

Exploring Fertilizer-Microbiome Interactions Through Next-Generation Sequencing (NGS): Insights for Sustainable Agriculture

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Abstract: With the global demand for food production escalating, concerns about the long-term sustainability and environmental impact of traditional farming practices, particularly the application of chemical fertilizers, have gained prominence. Recognizing the importance of addressing these issues, this review explores the evolving dynamic between fertilizers and microbial communities, emphasizing the need for a more profound understanding of these interactions. The transformative impact of next-generation sequencing (NGS) technologies in unraveling microbial intricacies within agricultural ecosystems is highlighted as a crucial tool for advancing this understanding. Investigation extends to discerning the nuanced effects of both chemical and non-chemical fertilizers on soil microbiomes, considering variations in soil type and crop specificity. Linking these findings to the Sustainable Development Goals (SDGs), the review highlights the critical connection between fertilizer use, microbial diversity, and the achievement of sustainability objectives. Despite the potential of NGS, the review acknowledges current limitations, sparking discussions on potential technological advancements and methodological improvements. Emphasizing the necessity for interdisciplinary collaboration, it advocates for comprehensive insights that bridge gaps between microbiology, agriculture, and sustainability. In conclusion, the article synthesizes historical perspectives, cutting-edge technologies, and sustainable development objectives to provide a holistic understanding of the intricate interplay between fertilizers, microbial diversity, and the imperative path toward a more sustainable agricultural future.

Keywords: Fertilizers, microbial diversity, sustainable agricultural practices, next-generation sequencing (NGS) technologies, sustainable development goals (SDGs)

1. Introduction

Background

Agriculture is the backbone of human civilization, providing sustenance, economic stability, and a foundation for societal development. Its global significance is indisputable, as it not only supports the basic human need for food but also fuels economies (Shen et al., 2023), shapes landscapes (Martello et al., 2023), and influences cultural identities (Zheng et al., 2023). The unprecedented growth of the world's population and the escalating demand for food resources have intensified the pressure on agricultural systems, necessitating innovative approaches to ensure sustainable food production.

At the core of modern agricultural practices lies the pivotal role of fertilizers. Fertilizers have become vital tools for farmers seeking to enhance crop yields and meet the escalating demands of a growing population (Mustafa et al., 2023). These chemical and non-chemical formulations offer valuable nutrients to plants, augmenting soil fertility and addressing nutrient deficiencies.

While fertilizers have played a critical role in boosting agricultural productivity, their widespread use has raised concerns about environmental impact, including soil acidification, greenhouse gas emissions, depletion of the ozone layer, and loss of biodiversity (Bai et al., 2020; Mustafa et al., 2023). As we delve into the intricate relationship among fertilizers and microbial communities, it becomes apparent that a nuanced understanding of these dynamics is essential for sustainably shaping the future of agriculture. This review investigates the transformative potential of Next Generation Sequencing (NGS) in unraveling the complex interplay between fertilizers and microorganisms, offering insights that can pave the way for achieving Sustainable Development Goals (SDGs) in agriculture.

Rationale for the Review

The escalating global demand for food production drives the imperative to reassess and optimize agricultural practices. With the world's population steadily climbing, the need for increased food yields places unprecedented pressure on the agricultural sector. This heightened demand necessitates a critical evaluation of existing farming methods to ensure both quantity and the quality and sustainability of our food supply. Simultaneously, the reliance on conventional fertilizers has sparked environmental and sustainability challenges that cannot be ignored. Runoff from these chemical inputs contributes to water pollution, adversely

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impacting aquatic ecosystems, while the accumulation of excess nutrients in the soil can lead to degradation and long-term loss of fertility (AbdelRahman et al., 2022; Li et al., 2022a). Addressing these challenges requires a major shift towards more environmentally conscious and sustainable agricultural practices.

Amidst these challenges, the introduction of NGS emerges as a transformative tool to deepen our understanding of microbial dynamics in agriculture. NGS technologies, including both shotgun and amplicon sequencing, enable researchers to investigate the genetic landscapes of microbial communities, offering unparalleled insights into their composition, diversity, and functional potential (Liu et al., 2021a; de Vries et al., 2023; Maretto et al., 2023; Tang et al., 2023). It holds the promise of unraveling the complex relationships between fertilizers and microorganisms and guiding the development of sustainable agricultural practices for the future (Chouhan et al., 2023). This review aims to investigate the synergy between fertilizer use, microbial dynamics, and NGS technologies, highlighting on the path toward achieving Sustainable Development Goals in agriculture.

