

Research Insight

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Effects of Organic Fertilizer Application on Soil Microbial Communities in Greenhouse Tomato Cultivation

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Abstract The application of organic fertilizers has become an important strategy for improving soil quality and promoting sustainable agricultural development in greenhouse tomato cultivation. Soil microbial communities play a critical role in maintaining soil fertility, nutrient cycling, and plant health. This study reviews the effects of organic fertilizer application on soil microbial communities in greenhouse tomato cultivation systems. Organic fertilizers can significantly improve soil physicochemical properties, increase soil organic matter content, and enhance nutrient availability, thereby creating a favorable environment for microbial growth and activity. Numerous studies have shown that organic fertilizer application can increase microbial abundance and diversity, alter the composition of dominant microbial taxa, and promote beneficial microorganisms involved in nutrient transformation and disease suppression. Furthermore, organic fertilizers influence microbial functional activities, including enzyme production and key processes in carbon and nitrogen cycling, which ultimately contribute to improved tomato growth, yield, and fruit quality. A case study of organic fertilizer substitution for chemical fertilizers demonstrates that appropriate organic fertilization practices can effectively optimize soil microbial community structure and improve soil ecological functions in greenhouse systems. Overall, the integration of organic fertilizers into fertilization management provides an effective approach to enhancing soil microbial diversity and maintaining soil health in greenhouse tomato production. Future research should further explore the long-term impacts of organic fertilizer application and its interactions with soil microbial networks to support sustainable greenhouse agriculture.

Keywords Organic fertilizer; Greenhouse tomato; Soil microbial community; Soil health; Sustainable agriculture

1 Introduction

Greenhouse tomato cultivation has become a vital component of protected agriculture worldwide due to its ability to provide stable and high yields throughout the year. However, intensive greenhouse production often leads to soil degradation problems such as nutrient imbalances, reduced microbial diversity, soil acidification, and accumulation of soil-borne pathogens, which threaten long-term sustainability and crop productivity. Overfertilization with synthetic chemicals is a common practice that exacerbates these issues by altering soil pH and microbial community structure, ultimately reducing tomato yield and soil health (Chen et al., 2022; Song et al., 2022). Addressing these challenges requires sustainable management practices that restore soil quality while maintaining high productivity.

The application of organic fertilizers in protected agriculture has gained attention for its ecological benefits in improving soil fertility and microbial function. Organic amendments such as bio-organic fertilizers, vermicompost, and composted organic matter enhance soil organic carbon, total nitrogen, and nutrient availability, which positively influence soil enzyme activities and microbial diversity. These fertilizers can also reduce the incidence of diseases like *Verticillium* wilt by reshaping rhizosphere fungal communities and increasing beneficial microbial interactions. Moreover, integrating organic fertilizers with optimized application rates can improve photosynthetic efficiency and fruit yield in greenhouse tomatoes by promoting nutrient cycling and microbiome-mediated pathways (Lu et al., 2025; Zhao et al., 2025).

Soil microbial communities play a crucial role in maintaining soil health and supporting crop growth through nutrient mineralization, disease suppression, and enhancement of plant physiological functions. Diverse bacterial

phyla such as Proteobacteria, Actinobacteria, and Bacteroidetes dominate healthy greenhouse soils under organic fertilization regimes. These microbes contribute to improved nitrogen availability, increased enzyme activities (e.g., urease, catalase), and greater resilience against pathogens. Studies show that soils managed with organic amendments harbor more complex microbial networks that foster plant growth compared to conventional chemical fertilization systems. Therefore, understanding how organic fertilizer application influences microbial community structure is essential for developing sustainable greenhouse tomato cultivation practices (Usero et al., 2021; Usero et al., 2023).

2 Characteristics of Soil Ecosystems in Greenhouse Tomato Cultivation

2.1 Changes in soil physicochemical properties under greenhouse cultivation

Greenhouse tomato cultivation often leads to significant alterations in soil physicochemical properties due to intensive management practices such as frequent irrigation and heavy fertilizer application. These changes typically include increased soil nutrient contents, especially nitrogen (N), phosphorus (P), and organic carbon (C), which initially improve soil fertility but may later contribute to nutrient imbalances and soil acidification. For example, studies have shown that total nitrogen and organic carbon levels tend to rise during the early years of greenhouse cultivation but can decline or stabilize after prolonged monoculture, reflecting a dynamic shift in soil nutrient status over time (Dang et al., 2022; Li et al., 2025). Additionally, soil pH often decreases under continuous greenhouse cropping, which can negatively affect nutrient availability and microbial activity (Figure 1) (Hao et al., 2019; Chen et al., 2022). Such physicochemical shifts are critical because they influence the overall soil environment and its capacity to support healthy crop growth.

The accumulation of nutrients like nitrogen and phosphorus is closely linked with changes in enzyme activities that regulate nutrient cycling in the soil. Enzymes such as urease, phosphatase, and catalase are sensitive indicators of soil biochemical functioning and are often affected by long-term greenhouse cultivation. Research indicates that enzyme activities may initially increase with moderate continuous cropping but tend to decline after extended periods, signaling deteriorating soil health (Fu et al., 2017; Lyu et al., 2025). Moreover, excessive fertilization can alter microbial metabolic patterns by shifting community-level physiological profiles, which further impacts nutrient transformations and availability (Hao et al., 2019). These physicochemical property changes underscore the need for balanced fertilization strategies to maintain sustainable soil fertility in greenhouse tomato systems.

