Molecular Soil Biology 2024, Vol.15, No.4, 183-192
http://bioscipublisher.com/index.php/msb

Research Insight Open Access

Impact of Bt Applicatins on Soil Micrbial Cmmunities

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Molecular Soil Biology, 2024, Vol.15, No.4 doi: 10.5376/msb.2024.15.0019
Received: 16 Jun., 2024
Accepted: 25 Jul., 2024
Published: 15 Aug., 2024

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Preferred citation for this article:
Wang J.J., Zhang X.M., and Liu H.W., 2024, Impact of Bt applicatins on soil micrbial cmmunities, Molecular Soil Biology, 15(4): 183-192 (doi: 10.5376/msb.2024.15.0019)

Abstract This study investigates the impact of *Bacillus thuringiensis* (Bt) applications on soil microbial communities, focusing on enzymatic activities, microbial diversity, and potential ecological risks. The findings indicate that Bt crop cultivation significantly alters soil enzymatic activities, with increases in dehydrogenase and urease activities, while neutral phosphatase activity decreases. Seasonal variations and differences between rhizosphere and non-rhizosphere soils were observed, but Bt maize did not generally change microbial populations or community activities. Bt proteins released into the soil through various plant residues can affect soil microbial diversity and physical-chemical properties. However, the presence of Bt proteins in earthworms showed no deleterious effects on their growth and reproduction. Environmental factors were found to have a more significant impact on microbial communities than Bt traits. The application of Bt crops and proteins can influence soil microbial communities and enzymatic activities, but the overall ecological risks appear to be minimal under field conditions. Continuous monitoring and comprehensive biosafety evaluations are recommended to ensure the long-term sustainability of Bt applications in agriculture.

Keywords *Bacillus thuringiensis*; Soil microbial communities; Enzymatic activities; Ecological risks; Bt crops; Microbial diversity

1 Introduction

Bacillus thuringiensis (Bt) is a Gram-positive, spore-forming bacterium known for its production of insecticidal proteins, particularly Cry and Cyt toxins, during sporulation. These proteins have been extensively utilized in agriculture due to their specificity and effectiveness against a wide range of insect pests, including lepidopteran, coleopteran, and dipteran larvae (Sanahuja et al., 2011; Sanchis and Bourguet, 2011; Liu, 2024). Bt's application in agriculture primarily involves the use of Bt transgenic plants and Bt biopesticides. Transgenic crops expressing Bt genes have been developed to provide inherent pest resistance, significantly reducing the need for chemical pesticides and contributing to higher crop yields (Sanahuja et al., 2011; Li et al., 2022). Additionally, Bt biopesticides are used as topical sprays to control pest populations, further highlighting the bacterium's versatility and importance in integrated pest management strategies (Sanchis and Bourguet, 2011).

Soil microbial communities play a crucial role in maintaining ecosystem health and functionality. These communities are involved in essential processes such as nutrient cycling, organic matter decomposition, and soil structure maintenance. They also contribute to plant health by promoting growth and protecting against pathogens (Almeida et al., 2021; Gomis-Cebolla and Berry, 2023). The introduction of Bt proteins into the soil environment, whether through transgenic plants or biopesticides, raises concerns about potential impacts on these vital microbial communities. Understanding how Bt applications affect soil microbial diversity and activity is essential for assessing the long-term sustainability and environmental safety of Bt-based agricultural practices (Li et al., 2019; 2022).

This study explores the impact of Bt applications on soil microbial communities, aiming to assess the effects of Bt proteins, released through transgenic plants and biopesticides, on soil microbial diversity and enzyme activity. It also evaluates the potential ecological risks associated with the persistence and bioactivity of Bt proteins in the soil environment. Furthermore, the study provides a comprehensive review of current research findings on the interactions between Bt applications and soil microbial communities, highlighting knowledge gaps and future research directions. The goal is to contribute to the scientific community's understanding of the environmental impact of Bt, promote biosafety assessments, and inform sustainable agricultural practices involving Bt technology.

2 Mechanism of Bt Action in Agricultural Applications

2.1 Bt as a biopesticide: mode of action on insect pests

Bacillus thuringiensis (Bt) produces insecticidal proteins, primarily Cry proteins, which are highly specific stomach poisons targeting insect pests. These proteins bind to receptors in the gut of susceptible insects, causing cell lysis and ultimately leading to the insect's death. Bt-based biopesticides have been widely used in agriculture due to their specificity and environmental safety compared to conventional chemical insecticides (Sanchis, 2011). The mode of action involves the ingestion of Bt spores or Cry proteins by the insect, followed by the activation of these proteins in the alkaline gut environment, leading to the formation of pores in the gut cells and causing septicemia (Hilbeck et al., 2018).