2. Fertilizers and Microbial Diversity: a Historical Perspective

Historically, the relationship between fertilizers and microorganisms has been focused primarily on nutrient provision to plants. Traditional agricultural practices often treated soil as a mere substrate for plant growth (Thompson, 1992; Lafaille, 2017), with little consideration for the intricate web of microbial life beneath the surface. While effective in boosting crop yields, this limited perspective failed to account for the broader ecological consequences of altering microbial ecosystems. It aimed to replenish essential nutrients without heavily understanding the dynamic interplay between these chemical inputs and the diverse microbial communities inhabiting the soil and plant root systems (Russel & Williams, 1977; Inubushi & Acquaye, 2004). However, the traditional mindset that viewed soil merely as a medium for plant growth is gradually transforming into a holistic approach that considers the soil as a living ecosystem (Ponge, 2015).

As agriculture evolved and intensified, the limitations of these traditional views became increasingly apparent that the indiscriminate use of chemical fertilizers led to soil degradation, negative impacts on microbial biodiversity, and environmental pollution (Feng et al., 2022; Mukhles et al., 2022; Wang et al., 2022; Nguyen et al., 2023). The emergence of these challenges underscored the need for a deeper understanding of the intricate relationships between fertilizers and microorganisms. The dynamic nature of microbial communities, their symbiotic relationships with plants, and their roles in nutrient cycling and soil health became focal points for researchers seeking sustainable agricultural solutions (Abid et al., 2021; Iqbal et al., 2023; Wei et al., 2023a; Zhang et al., 2023a). Acknowledging the complexity of these interactions is vital for mitigating the unintended consequences of fertilization and steering agricultural practices toward greater ecological resilience.

The historical shift towards environmentally-friendly agricultural practices, as highlighted in the Sustainable Development Goals (SDGs) and underscored by scholars like Dubey et al. (2021) and Shahmohammadloo et al. (2021), reflects a growing awareness of the complex interplay between human activities and the environment. This departure from historical practices, often focused solely on maximizing yields with little regard for long-term ecological consequences, is now characterized by a commitment to biodiversity conservation, climate resilience, and reducing ecological footprint. Aligning agricultural strategies with sustainable development objectives underscores a historical trajectory towards a more responsible and conscientious approach, recognizing the importance of integrating sustainable practices to ensure the long-term well-being of ecosystems and harmony between food production and ecological health preservation.

3. Next Generation Sequencing: Revolutionizing Agricultural Microbiology

The aim of sustainable agriculture relies on microbial diversity. These microscopic organisms, including bacteria, fungi, and archaea, contribute to soil fertility, nutrient cycling, and plant health (May et al., 2023; Xiang et al., 2023). A rich and diverse microbial community can enhance plant tolerance to soil salinity (Feng et al., 2023; Wang et al., 2023), suppress pathogenic organisms (Ehau-Taumaunu & Hockett, 2023; Yang et al., 2023a), and improve nutrient availability for plants (Arunrat et al., 2023; Li et al., 2023a). As the agricultural community recognizes the multifaceted benefits of microbial diversity, there is a growing shift towards holistic and regenerative practices that aim to maximize crop yields and foster long-term soil health.

NGS technologies have emerged as powerful tools, propelling agricultural microbiology into a new era of precision and depth. Two primary methodologies, shotgun and amplicon sequencing, form the cornerstone of NGS applications in studying microbial communities. Shotgun Sequencing involves the random sequencing of DNA fragments, providing a comprehensive snapshot of the entire genetic content present in a sample (Doni et al., 2023). Amplicon sequencing, on the other hand, targets specific gene regions such as 16S ribosomal RNA (16S) and Internal Transcribed Spacer (ITS), allowing for a more focused analysis of microbial diversity (Liu et al., 2021a). These approaches help researchers to investigate the genetic complexities of microbial communities with unprecedented resolution, shedding light on the composition, functional potential, and dynamics of these microscopic ecosystems (Liu et al., 2021a; de Vries et al., 2023; Maretto et al., 2023; Tang et al., 2023).