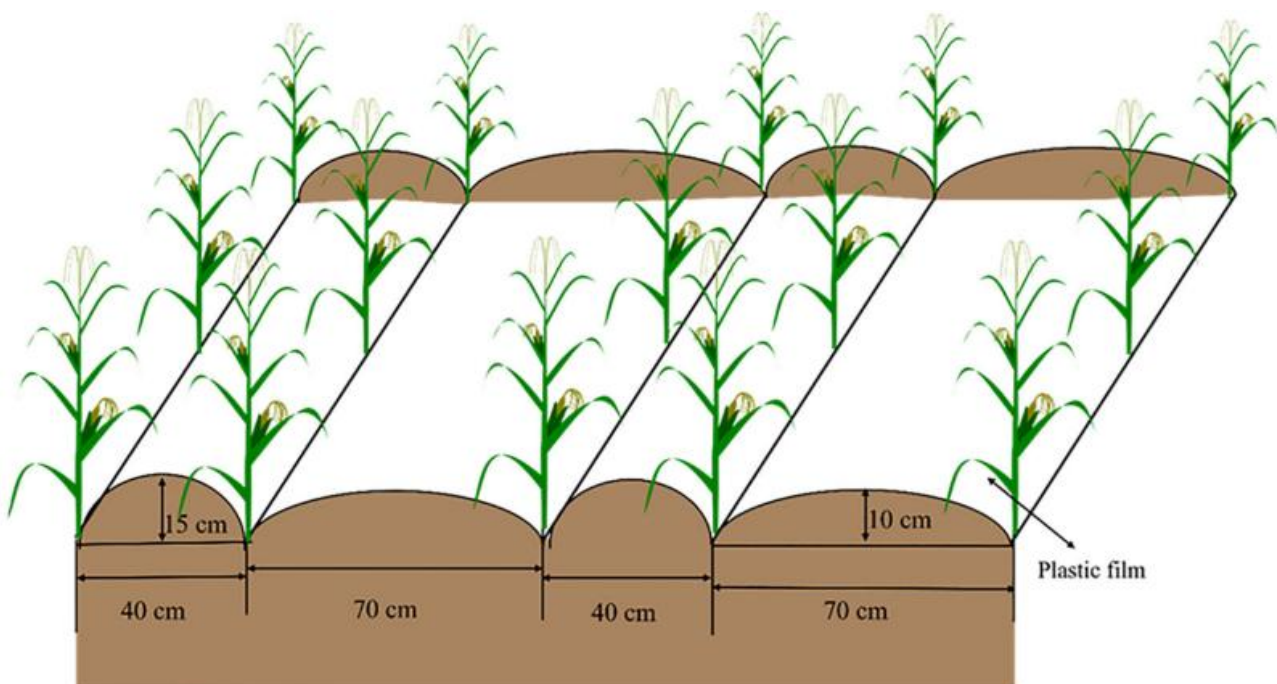


Figure 1 Graph of the whole film of double ridge furrow planting technology (Adopted from Hao et al., 2019)

2.2 Effects of long-term greenhouse cultivation on soil microbial diversity

Long-term greenhouse tomato monoculture has been consistently associated with declines in soil microbial diversity and richness, particularly affecting bacterial and fungal communities essential for maintaining soil ecosystem functions. Studies tracking fields with 5 to over 20 years of continuous tomato cropping report significant reductions in both bacterial and fungal diversity indices compared to non-cultivated or rotational cropland soils (Fan et al., 2024; Hu et al., 2025). This loss of microbial diversity is often accompanied by shifts in dominant taxa; for instance, Actinobacteria abundance tends to decrease while certain pathogenic fungi such as *Fusarium* increase with prolonged monoculture (Chen et al., 2022). Such changes reduce the resilience of the microbial community against environmental stresses and pathogen invasion.

Microbial community structure also undergoes notable alterations under long-term greenhouse conditions. Beta diversity analyses reveal distinct clustering patterns corresponding to different durations of monoculture, indicating progressive divergence from healthy baseline communities (Fan et al., 2024). Network complexity within bacterial communities may initially increase but eventually declines after extended cropping periods, reflecting reduced microbial interactions and stability (Dang et al., 2022; Hu et al., 2025). These structural shifts are linked with functional consequences such as impaired nutrient cycling and diminished disease suppression capacity. Maintaining or restoring microbial diversity through management interventions is therefore critical for sustaining productive greenhouse tomato cultivation.

2.3 Microbial mechanisms underlying soil degradation and continuous cropping obstacles

Soil degradation under continuous greenhouse tomato cultivation is driven largely by microbial community imbalances that disrupt key ecological processes supporting soil health. One major mechanism involves the accumulation of pathogenic fungi like *Fusarium*, *Alternaria*, and *Cladosporium* that cause diseases such as wilt and leaf mold; their relative abundance increases significantly with longer monoculture duration (He et al., 2025; Hu et al., 2025). Concurrently, beneficial bacteria genera including *Bacillus*, *Paenibacillus*, and *Streptomyces* decline, weakening natural disease suppression mechanisms. This shift from a balanced microbiome toward pathogen dominance contributes directly to continuous cropping obstacles.