2.2 Forms of Bt application: sprays vs. genetically modified (GM) Bt crops

Bt can be applied in two primary forms: as microbial sprays or through genetically modified (GM) crops. Bt sprays involve the application of Bt spores and Cry proteins directly onto crops, providing immediate but short-term pest control due to environmental degradation factors such as UV light and rain (Sanchis, 2011). On the other hand, GM Bt crops are engineered to express Cry proteins throughout the plant tissues, offering continuous protection against pests. This method has been shown to reduce the reliance on chemical insecticides and improve pest management efficiency (Tabashnik et al., 2023). However, the persistence and potential ecological impacts of Cry proteins released from GM Bt crops into the soil through root exudates and plant residues are areas of ongoing research (Icoz and Stotzky, 2008; Li et al., 2022).

2.3 Persistence of Bt toxins in soil

The persistence of Bt toxins in soil is influenced by both biotic and abiotic factors. Bt toxins can be released into the soil through root exudates, pollen, and decomposing plant residues from GM Bt crops (Li et al., 2022). The degradation of these toxins in soil is affected by microbial activity and physicochemical interactions with soil components. Studies have shown that Bt toxins can persist in soil for several months, with their persistence being soil-dependent and influenced by factors such as soil type, pH, and microbial activity (Icoz and Stotzky, 2008; Helassa et al., 2011). While some studies indicate that Bt toxins do not significantly alter soil microbial communities, the long-term ecological impacts require further investigation (O’Callaghan et al., 2005; Zhaolei et al., 2018).

3 Influence of Bt Applications on Soil Microbial Communities

3.1 Immediate effects on microbial diversity and composition

The immediate effects of Bt applications on soil microbial communities have been a subject of extensive research. Studies have shown that the introduction of Bt crops, such as Bt maize, can lead to changes in the abundance of certain soil microorganisms. For instance, the presence of Bt traits in maize resulted in an increase in soil nematodes and protozoa (amoebae) (Griffiths et al., 2006). However, these changes were relatively minor compared to the effects of chemical insecticides. Another study found that the application of Cry1Ac protein, a common Bt toxin, did not significantly alter the diversity or population sizes of bacteria, fungi, and archaea in the soil over a 100-day period (Zhaolei et al., 2018). These findings suggest that while Bt applications can have immediate effects on specific microbial populations, the overall impact on microbial diversity and composition is limited.

3.2 Long-term impacts: shifts in microbial community structure

Long-term studies on the impact of Bt crops on soil microbial communities indicate that there may be shifts in microbial community structure over time. For example, research on the long-term application of glyphosate, a common herbicide used in conjunction with Bt crops, showed an increase in the relative abundance of Proteobacteria and a decrease in Acidobacteria in the rhizosphere (Newman et al., 2016). This shift could have significant implications for nutrient cycling and soil health. Additionally, long-term nitrogen fertilization, often used in Bt crop cultivation, has been shown to decrease bacterial diversity and favor the growth of Actinobacteria and Proteobacteria (Dai et al., 2018). These shifts in microbial community structure highlight the need for ongoing monitoring to understand the long-term ecological impacts of Bt crop cultivation.

3.3 Comparative effects of Bt crops vs. chemical pesticides

When comparing the effects of Bt crops to chemical pesticides, studies have generally found that Bt crops have a less pronounced impact on soil microbial communities. For instance, a glasshouse experiment comparing Bt maize and pyrethroid insecticide (deltamethrin) found that the insecticide had a more significant effect on altering nematode community structure and increasing plant Bt concentration than the Bt trait itself (Griffiths et al., 2006). Similarly, a study on the impact of various pesticides on soil microbial functions related to carbon cycling found that insecticides like fipronil had a more substantial effect on stimulating cellulolytic and chitinolytic microorganisms compared to Bt applications (Sim et al., 2022). These findings suggest that while Bt crops do influence soil microbial communities, their impact is generally less severe than that of chemical pesticides, making them a potentially more sustainable option for pest management in agriculture.