By unlocking the genetic codes of diverse microorganisms through NGS technologies, researchers can elucidate their roles in nutrient cycling, plant-microbe interactions, and overall soil health (Windisch et al., 2021; Kim et al., 2022; Xiang et al., 2023). NGS aids in the identification and quantification of microbial species, offering insights into their functional capabilities and interactions. By exploring the microbial ecosystems, NGS enables

the comprehensive analysis of microbial diversity at a scale and precision unattainable by traditional methods such as PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), Terminal Restriction Fragment Length Polymorphism (T-RFLP), and Fluorescent in Situ Hybridization (FISH) (Wolsing & Priemé, 2004; Caracciolo et al., 2005; Wenhui et al., 2007; Bokulich & Mills, 2012). This transformative capability extends beyond taxonomy, allowing a deeper understanding of the functional attributes governing microbial contributions to agricultural ecosystems.

The application of NGS in agricultural microbiology goes beyond mere taxonomic identification and explores the functional dynamics of microbial communities. Metagenomics, a primary application of NGS, enables the direct study of genomic material extracted from environmental samples, providing valuable insights into the functional potential of microbial communities (Tas et al., 2021). Metatranscriptomics further extends this capability by analyzing the actively expressed genes, allowing researchers to analyze real-time functional activities within microbial ecosystems (Mukherjee & Reddy, 2020). This understanding of genetic composition and functional activities empowers scientists to identify microbial contributions to nutrient cycling, disease suppression, and plant resilience (Chuckran et al., 2021; Mendes et al., 2023; Pande et al., 2023). The integration of metagenomic and metatranscriptomic data opens avenues for targeted interventions, allowing for the development of precision agricultural practices that leverage the specific functions of microbial communities to improve crop health and yield.

The shift from traditional to NGS-based approaches represents a paradigmatic leap in our capacity to assess microbial diversity. NGS offers several advantages over outdated conventional methods, including higher throughput, reduced cost per base pair, and the ability to detect rare and novel microbial taxa (Zhao et al., 2020; Adhikari et al., 2021; Greay et al., 2021). The technology's capacity to provide quantitative data on microbial abundances, community structures, and functional potentials in a single experiment is unparalleled (Hiiesalu et al., 2012; Azarbad et al., 2022). NGS minimizes biases inherent in cultivation-dependent methods, allowing for the identification of previously unculturable microorganisms (Chaudhary et al., 2019; Qaisrani et al., 2019; Sessou et al., 2023; Deinert et al., 2023). It is a transformative tool that broadens our understanding of microbial ecosystems and guides the development of targeted strategies for enhancing agricultural productivity and resilience toward sustainable practices.

4. Fertilizer Impact on Microbial Communities: Insights from NGS Studies

Chemical Fertilizers and Their Effects on Microbiomes

The widespread application of chemical fertilizers in modern agriculture has significantly changed the dynamics of soil microbial communities, prompting a closer examination of their impacts through the NGS. Chemical fertilizers, rich in nitrogen (N), phosphorus (P), and potassium (K), are known to influence soil pH, nutrient availability, and overall soil structure (Li et al., 2020a;

Liu et al., 2020). However, the consequences of these alterations on microbial diversity and function have become increasingly apparent with the application of NGS technologies.

Apart from demonstrating an increase in the abundance of the denitrification-related gene *nirK* (Carrascosa et al., 2023), chemical fertilization exhibited an increased abundance of genes associated with methane oxidation, soil nitrogen degradation, nitrification, and anammox, emphasizing a distinct influence on nutrient cycling as shown by the 16S amplicon studies (Hu et al., 2022a). The NPK fertilization also positively influenced acidophilic groups, such as nitrifiers and denitrifiers, based on 16S and ITS amplicon sequencing, and concurrently increased the alpha diversity of arbuscular mycorrhizal (AM) fungi in the root endosphere (Semenov et al., 2020; Ma et al., 2021). According to Hu et al. (2022b), nitrogen (N) fertilization induced the saprotroph fungal functional group, which may suggest its potential role in decomposing and absorbing organic matter from dead or decaying organisms using the ITS sequencing approach.