In addition to pathogen proliferation, long-term monoculture alters microbial functional potential related to carbon and nitrogen cycling. Functional gene predictions indicate decreased expression of genes involved in organic matter decomposition and nutrient transformation after extended cropping periods (Dang et al., 2022; Li et al., 2025). This results in impaired metabolic activity within the microbial community, reducing nutrient availability for plants despite high total nutrient contents in the soil. Furthermore, increased stress resistance genes suggest that microbes experience environmental pressures such as nutrient imbalances or toxic accumulations under continuous cropping (Dang et al., 2022). Understanding these microbial mechanisms provides a foundation for developing strategies-such as organic amendments or crop rotation -to mitigate soil degradation and enhance sustainability in greenhouse tomato production systems.

3 Types of Organic Fertilizers and Their Mechanisms in Soil Improvement

3.1 Common types of organic fertilizers and their nutrient characteristics

Organic fertilizers commonly used in agriculture include manure, compost, biochar, and insect-derived fertilizers such as those from black soldier fly larvae. Manure and compost are rich in organic matter and provide a broad spectrum of nutrients including nitrogen (N), phosphorus (P), and potassium (K), which are essential for plant growth. Biochar, a carbon-rich product derived from pyrolyzed biomass, is valued for its ability to improve soil structure and nutrient retention but generally contains lower nutrient concentrations compared to manure or compost (Khan et al., 2024; Wang et al., 2024). Recently, black soldier fly (BSF) organic fertilizer has gained attention due to its high nutrient content and ability to enhance soil fertility through the bioconversion of organic waste into nutrient-rich amendments (Zhao et al., 2025). These diverse organic fertilizers differ not only in nutrient composition but also in their effects on soil microbial communities and nutrient cycling processes.

The nutrient release patterns of these organic fertilizers vary significantly. Manure and compost typically release nutrients more slowly than chemical fertilizers, providing a sustained supply that supports long-term soil fertility. Biochar's primary role is improving soil physical properties such as porosity and water retention rather than

directly supplying nutrients (Khan et al., 2024; Cao et al., 2025). BSF organic fertilizer combines rapid nutrient availability with beneficial microbial stimulation, enhancing both carbon (C) and nitrogen (N) cycling in soils (Figure 2) (Zhao et al., 2025). Understanding these nutrient characteristics is crucial for selecting appropriate organic amendments tailored to specific crop needs and soil conditions.



Figure 2 Major categories of organic fertilizers used in agriculture, including manure, compost, biochar, and black soldier fly-derived fertilizers, and their primary functions in soil fertility management (Adopted from Zhao et al., 2025)

3.2 Regulatory effects of organic fertilizers on soil physicochemical properties

Application of organic fertilizers generally improves key soil physicochemical properties including soil organic carbon (SOC), total nitrogen (TN), available phosphorus (AP), pH, and soil structure. Studies have demonstrated that partial substitution of chemical fertilizers with organic amendments increases SOC fractions such as easily oxidizable organic carbon (EOC) and particulate organic carbon (POC), which enhance soil fertility and aggregate stability (Wang et al., 2024; Chen et al., 2025). Organic fertilizer application also tends to increase total phosphorus content and available phosphorus by promoting phosphorus cycling genes, thereby improving phosphorus bioavailability (Ying et al., 2023; Wang et al., 2025). Moreover, the addition of biochar combined with organic fertilizer can significantly raise SOC and total phosphorus levels while moderating soil pH changes that often occur under intensive cropping systems (Cao et al., 2025; Yang et al., 2025).

Organic amendments also influence enzyme activities related to nutrient cycling. For example, β -glucosidase activity, important for carbon cycling, can increase dramatically following combined biochar-organic fertilizer treatments, indicating enhanced microbial metabolic function (Yang et al., 2025). However, some phosphatase activities may decrease with certain co-applications of chemical and organic materials due to shifts in microbial community functions (Wang et al., 2025). Additionally, improvements in soil porosity and water retention have been observed with moderate rates of organic fertilizer application, which help reduce water consumption during critical crop growth stages (Chen et al., 2025). These regulatory effects collectively contribute to improved soil health and sustainable crop production.

3.3 Effects of organic fertilizers on soil carbon-nitrogen cycling and nutrient transformation

Organic fertilizers play a pivotal role in enhancing soil carbon and nitrogen cycling by stimulating microbial communities responsible for these processes. Metagenomic analyses reveal that treatments with black soldier

fly-derived fertilizer or biochar-organic fertilizer mixtures upregulate genes involved in carbon fixation pathways such as the Calvin-Benson-Bassham cycle while suppressing genes related to carbon degradation, promoting net carbon sequestration in soils (Yang et al., 2025; Zhao et al., 2025). In terms of nitrogen cycling, the abundance of functional genes associated with nitrification, denitrification (e.g., nirK, nirS), ammonification, and nitrogen fixation increases significantly after organic fertilizer application, accelerating nitrogen turnover efficiency beyond additive effects seen with chemical fertilization alone (Chu et al., 2025).

