J.M., and B. Vernooij. 1998. Transgenic approaches to microbial disease resistance in crop plants. *Curr. Opin. Plant Biol.* 1:347–352.
- Sands, J.S., D. Ragland, C. Baxter, B.C. Joern, T.E. Sauber, and O. Adeola. 2001. Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. *J. Anim. Sci.* 79:2134–2142.
- Saxena, D., S. Flores, and G. Stotzky. 1999. Insecticidal toxin in root exudates from Bt corn. *Nature (London)* 402:480.
- Saxena, D., S. Flores, and G. Stotzky. 2002. Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. *Soil Biol. Biochem.* 34:133–137.
- Saxena, D., and G. Stotzky. 2001a. *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. *Soil Biol. Biochem.* 33:1225–1230.
- Saxena, D., and G. Stotzky. 2001b. Bt corn has a higher lignin content than non-Bt corn. *Am. J. Bot.* 88:1704–1706.
- Schnepf, E., N. Crickmore, J.V. Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62:775–806.
- Takahashi, M., H. Nakanishi, S. Kawasaki, N.K. Nishizawa, and S. Mori. 2001. Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. *Nat. Biotechnol.* 19:466–469.
- Tapp, H., L. Calamai, and G. Stotzky. 1994. Adsorption and binding of the insecticidal proteins from *Bacillus thuringiensis* subsp. *kurstaki* and subsp. *tenebrionis* on clay minerals. *Soil Biol. Biochem.* 26:663–679.
- Trevors, J.T., P. Kuikman, and B. Watson. 1994. Transgenic plants and biogeochemical cycles. *Mol. Ecol.* 3:57–64.
- Trinsoutrot, I., S. Recous, B. Bentz, M. Linères, D. Chèneby, and B. Nicolardot. 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. *Soil Sci. Soc. Am. J.* 64:918–926.
- Zwahlen, C., A. Hilbeck, P. Gugerli, and W. Nentwig. 2003. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Mol. Ecol.* 12:765–775.