Recent studies using shotgun metagenomic sequencing are further discussed and have revealed novel insights into how the taxonomic makeup of soil microorganisms changes when exposed to chemical fertilizers. High inorganic nitrogen fertilizer led to higher relative abundance in Bacillaceae and Carnobacteriaceae families, while certain bacterial families like Pseudonocardiaceae, Clostridiaceae, Cytophagaceae, Micromonosporaceae, and others were relatively less abundant (Enebe & Babalola, 2021). Apart from increasing soil nutrient availability, excessive K₂SO₄ application in tobacco-planting soil increased copiotrophic groups such as Burkholderiaceae and Rhodospirillaceae families, as well as *Ellin6067* genus, while negatively impacting oligotrophic taxa (Lu et al., 2022). Li et al. (2020b) found that applying NPK fertilizer reduced Proteobacteria and Bradyrhizobium at the genus level and increased Acidobacteria at the phylum level. In a separate study, Li et al. (2022b) reported that NPK fertilizer resulted in decreased Proteobacteria abundance, increased Actinobacteria abundance, the lowest Acidobacteria abundance, and the highest Chloroflexi abundance. Fadji et al. (2020) found that using inorganic fertilizer (NK) led to the dominance of Dothideomycetes at the class level and endophytic Leptosphaeria at the genus level. Leptosphaeria sp. has been reported to promote plant growth by increasing root volume and plant tolerance to salinity and drought (Poveda, 2022; Zhao et al., 2024). These findings highlight the impact of nutrient changes on microorganisms, influencing specific groups that, in turn, can promote plant growth, emphasizing the potential for targeted approaches to enhance overall plant health and productivity.

These studies uncover previously overlooked microorganisms, revealing details of nutrient cycling, organic matter decomposition, and plant-microbe interactions. By integrating data from diverse ecosystems and crops, these investigations offer a complete understanding of the intricate connections between chemical fertilizers and microbial communities. This awareness aids in refining fertilizer applications, lessening environmental impact, and fostering robust and productive farming practices. The research also takes economic factors into

account, directing efforts toward cost-effective and sustainable approaches. Moreover, it addresses social considerations by advocating for microbiome-conscious agriculture, contributing to a more promising future.

Non-Chemical Fertilizers and Their Influence on Microbial Diversity

In contrast to chemical fertilizers, non-chemical alternatives, such as organic or bio-based fertilizers, have gained prominence in agricultural systems aiming for sustainability and reduced environmental impact. The impact of these non-chemical fertilizers on microbial diversity has become a focal point of the investigation, leveraging the capabilities of NGS to delve into the subtleties of microbial responses.

NGS studies have facilitated a signified comparative analysis of the impacts of non-chemical and chemical fertilizers on microbial diversity. By adopting a comparative approach, researchers have unraveled the intricate interactions between diverse fertilizer types and the soil microbiome, with multiple studies highlighting the beneficial effects of both organic matter and fertilizers on agriculture. Zhu et al. (2023a) found that composting enhances microbial diversity, promoting the activity of beneficial genera like *Corynebacterium* and *Lactobacillus*, while influencing nutrient cycling and emissions. Composting also showcases *Marinimicrobium* and *Thermobifida*'s crucial roles in ammonia assimilation, highlighting the diverse microbial contributions (Zhu et al., 2023b). Moreover, bark compost boosts beneficial fungi, including *Penicillium* and *Inocybe*, with potential benefits for nutrient uptake and plant health (Malewski et al., 2023) whereas the combination of compost and citrus rootstocks significantly influences rhizobiome bacterial abundance and diversity, correlating with essential nutrient concentrations for plant health and productivity (Castellano-Hinojosa et al., 2023). *Sophora alopecuroides* L., or Kudouzi in China, traditionally used for medicinal purposes, is also employed as an organic fertilizer, enhancing sugar content and promoting beneficial microorganisms, as indicated by Hua et al. (2023).