Furthermore, long-term application of manure-based or integrated chemical-organic fertilization enhances microbial diversity and stability while slowing down rapid nutrient transformation processes typical under exclusive chemical fertilization regimes (Ying et al., 2023; Chen et al., 2025). This balance helps maintain sustained nutrient availability without excessive losses through leaching or gaseous emissions. Structural equation modeling indicates that improved microbial functional potential mediated by enhanced bacterial diversity directly contributes to increased ecosystem multifunctionality under combined fertilization strategies (Ying et al., 2023). Overall, the use of organic fertilizers fosters a more resilient soil microbial ecosystem that supports efficient carbon-nitrogen cycling critical for sustainable greenhouse tomato cultivation.

4 Effects of Organic Fertilizer Application on Soil Microbial Community Structure

4.1 Influence of organic fertilizers on microbial abundance and diversity

Organic fertilizer application generally enhances soil microbial abundance and diversity compared to conventional chemical fertilization. Studies have shown that partial substitution of chemical fertilizers with organic ones significantly increases bacterial richness and diversity indices such as ACE and Chao1, alongside improvements in soil pH, organic matter, and nutrient availability (Guo et al., 2025). These changes create a more favorable environment for microbial growth, leading to denser and more complex microbial networks. For example, the abundance of dominant bacterial phyla like Proteobacteria and fungal groups such as Basidiomycota can increase substantially under organic fertilization regimes (Pan et al., 2025). This enhanced microbial diversity is critical for maintaining soil health and resilience in intensive cropping systems.

Moreover, the degree of organic fertilizer substitution influences microbial community evenness and richness differently depending on soil cultivation duration. In soils with longer cultivation histories, moderate to high organic substitution ratios (25%-75%) tend to optimize microbial diversity and evenness, while very low or very high substitution rates may be less effective (Guo et al., 2025). Organic amendments stimulate enzyme activities related to nutrient cycling, further supporting microbial proliferation (Lazcano et al., 2013). These findings suggest that tailored organic fertilizer applications can sustainably improve microbial abundance and diversity in greenhouse soils.

4.2 Regulatory effects of organic fertilizers on dominant microbial groups and functional microorganisms

Organic fertilizers selectively promote beneficial microbial taxa while suppressing some oligotrophic or pathogenic groups. Long-term manure application has been shown to increase the relative abundance of copiotrophic bacteria such as Bacillales, Gaiellales, and fungal orders like Pezizales, which are associated with efficient organic matter decomposition and nutrient cycling (Lin et al., 2019). Conversely, soils without manure tend to harbor more oligotrophic taxa like Acidobacteria that thrive in nutrient-poor conditions (Francioli et al., 2016). Organic amendments also enhance populations of plant-beneficial microbes including Actinobacteriota and Glomeromycota fungi, which contribute to nutrient uptake and disease resistance (Shu et al., 2022; Abudurezike et al., 2025).

Functional microorganisms involved in carbon and nitrogen cycling respond positively to organic fertilizer inputs. For instance, genes linked to urease activity and catalase are stimulated by organic amendments, improving nitrogen mineralization processes (Lazcano et al., 2013; Guo et al., 2025). Additionally, shifts in fungal communities toward Ascomycota and Basidiomycota dominance under organic fertilization enhance decomposition of complex organics (Figure 3) (Abudurezike et al., 2025; Cong et al., 2025). These regulatory effects help restore balanced microbial functions critical for sustaining soil fertility under intensive greenhouse cultivation.