4 Microbial Functions Affected by Bt Applications

4.1 Impact on nitrogen-fixing bacteria

Bt applications can significantly influence nitrogen-fixing bacteria in the soil. Studies have shown that the presence of nitrogen-fixing bacteria, such as *Azospirillum* sp. and *Nostoc edaphicum*, is crucial for plant growth and soil health. However, the impact of Bt applications on these bacteria can vary. For instance, single bacterial inoculation without mycorrhiza does not positively affect plant growth, while co-inoculation with arbuscular mycorrhizal fungi (AMF) improves plant growth and vitality (Ogar et al., 2015). Additionally, nitrogen fertilization, which can be influenced by Bt applications, has been shown to reduce the abundance of nif genes, indicating a decrease in nitrogen fixation potential (Liao et al., 2021).

4.2 Effects on decomposers and nutrient cycling

Bt applications can also affect decomposers and nutrient cycling processes in the soil. Decomposers, such as saprotrophic fungi, play a critical role in breaking down organic matter and recycling nutrients. Nitrogen enrichment, which can be a consequence of Bt applications, has been shown to decrease microbial biomass carbon (MBC) and alter the composition of decomposer communities (Jia et al., 2020). Furthermore, the presence of AMF can modify the soil bacterial community and nitrogen cycling during litter decomposition, indicating that Bt applications that affect AMF can subsequently influence decomposer activity and nutrient cycling (Nuccio et al., 2013). The addition of fungicides and bactericides, often used alongside Bt applications, can also have mixed effects on soil respiration and nitrogen cycling, further complicating the impact on decomposers (Ullah and Dijkstra, 2019).

4.3 Changes in microbial symbiosis with plants (e.g., mycorrhizal fungi)

Bt applications can lead to significant changes in microbial symbiosis with plants, particularly with mycorrhizal fungi. Mycorrhizal fungi, such as AMF, form symbiotic relationships with plant roots, enhancing nutrient uptake and plant growth. However, nitrogen enrichment and other factors associated with Bt applications can reduce the abundance of AMF and alter their community composition (Zhang et al., 2018; Jia et al., 2020). Studies have shown that AMF can shift the community composition of nitrogen-cycling microbes and suppress soil N2O emissions, indicating that changes in AMF populations due to Bt applications can have broader ecological impacts (Zhang et al., 2022). Additionally, the presence of AMF has been shown to buffer the impact of varying nutrient inputs on the decomposer community, highlighting the importance of maintaining healthy mycorrhizal symbioses in the face of Bt applications (Schmidt et al., 2017).

5 Soil Health and Ecological Balance

5.1 Bt applications and soil fertility

Bt crops, which are genetically modified to express *Bacillus thuringiensis* (Bt) toxins, have been widely adopted in agriculture. These crops can influence soil fertility through various mechanisms. For instance, Bt proteins released into the soil via root exudates, pollen, and plant residues can interact with soil microorganisms, which play crucial roles in nutrient cycling and energy flow (Li et al., 2022) (Figure 1). Studies have shown that Bt crops can alter soil enzymatic activities, which are essential for maintaining soil health. For example, dehydrogenase and urease activities significantly increased under Bt crop cultivation, indicating potential positive effects on soil fertility (Li et al., 2019). However, the overall impact on soil fertility can vary depending on factors such as soil type, crop species, and agricultural practices (Icoz and Stotzky, 2008).