Manure serves as an alternative to compost for organic fertilizer. Khatri et al. (2023) claim that green manure applications lead to higher microbial abundance, reduced disease development, and increased abundance of beneficial phyla, thereby contributing to sustainable agriculture. *Erythrobacter* sp. YH-07-inoculated organic manure enhances microbial diversity, suppresses *Fusarium* wilt, and alters community composition. This demonstrates its potential for disease control, as Tang et al. (2023) highlighted. Another finding reveals that Hanwoo manure positively impacts soil organic matter, crude ash, and phosphorus, providing a valuable alternative to chemical fertilizers (Lee et al., 2023). Lastly, implementing reduced tillage with green manure for a decade enhances soil organic carbon and total nitrogen, maintaining almond yields and promoting sustainability, albeit with changes in the bacterial community structure (Özbolat et al., 2023). These findings showcase their significant potential in fostering sustainable agriculture, enhancing soil health, and

mitigating disease risks, emphasizing a promising shift towards eco-friendly farming practices.

One notable trend from the comparative studies is the potential for non-chemical fertilizers to foster greater microbial diversity than their chemical counterparts. Organic amendments, for instance, introduce a spectrum of organic matter that serves as a substrate for a diverse array of microorganisms (Saunders et al., 2012; Verma et al., 2020). This diversity, in turn, contributes to enhanced nutrient cycling, disease suppression, and overall soil health as discussed previously. The comparative insights gained from NGS studies not only inform on the immediate impacts of fertilizer choices but also provide a foundation for developing strategies that optimize microbial diversity in ways that align with sustainable development goals in agriculture (Meuniern & Bayir, 2021; Ashraf et al., 2022).

The Role of Soil Type and Crop Specificity in Shaping Microbial Communities

The relationships between fertilizers and microbial communities are not only influenced by the type of fertilizer. However, they are equally shaped by the unique characteristics of soil types and the specific crops being cultivated. NGS studies have been instrumental in elucidating the complex interplay between soil types, crop specificity, and the dynamic microbial ecosystems they harbor.

Understanding the impact of soil type on microbial communities is crucial, as various soils possess distinct physical and chemical properties that influence microbial life. NGS technologies have unveiled how microbial compositions respond differently to fertilizers in clayey, loamy, and sandy soils. High clay content in the soil offered enhanced physicochemical protection for microbial biomass pools and mitigated water stress, impacting the efficacy of N fertilization on soil microbial communities (Kallenbach & Grandy, 2011; Fierer et al., 2012; Yu et al., 2019). The 16S rRNA sequencing results indicated shifts in bacterial composition post-fertilization, which led to a decrease in the relative abundance of *Chloroflexi*, *Acidobacteria*, and *Nitrospirae* at the phylum level while increasing *Proteobacteria* and *Actinobacteria* (Liu et al., 2021b). The relative abundance of *Gemmatimonadota* and *Firmicutes* significantly increased with NPK treatments, accompanied by a decrease in *Bacteroidetes* (Chen et al., 2023).

On the contrary, sandy loam soils demonstrate a well-rounded capacity for water retention, stable structure formation, adequate aeration, and maintenance of a moderate soil temperature (Lakesh et al., 2022). Fertilizer applications resulted in a major shift in microbial composition, with a lower relative abundance of *Bacteroidetes* and elevated levels of *Proteobacteria* and *Verrucomicrobia* compared to the control (Zhang et al., 2022a). Meanwhile, fertilized sandy loam soils exhibit significant increases in *Ambispora*, *Funneliformis*, and *Glomus*, accompanied by a marked decline in *Paraglomus* at the genus level of the arbuscular mycorrhizal fungal community (Liu et al., 2022). These findings collectively highlight the multifaceted impact of

fertilization on microbial attributes, influencing bacterial and fungal communities in different soil types.

In addition, the crop specificity of microbial communities adds another layer of complexity to the fertilization equation in the NGS studies. A study by Zhang et al. (2023b) reveals that rotating rice and oilseed rape crops significantly enhances soil microbes, particularly those associated with nitrogen, phosphorus, and overall soil quality. A similar study on crop rotation of peppers and eggplants created helpful microbes that protected bananas from diseases, aiding in sustainable banana farming (Hong et al., 2023). Compared to annually rotated crops, corn and soybean crops influenced bacterial communities differently, with grass cover crops having minimal impact, emphasizing the greater role of soil pH and organic matter for bacteria (Chamberlain et al., 2020).