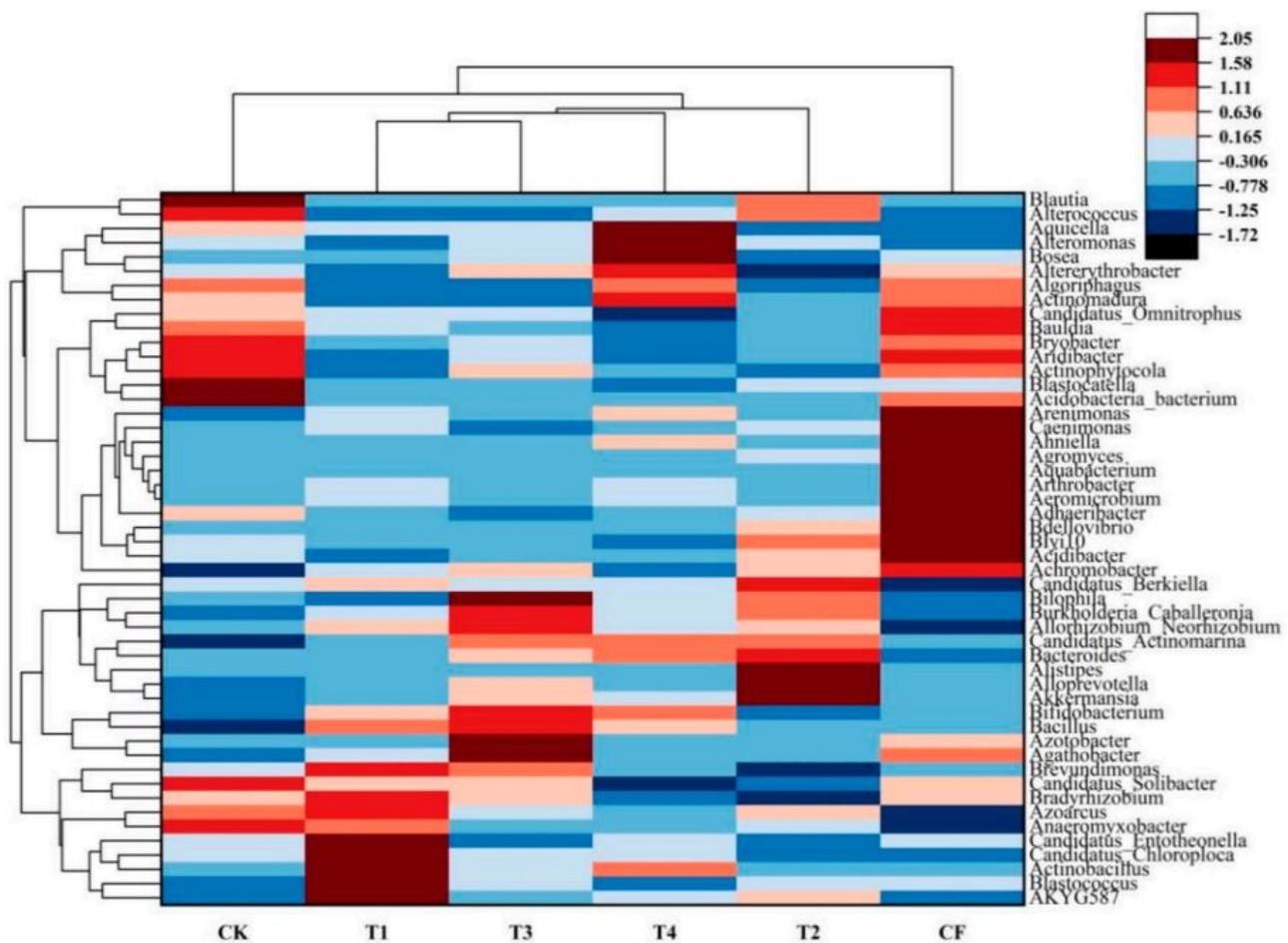


Figure 3 Cluster analysis of the top 50 soil bacteria at the genus level (Adopted from Abudurezike et al., 2025)

Image caption: CK -no fertilization; CF -only the chemical fertilizer; T1 -25% organic replacement; T2 -50% organic replacement; T3 -75% organic replacement; and T4 -100% organic replacement. The color bar represents the relative abundance of genera across treatments (Adopted from Abudurezike et al., 2025)

4.3 Differences in microbial community structure under different fertilization regimes

Distinct fertilization regimes produce markedly different soil microbial community structures. Long-term studies comparing mineral-only, organic-only, and combined fertilization reveal that organic treatments increase total organic carbon (TOC), pH, and nutrient pools more than mineral fertilizers alone, driving shifts toward copiotrophic bacterial groups such as Proteobacteria and Firmicutes (Francioli et al., 2016; Lin et al., 2019). In contrast, mineral-fertilized soils often show enrichment of oligotrophic taxa adapted to low-nutrient environments. Fungal communities also differ; organic amendments favor Zygomycota and Basidiomycota while mineral treatments may support higher chitinase activity but lower overall fungal diversity (Francioli et al., 2016).

Network analyses indicate that organic fertilization fosters more complex co-occurrence patterns among microbes compared to chemical fertilization, reflecting stronger synergistic interactions within the community (Lin et al., 2019; Pan et al., 2025). Moreover, aggregate size influences community composition; microaggregates harbor distinct keystone taxa sensitive to fertilization type (Lin et al., 2019). These structural differences translate into functional variations affecting nutrient cycling efficiency and disease suppression capacity. Overall, integrating organic fertilizers into greenhouse tomato cultivation promotes a more diverse, stable, and functionally robust soil microbiome than exclusive reliance on chemical fertilizers.

5 Effects of Organic Fertilizer Application on Soil Microbial Functions

5.1 Effects of organic fertilizers on microbial metabolic functions and enzyme activities

Organic fertilizer application significantly enhances soil microbial metabolic functions and enzyme activities, which are critical for nutrient cycling and soil health. Meta-analyses show that organic amendments increase

microbial diversity and functionality more than mineral-only fertilization, with positive effects on enzymes involved in carbon, nitrogen, and phosphorus cycling (Shu et al., 2022; Liu et al., 2023). For example, β -glucosidase, urease, and phosphatase activities often increase following organic fertilizer application, reflecting enhanced microbial capacity to decompose organic matter and mineralize nutrients (Lazcano et al., 2013; Ren et al., 2021). These enzymatic changes improve soil biochemical processes that support plant growth.

Furthermore, combined applications of biochar and organic fertilizers have been found to boost the abundance of functional microbial taxa related to carbon degradation, nitrification, and phosphorus mineralization more effectively than either amendment alone (Hu et al., 2024). This synergy leads to increased ecosystem multifunctionality by enhancing multiple soil functions simultaneously. The improvements in enzyme activities are closely linked to shifts in microbial community structure and increased nutrient availability, highlighting the integral role of organic fertilizers in stimulating microbial metabolism (Ren et al., 2021).