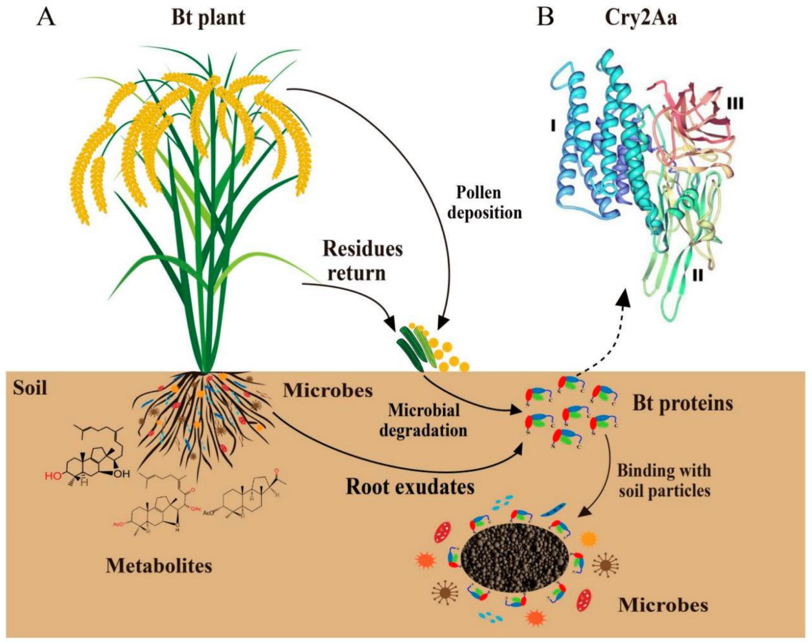


Figure 1 Environmental behaviors of Bt protein (A) and its three-dimensional structures (B). I, II, and III: domains I, II, and III (Adopted from Li et al., 2022)

5.2 Risk of Bt toxin accumulation and microbial resistance development

The persistence and accumulation of Bt toxins in the soil are critical concerns for environmental safety. Bt proteins can remain in the soil environment for extended periods, depending on factors like microbial activity, soil type, and climatic conditions (Icoz and Stotzky, 2008). While some studies have reported no significant adverse effects of Bt proteins on soil microbial communities (Zhaolei et al., 2018), others have highlighted the potential for Bt toxin accumulation to alter microbial dynamics and biodiversity. The risk of microbial resistance development is another concern, as continuous exposure to Bt toxins could lead to the selection of resistant microbial strains, potentially disrupting soil ecological balance (Li et al., 2022).

5.3 Ecological significance of microbial shifts on plant health and productivity

Microbial communities in the soil are integral to plant health and productivity. Bt crops can induce shifts in these communities, which may have ecological implications. For instance, changes in the diversity and composition of bacterial and fungal communities in the rhizosphere of Bt maize have been observed, although these effects were often temporary and influenced more by seasonal factors than by the Bt trait itself (Wang et al., 2022). Additionally, Bt crops have been shown to affect beneficial mycorrhizal symbiosis, which is crucial for nutrient uptake and plant growth (Castaldini et al., 2005). While some studies suggest that Bt crops do not cause significant long-term changes in soil microbial communities (Zhaolei et al., 2018), the potential for subtle shifts that could impact plant health and productivity cannot be ignored (Castaldini et al., 2005; Icoz and Stotzky, 2008).

6 Interactions Between Bt Toxins and Other Soil Microorganisms

6.1 Antagonistic and synergistic interactions with beneficial soil bacteria

Bt crops, which produce insecticidal proteins, have been scrutinized for their potential impacts on soil microbial communities. Studies have shown that Bt toxins can interact with beneficial soil bacteria, but these interactions are often complex and context-dependent. For instance, research indicates that Bt cotton does not adversely affect microbial populations or enzymatic activities in the soil, suggesting a neutral or potentially synergistic relationship with beneficial bacteria (Yasin et al., 2016). Additionally, soil microbial diversity and evenness indices did not significantly shift with the addition of Cry1Ac protein, indicating that Bt proteins might not disrupt beneficial bacterial communities (Zhaolei et al., 2018).

6.2 Impacts on soil pathogens and microbial disease suppression

The presence of Bt toxins in the soil can influence soil pathogens and the microbial suppression of diseases. For example, Bt crops have been shown to increase the activities of certain soil enzymes, such as dehydrogenase and urease, which are involved in nutrient cycling and can indirectly affect soil pathogen dynamics (Li et al., 2019). However, the overall impact on soil pathogens remains inconclusive, as some studies report no significant changes in microbial community structures, including those of pathogenic fungi, due to Bt crop residues (Lu et al., 2010). This suggests that while Bt toxins may alter some aspects of microbial activity, they do not necessarily enhance or suppress soil-borne diseases in a consistent manner.

6.3 Effects on microbial-driven nutrient availability and uptake by plants

Bt crops can also affect nutrient availability and uptake by plants through their interactions with soil microorganisms. The cultivation of Bt cotton, for instance, has been associated with higher cation exchange capacity and increased levels of essential nutrients such as nitrogen, phosphorus, and potassium in the soil, compared to non-Bt cotton. This enhancement in nutrient dynamics is likely mediated by microbial processes, as Bt crops have been shown to increase the population of beneficial bacteria involved in nutrient cycling (Yasin et al., 2016). Moreover, the decomposition of Bt crop residues does not significantly alter the microbial community composition, suggesting that nutrient availability driven by microbial activity remains stable (Lu et al., 2010).