Biochar had different effects on wheat and mash bean soils, increasing specific genes in both, with wheat showing an additional gene increase; the crop type played a major role in how biochar and fertilizer influenced microbes in mash bean soils (Azeem et al., 2020). A study by Woo et al. (2022) found that pea farming clearly reduced microbe diversity compared to wheat, pea-wheat rotations, and fallow fields, impacting diverse fungi, and influencing nitrogen, water, as well as both beneficial and harmful fungi in the Canadian prairies. Consequently, the choice of fertilizer can exert variable effects on microbial communities based on the specific crop it is intended for (Dincă et al., 2022; Gupta et al., 2022; Williams et al., 2023). Integrating crop-specific insights from NGS studies into fertilizer management can enhance nutrient uptake, disease resistance, and overall crop performance. These studies offer a roadmap for customizing agricultural practices based on the intricate relationships among fertilizers, soil types, and crop specificity. This holistic approach acknowledges unique microbial dynamics in diverse agroecosystems, which are crucial for creating sustainable and resilient agricultural systems in the face of global challenges.

5. Sustainable Development Goals (SDGs) and Agricultural Microbial Diversity

Linking Fertilizer Use, Microbial Diversity, and SDGs

The intersection of fertilizer use, microbial diversity, and the ambitious targets set by the SDGs forms a critical nexus in pursuing a more sustainable and equitable global agriculture (Figure 1). Fertilizers, both chemical and non-chemical, play a pivotal role in meeting the SDG mandate to eradicate hunger (SDG 2) by bolstering crop yields (Heidkamp et al., 2021; Saqib et

al., 2020; Ishfaq et al., 2023). However, the implications of fertilizer use extend beyond mere productivity, resonating with several other SDGs that collectively underscore the need for holistic and environmentally responsible agricultural practices (United Nations, 2023).

Microbial diversity emerges as a linchpin in achieving these broader sustainability goals. NGS studies have illuminated the intricate relationships between fertilizers and microbial communities, revealing their profound impact on soil health, nutrient cycling, and ecosystem resilience (Ma et al., 2023; May et al., 2023; Xiang et al., 2023). Recognizing the symbiotic alliances between plants and microorganisms facilitated by appropriate fertilization (Li et al., 2023b) aligns with SDG 15 (Life on Land), promoting the conservation, restoration, and sustainable use of terrestrial ecosystems (Kumawat et al., 2023). Moreover, fostering microbial diversity through judicious fertilizer management contributes to SDG 13 (Climate Action) by enhancing soil carbon sequestration and mitigating greenhouse gas emissions (Jiang et al., 2021; Zhou et al., 2022). The potential to reduce agriculture's environmental footprint while simultaneously increasing its productivity positions microbial-conscious fertilization as a key player in the global effort to combat climate change.

Furthermore, the relationship between fertilizer use, microbial diversity, and the SDGs extends into water resource management, aligning with SDG 6 (Clean Water and Sanitation). Proper fertilization practices that promote microbial diversity contribute to enhanced water retention in soils (Wang et al., 2021; Xu et al., 2023a), reducing the risk of nutrient runoff and contamination of water bodies (Menziez Puer et al., 2020). By mitigating water pollution and ensuring sustainable water use in agriculture, this integrated approach addresses a critical aspect of the SDGs related to clean and accessible water. Implementing microbial-conscious fertilization supports agricultural productivity and safeguards vital water resources, marking a significant stride towards achieving the broader agenda of sustainable development.

Linking fertilizer application, microbial diversity, and SDGs demonstrates the need for an integrative approach to agricultural practices. Harnessing the insights gleaned from NGS studies, this linkage provides a pathway for sustainable intensification, ensuring that the gains in crop production are harmonized with environmental conservation, social equity, and the overarching objectives of the Sustainable Development Agenda.