5.2 Influence of organic fertilizers on microorganisms involved in soil nutrient cycling

Organic fertilizers promote the proliferation of microorganisms that drive key nutrient cycling processes such as carbon fixation, nitrogen transformation, phosphorus solubilization, and sulfur cycling. Studies report increased abundances of functional genes related to these cycles under organic amendment treatments compared to chemical fertilization (Cui et al., 2023; Hu et al., 2024). For instance, copiotrophic bacterial groups like Proteobacteria and Bacteroidetes -known for their roles in nutrient turnover -are enriched by organic inputs (Tang et al., 2022). Similarly, fungal phyla such as Basidiomycota contribute to complex organic matter decomposition under these regimes.

The enhanced presence of microbes involved in nitrogen cycling -including nitrifiers, denitrifiers, and nitrogen fixers -improves nitrogen availability while potentially reducing losses through leaching or gaseous emissions (Hu et al., 2024; Guo et al., 2025). Organic amendments also stimulate phosphorus-mineralizing microbes that increase phosphorus bioavailability critical for tomato growth (Ren et al., 2021; Cui et al., 2023). These functional shifts collectively optimize nutrient transformations in greenhouse soils, supporting sustainable crop production.

5.3 Impacts of microbial functional changes on tomato growth and yield

Improvements in soil microbial functions driven by organic fertilizer application translate into enhanced tomato growth and yield outcomes. Increased microbial diversity and enzyme activities correlate positively with higher photosynthetic efficiency and fruit production under greenhouse conditions (Shu et al., 2022). For example, bio-organic fertilizer applied at optimal rates significantly raised net photosynthesis by nearly 30% and fruit yield by over 40%, linked to improved soil organic matter content and total nitrogen levels mediated by beneficial microbes (Lu et al., 2025).

Moreover, partial substitution of chemical fertilizers with organic manure enhances soil multifunctionality through increased bacterial-fungal network complexity and keystone taxa abundance, which supports nutrient availability and plant health (Ren et al., 2021; Tang et al., 2022). These microbiome-mediated pathways contribute to more resilient agroecosystems capable of sustaining high yields while reducing reliance on synthetic inputs. Overall, the positive feedback between microbial functional enhancement and tomato productivity underscores the value of integrating organic fertilizers into greenhouse cultivation systems.

6 Case Study: Application Effects of Organic Fertilizer Substitution for Chemical Fertilizer in Greenhouse Tomato Soil

6.1 Experimental design and research methods

Several studies have investigated the effects of substituting chemical fertilizers with organic fertilizers in greenhouse tomato cultivation using controlled field and pot experiments. One common approach involves setting up multiple treatments with varying substitution ratios, such as 25%, 50%, and 75% replacement of chemical fertilizer by microbial or bio-organic fertilizers, alongside a full chemical fertilizer control (Tran et al., 2025). These experiments typically measure soil chemical properties, microbial community changes, tomato growth parameters, yield, and fruit quality over one or more growing seasons. Some studies also incorporate microbial

inoculants like *Trichoderma* to enhance the bio-organic fertilizer effect (Figure 4) (Ye et al., 2020). Soil enzyme activities and microbial biomass are frequently assessed to understand functional changes induced by fertilization regimes (Rong et al., 2018; Li et al., 2023).

Experimental designs often use randomized complete block designs (RCBD) or similar statistical frameworks to ensure reliable comparisons among treatments (Liu et al., 2025; Tran et al., 2025). Measurements include soil nutrient contents (N, P, K), pH, organic matter, microbial diversity via sequencing or phospholipid fatty acid analysis, and enzyme activities such as urease and catalase (Rong et al., 2018; Fan et al., 2023). Tomato agronomic traits like plant height, leaf number, photosynthetic rate, fruit number, weight, and quality indicators (soluble sugars, vitamin C, nitrate content) are recorded to link soil changes with crop performance (Stoleru et al., 2020; Li et al., 2023). These comprehensive methods enable evaluation of how partial organic substitution affects both soil health and tomato productivity.

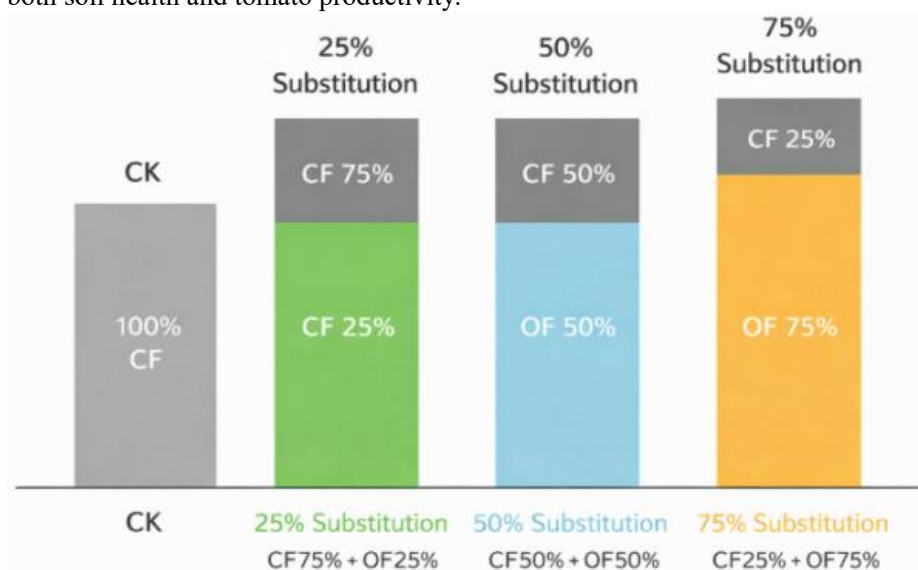


Figure 4 Experimental treatment structure illustrating different substitution ratios of chemical fertilizer with organic or bio-organic fertilizers in greenhouse tomato cultivation experiments (Adopted from Ye et al., 2020)