7 Case Studies and Comparative Research

7.1 Field studies on Bt crops: key findings

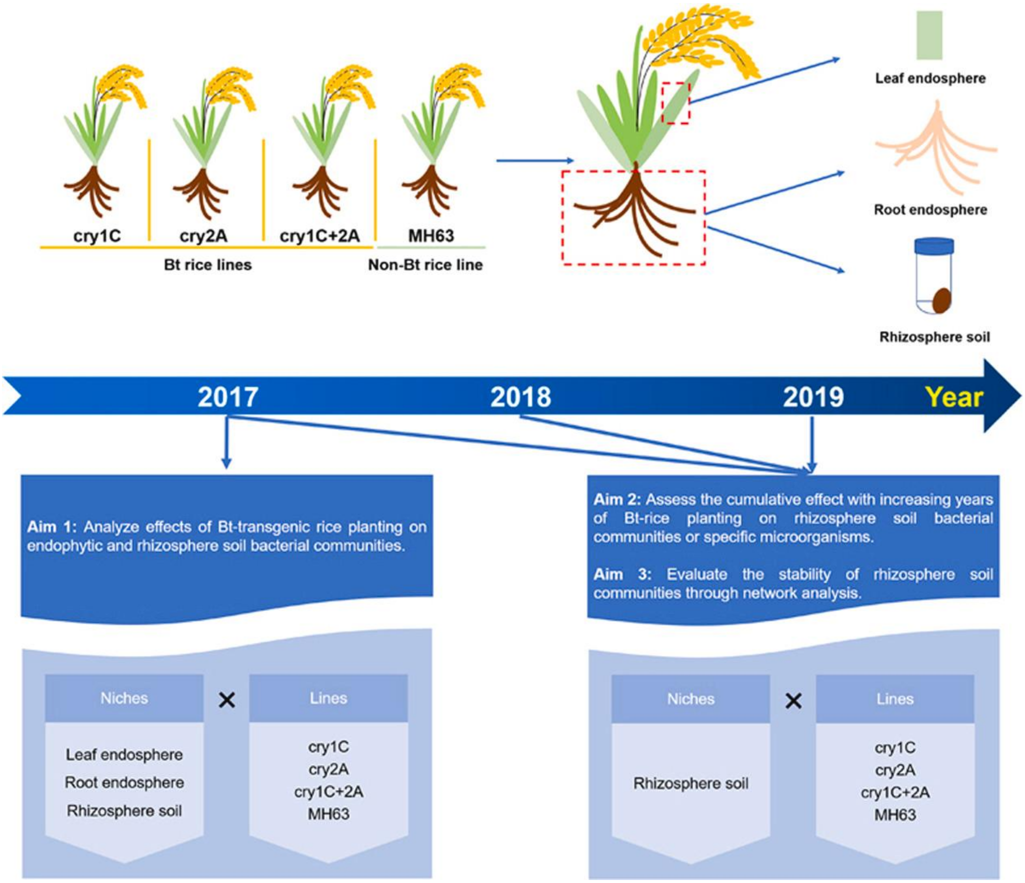
Field studies have provided significant insights into the impact of Bt crops on soil microbial communities. For instance, a global meta-analysis revealed that Bt crops can alter soil enzymatic activities, with specific enzymes like dehydrogenase and urease showing significant increases, while neutral phosphatase decreased under Bt crop cultivation without Bt residues incorporation (Li et al., 2019). Another study conducted in tropical western India found that Bt cotton significantly altered microbial community composition and functional diversity in rhizospheric soil compared to non-Bt cotton (Saha et al., 2021). Additionally, a systematic review and meta-analysis highlighted that while there is considerable variation among soil invertebrate orders, there was no significant overall effect of Cry proteins on soil invertebrates (Krogh et al., 2020). These findings underscore the complexity and variability of Bt crop impacts on soil ecosystems.

7.2 Laboratory vs. field conditions: variability in outcomes

The outcomes of Bt crop studies can vary significantly between laboratory and field conditions. Laboratory studies, such as those involving soil incubation with Cry1Ac protein, have shown no significant changes in soil microbial diversities and population sizes over a 100-day period (Zhaolei et al., 2018). In contrast, field studies often reveal more nuanced effects. For example, field research on Bt rice indicated that while bacterial communities in the rhizosphere did not show significant changes, the root and leaf endospheres exhibited mild and transient differences compared to non-Bt rice lines over three consecutive years (Wu et al., 2021) (Figure 2). This variability highlights the importance of considering environmental factors and the complexity of natural ecosystems when evaluating the impacts of Bt crops.

7.3 Global perspectives: regional studies and differing environmental conditions

Regional studies have shown that the impact of Bt crops on soil microbial communities can vary widely depending on environmental conditions. For instance, a study in tropical western India found that Bt cotton significantly altered microbial community composition and functional diversity in rhizospheric soil (Saha et al., 2021). In contrast, a global meta-analysis of soil enzymatic activities under Bt crops found that the response ratios of soil enzymes varied with Bt crop types and growth periods, with the strongest effects observed under Bt cotton (Li et al., 2019). Additionally, a review of global resistance monitoring data for Bt crops revealed that practical resistance has been documented in some pest populations across seven countries, affecting various Bt toxins (Tabashnik et al., 2023). These regional differences underscore the need for localized studies to understand the specific environmental impacts of Bt crops in different contexts.



Aim 1: Analyze effects of Bt-transgenic rice planting on endophytic and rhizosphere soil bacterial communities.

Aim 2: Assess the cumulative effect with increasing years of Bt-rice planting on rhizosphere soil bacterial communities or specific microorganisms.

Aim 3: Evaluate the stability of rhizosphere soil communities through network analysis.

Figure 2 Long-term effects of Bt-transgenic rice on bacterial communities in different plant niches: a three-year study (Adapted from Wu et al., 2021)

8 Regulatory and Environmental Considerations

8.1 Current regulatory guidelines on Bt use and microbial safety

Current regulatory guidelines for the use of *Bacillus thuringiensis* (Bt) and its associated products, such as Bt transgenic crops and Bt biopesticides, emphasize the need for comprehensive biosafety evaluations before their approval for field tests and commercial production. These evaluations are crucial due to the potential ecological risks posed by Bt proteins, which are considered environmentally exogenous substances with insecticidal toxicity. Regulatory frameworks typically require detailed assessments of the adsorption, retention, and degradation behaviors of Bt proteins in soils, as well as their impacts on soil physical and chemical properties and microbial diversity (Li et al., 2022). Additionally, the guidelines mandate the monitoring of non-target organisms, including soil microorganisms, to ensure that Bt applications do not adversely affect soil biodiversity and ecosystem services (Castaldini et al., 2055; Kostov et al., 2014).

8.2 Environmental monitoring practices for Bt toxin residues

Environmental monitoring practices for Bt toxin residues involve systematic field and laboratory studies to track the persistence and effects of Bt proteins in soil environments. These practices include the use of various analytical techniques to measure Bt protein concentrations in soil and assess their impact on soil microbial communities. For instance, studies have employed ribosomal DNA-fingerprinting methods and real-time PCR to monitor changes in soil bacterial, fungal, and archaeal diversities and community structures over time (Zhaolei et al., 2018). Additionally, the use of litterbag methods in field trials helps evaluate the decomposition of Bt crop residues and their effects on soil microbial composition (Zwahlen et al., 2007; Lu et al., 2010). These monitoring practices are essential for understanding the long-term ecological impacts of Bt crops and ensuring that any potential risks are identified and mitigated.

8.3 Public concerns and ecological risk assessments

Public concerns regarding the use of Bt crops and biopesticides primarily revolve around their potential impacts on non-target organisms and overall soil health. Ecological risk assessments are conducted to address these concerns by evaluating the direct and indirect effects of Bt applications on soil microbial communities and other non-target organisms. For example, studies have shown that while Bt proteins can affect soil microbial diversity and enzymatic activities, the observed changes are often small and not significantly different from those caused by conventional agricultural practices, such as the use of chemical insecticides (Griffiths et al., 2006; Li et al., 2019). However, some studies have reported significant differences in soil fungal community composition during the initial stages of Bt crop residue decomposition, highlighting the need for ongoing research and monitoring (Lu et al., 2010). These assessments help build public trust by ensuring that Bt applications are safe and sustainable for the environment.