6.2 Analysis of changes in soil microbial community structure after organic fertilizer application

Partial substitution of chemical fertilizers with organic amendments consistently alters soil microbial community structure by increasing microbial abundance and shifting dominant taxa. Studies report that moderate substitution rates (around 25%-30%) enhance bacterial richness and promote copiotrophic groups such as Proteobacteria and Actinobacteria while reducing oligotrophic taxa like Acidobacteria commonly found under pure chemical fertilization (Liu et al., 2025; Tran et al., 2025). Fungal communities also shift toward beneficial groups including Basidiomycota and Glomeromycota that support nutrient cycling and plant health (Ye et al., 2020; Fan et al., 2023). These changes are often accompanied by increased microbial biomass carbon and nitrogen as well as elevated enzyme activities related to nutrient mineralization (Rong et al., 2018; Liu et al., 2025).

Metagenomic analyses reveal that organic substitution enriches genes involved in nitrogen cycling processes such as denitrification (*nirK*, *nirS*) and nitrogen fixation, indicating enhanced functional potential of the microbiome (Liu et al., 2025). Network complexity within the microbial community increases under organic amendments due to improved soil pH and organic matter content, fostering stronger interactions among microbes (Han et al., 2025). This restructuring supports a more resilient soil ecosystem capable of sustaining nutrient availability for tomatoes. However, excessive substitution beyond moderate levels may reduce yield despite further microbiome shifts, highlighting the importance of balanced fertilization strategies.

6.3 Comprehensive effects on tomato yield, quality, and soil health

The integration of organic fertilizers partially replacing chemical inputs generally improves tomato yield stability while enhancing fruit quality attributes such as soluble sugars and vitamin C content. For example, combining

reduced chemical fertilizer rates (75%) with bio-organic fertilizers containing beneficial microbes like *Trichoderma* maintained yields equivalent to full chemical fertilization but significantly increased fruit sugar (+24%) and vitamin C (+57%) while reducing nitrate accumulation (-62%) in tomatoes (Ye et al., 2020). Similarly, digestate-based organic fertilizers combined with chemical fertilizers increased tomato yield by up to 26% compared to chemical-only treatments under greenhouse conditions while improving fruit protein content and sugar-acid balance (Li et al., 2023).

Soil health benefits include increased total nitrogen, organic carbon content, improved pH balance in acidic soils, enhanced enzyme activities (urease, catalase), and reduced bulk density following partial organic substitution (Dong et al., 2019; Fan et al., 2023). These improvements correlate positively with better plant growth parameters such as chlorophyll content and photosynthetic rate (Stoleru et al., 2020; Li et al., 2023). However, long-term studies suggest that moderate substitution ratios (~30%-60%) optimize sustainability by balancing yield gains with environmental impacts; full organic substitution may reduce yield despite improving some soil fertility indices due to nutrient imbalances or slower nutrient release rates (Han et al., 2025; Liu et al., 2025). Overall, partial replacement of chemical fertilizers with organic amendments offers a promising strategy for sustainable greenhouse tomato production by simultaneously enhancing soil microbial functions, crop quality, and environmental outcomes.

7 Research Methods and Analytical Techniques

7.1 Application of high-throughput sequencing in soil microbial studies

High-throughput sequencing (HTS) has become a pivotal tool for investigating soil microbial communities due to its ability to provide comprehensive and high-resolution data on microbial composition and function. HTS techniques, including amplicon sequencing of marker genes (e.g., 16S rRNA for bacteria and ITS for fungi) and shotgun metagenomics, enable researchers to capture the vast diversity of soil microbes, many of which are unculturable by traditional methods (Zhang et al., 2021; Reid et al., 2025). These approaches allow detailed profiling of microbial taxa and functional genes, facilitating insights into how microbial communities respond to environmental changes such as organic fertilizer application in greenhouse soils.

Moreover, HTS can be integrated with RNA sequencing to assess active microbial populations and their metabolic potential, providing a dynamic view of soil microbiomes (Reid et al., 2025). The use of co-assembly strategies in shotgun metagenomics further enhances genome recovery from complex soil samples, improving detection of rare taxa and functional genes (Johansen et al., 2025). This depth of sequencing is essential for understanding the intricate interactions within soil microbial communities that influence nutrient cycling and plant health in tomato cultivation systems.

7.2 Microbial diversity analysis and bioinformatics methods

Analyzing microbial diversity from HTS data involves calculating alpha diversity metrics (e.g., species richness, Shannon index) to assess within-sample diversity, as well as beta diversity measures to compare community composition across samples (Xia et al., 2020; He et al., 2023). Bioinformatics pipelines typically include quality filtering, sequence clustering or denoising into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs), taxonomic assignment using reference databases, and statistical analyses to identify significant differences among treatments or environmental gradients (Zhang et al., 2021; Edwin et al., 2025). These methods reveal how factors like organic fertilizer substitution alter bacterial and fungal community structures in greenhouse soils.

Advanced analyses also incorporate network construction to explore microbial co-occurrence patterns and identify keystone taxa that drive ecosystem functions (Zhao et al., 2025). Functional prediction tools infer metabolic capabilities based on taxonomic profiles, linking community shifts to potential changes in nutrient cycling processes (Xia et al., 2020). The choice between amplicon versus shotgun sequencing depends on project goals; amplicon sequencing offers cost-effective community profiling while shotgun approaches provide deeper functional insights but require more computational resources (Edwin et al., 2025).

7.3 Correlation analysis between soil physicochemical properties and microbial data

Integrating soil physicochemical measurements with microbial community data is critical for elucidating the drivers of microbial diversity and function. Statistical techniques such as redundancy analysis (RDA), canonical correspondence analysis (CCA), Spearman correlation, and partial least squares path modeling (PLS-PM) are commonly used to link variables like pH, organic matter content, moisture, nutrient levels, and texture with shifts in bacterial and fungal communities (He et al., 2023; Yang et al., 2024). These analyses demonstrate that soil pH often emerges as a primary factor shaping microbial composition, followed by texture components such as sand or clay fractions that influence habitat structure.

Studies show that changes in enzyme activities and nutrient availability induced by organic fertilizers correlate with alterations in specific microbial taxa involved in carbon degradation, nitrogen cycling, and phosphorus solubilization (Chen et al., 2021; Lv et al., 2024). Such correlations help clarify how management practices affect both abiotic conditions and biotic responses in greenhouse tomato soils. Understanding these relationships supports targeted interventions to optimize soil health and crop productivity through informed fertilizer application strategies.

8 Conclusions and Future Research Prospects

Organic fertilizer application in greenhouse tomato cultivation consistently enhances soil microbial diversity, abundance, and functional potential compared to chemical fertilization alone. Studies show that bio-organic fertilizers increase the relative abundance of beneficial microbial taxa such as Proteobacteria and Actinobacteria, which are closely linked to nutrient cycling and plant growth promotion. These microbial shifts are accompanied by improvements in soil properties including organic matter content, total nitrogen, and enzyme activities like urease and catalase, which collectively support healthier soil ecosystems. Enhanced microbial network complexity and interspecies interactions under organic amendments further contribute to soil resilience and nutrient availability. These microbiome changes translate into agronomic benefits such as increased photosynthetic efficiency, higher tomato yields, and improved fruit quality parameters including soluble sugars and vitamin C content. Organic fertilizers also reduce nitrate accumulation in fruits, addressing food safety concerns. However, the magnitude of these benefits depends on the type and application rate of organic fertilizer; moderate substitution rates (around 25%-50%) often optimize both microbial function and crop productivity. Overall, integrating organic fertilizers into greenhouse tomato systems promotes sustainable intensification by simultaneously enhancing soil health, microbial ecology, and crop performance.

Despite advances in understanding organic fertilizer impacts on soil microbiomes, several limitations constrain current knowledge. Many studies focus on short-term experiments or single growing seasons, limiting insights into long-term sustainability and cumulative effects on soil microbial communities. Additionally, variability in organic fertilizer types, compositions, and application rates complicates direct comparisons across studies. The complex interactions between soil physicochemical properties, microbial taxa, and plant responses remain incompletely resolved due to limited multi-omics integration and mechanistic investigations. Another challenge lies in translating microbial community shifts into functional outcomes relevant for disease suppression or nutrient use efficiency. For example, while some research links organic amendments to reduced pathogen incidence through enhanced fungal diversity or key taxa enrichment, causal relationships require further validation. Moreover, environmental factors such as irrigation regimes or soil acidification can modulate fertilizer effects but are often underexplored in greenhouse contexts. Addressing these gaps demands standardized methodologies, longer-term field trials, and interdisciplinary approaches combining microbiology with agronomy.

Future research should prioritize optimizing integrated fertilization strategies that balance chemical inputs with tailored organic amendments to maximize microbial benefits while maintaining yield stability. Precision application guided by high-throughput sequencing data combined with soil physicochemical monitoring can enable site-specific management adapting to dynamic greenhouse conditions. Developing bio-organic fertilizers enriched with beneficial microbes like *Trichoderma* offers promising avenues for enhancing nutrient cycling and disease resistance synergistically with reduced chemical fertilizer use. Long-term studies assessing cumulative

impacts on soil carbon sequestration, greenhouse gas emissions, and microbiome resilience under varying climate scenarios are essential for sustainable intensification goals. Advances in multi-omics technologies (metagenomics, metatranscriptomics) integrated with machine learning will improve functional predictions of microbial communities driving ecosystem services in protected agriculture. Finally, expanding research on interactions between water management practices and fertilization will refine holistic approaches to optimize resource use efficiency while safeguarding soil health in intensive tomato production systems. These directions collectively support the transition toward environmentally sound and productive greenhouse horticulture.

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Conflict of Interest Disclosure

The author affirms 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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