9 Future Research Directions

9.1 Advances in microbial metagenomics for better understanding soil responses

Advances in microbial metagenomics offer a promising avenue for gaining deeper insights into how Bt applications affect soil microbial communities. Metagenomic techniques can provide comprehensive profiles of microbial diversity and functional potential, which are crucial for understanding the ecological impacts of Bt crops. For instance, high-throughput sequencing has been used to analyze bacterial communities in different niches of Bt rice over consecutive years, revealing significant but transient differences in microbial populations (Wu et al., 2021). Similarly, metagenomic approaches could help elucidate the complex interactions between Bt proteins and soil microorganisms, as seen in studies where Cry1Ac protein did not significantly alter microbial diversity over a 100-day period (Zhaolei et al., 2018). Future research should focus on leveraging these advanced techniques to monitor long-term changes and functional shifts in microbial communities in response to Bt applications.

9.2 Long-term studies on Bt applications and ecological resilience

Long-term studies are essential to assess the ecological resilience of soil microbial communities to Bt applications. While short-term studies have shown minimal or transient effects on microbial diversity and community structure (Griffiths et al., 2006; Lu et al., 2010), the long-term ecological consequences remain underexplored. For example, a meta-analysis highlighted the need for long-term field studies to document the responses of soil microbial communities to biochar application, which could be analogous to Bt applications (Deshoux et al., 2023). Additionally, research on the long-term impact of Bt crops on soil enzymatic activities has shown variable responses, indicating the need for extended monitoring to understand the full ecological implications (Li et al., 2019). Future research should aim to conduct multi-year field trials to evaluate the sustainability and resilience of soil ecosystems under continuous Bt crop cultivation.

9.3 Sustainable management of Bt in agricultural practices

Sustainable management practices are crucial for mitigating any potential adverse effects of Bt applications on soil microbial communities. Studies have shown that while Bt crops can affect soil microbial and faunal communities, these effects are often comparable to those of conventional agricultural practices, such as insecticide application (Griffiths et al., 2006). However, the variability in microbial responses to Bt crops suggests that management practices should be tailored to specific soil types and environmental conditions (Kostov et al., 2014). For instance, incorporating crop residues and optimizing Bt protein expression levels could help maintain soil health and microbial diversity (Castaldini et al., 2005; Li et al., 2022). Future research should focus on developing best management practices that integrate Bt crops into sustainable agricultural systems, ensuring minimal disruption to soil microbial communities and overall ecosystem health.

10 Concluding Remarks

The research on the impact of Bt (*Bacillus thuringiensis*) crops on soil microbial communities has yielded mixed results. Several studies have shown that Bt crops do not significantly alter the overall microbial biomass or diversity in the soil. For instance, a study on Bt corn found no significant effects on soil microbial biomass, enzyme activity, or bacterial functional diversity over multiple seasons. Similarly, another study indicated that the bacterial communities in the rhizosphere soil of Bt rice did not show significant differences compared to non-Bt rice over three years. However, some studies have reported specific changes in microbial community composition. For example, Bt rice roots were found to affect soil fungal communities during the initial stages of decomposition. Additionally, a global meta-analysis revealed that certain soil enzymatic activities, such as dehydrogenase and urease, increased under Bt crop cultivation.

The findings suggest that Bt crops can be integrated into agricultural systems without causing major disruptions to soil microbial communities, which are crucial for nutrient cycling and soil health. The lack of significant adverse effects on microbial biomass and diversity indicates that Bt crops may be a sustainable option for pest management, reducing the need for chemical insecticides and their associated environmental impacts. However, the observed changes in specific microbial communities and enzymatic activities highlight the need for ongoing monitoring to ensure that long-term cultivation of Bt crops does not lead to unforeseen ecological consequences. Maintaining soil health is essential for sustainable agriculture, and the current evidence suggests that Bt crops can be part of a balanced approach to achieving this goal.

Future agricultural practices should continue to incorporate Bt crops as part of integrated pest management strategies, given their potential to reduce chemical pesticide use and associated environmental impacts. However, it is crucial to implement long-term monitoring programs to track any subtle changes in soil microbial communities and functions over time. Research should focus on understanding the mechanisms behind the observed changes in specific microbial groups and enzymatic activities, as well as their potential implications for soil health and crop productivity. Additionally, studies should explore the combined effects of Bt crops with other sustainable agricultural practices, such as crop rotation, organic amendments, and reduced tillage, to develop holistic approaches that promote soil health and agricultural sustainability.

Acknowledgments

We would like to express our sincerest gratitude to Professor Z. Wu from Institute of Life Science, Jiyang College of Zhejiang A&F University for his critical reading and revising suggestions.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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