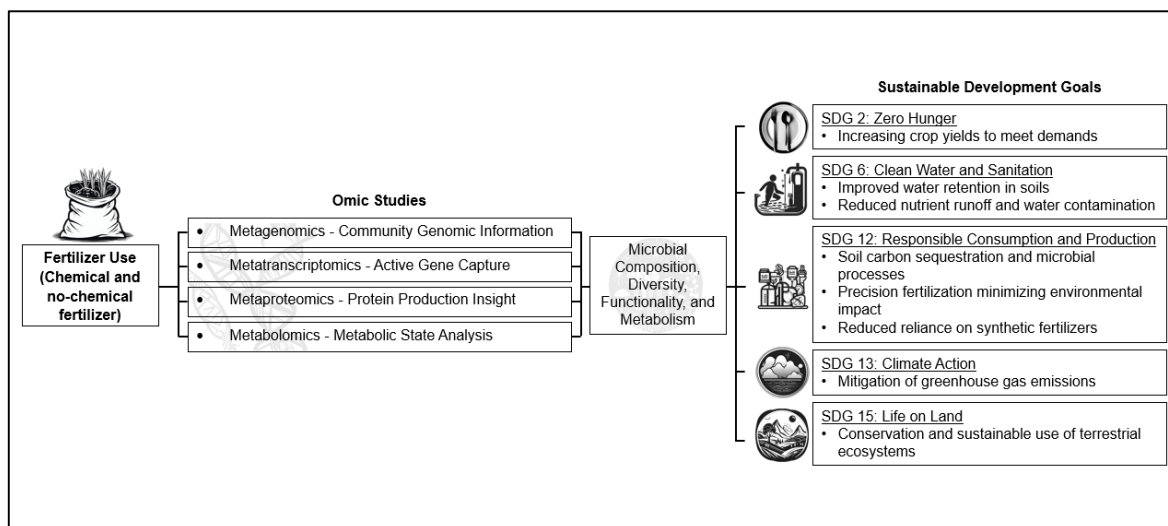


Figure 1. An illustration showing how fertilizer applications and multi-omics studies contribute to the achievement of Sustainable Development Goals

NGS as a Tool for Aligning Agricultural Practices with Sustainability Goals

NGS is a transformative tool that dissects the complex relationships between fertilizers, microbial diversity, and agricultural ecosystems and aligns agricultural practices with the broader canvas of sustainability goals. In the context of the SDGs, NGS acts as a linchpin by providing a comprehensive understanding of microbial communities' responses to different fertilizers and their implications for sustainable agriculture.

NGS offers a holistic view of the soil microbiome, enabling researchers and practitioners to identify microbial signatures associated with sustainable practices. By delineating the impacts of various fertilizers on microbial diversity, NGS facilitates the development of precision fertilization strategies that optimize crop yields efficiently while minimizing environmental harm (Kurzemann et al., 2020; Iqbal et al., 2022; Wyszowska et al., 2022). This aligns with SDG 12 (Responsible Consumption and Production) by reducing the need for excessive resource consumption, minimizing environmental degradation, and supporting the goal of ensuring sustainable consumption and production patterns for a healthier planet.

Furthermore, NGS-driven insights contribute to achieving SDG 2 (Zero Hunger) by fostering resilient agricultural systems. Better understanding the microbial dynamics in response to fertilizers enables the design of strategies that enhance nutrient availability, improve soil health, and, consequently, increase food security (Kurzemann et al., 2020; Nwachukwu & Babalola, 2022; Arunrat et al., 2023). NGS's capacity to uncover microbial contributions to carbon sequestration (Zhou et al., 2022) aligns with SDG 13 (Climate Action), offering methods to reduce climate change through sustainable agriculture.

Moreover, NGS contributes significantly to the pursuit of SDG 15 (Life on Land) by shedding light on the impact of fertilizers on microbial diversity and its cascading effects on terrestrial ecosystems. The ability of NGS to uncover the intricate relationships between microbial communities and the

surrounding environment enables a nuanced understanding of the role of these microorganisms in promoting biodiversity and ecosystem resilience (Wang & Xiong, 2022; Meng et al., 2023; Xu et al., 2023b). This aligns with the broader objective of conserving land ecosystems, restoration, and sustainable use (Küfeoğlu, 2022). Through soil analysis, NGS helps farmers boost agricultural productivity and support ecosystem health. Integrating NGS data into land management strategies preserves biodiversity, prevents soil degradation, and promotes species coexistence. NGS is a powerful tool aligning agriculture with sustainability goals by understanding fertilizer-microbe interactions, enabling precision agriculture for enhanced productivity, environmental protection, and fulfillment of SDGs.

Addressing Global Challenges Through a Microbial Perspective

Embracing a microbial perspective in agriculture is pivotal for addressing the multifaceted global challenges outlined in the SDGs. Using NGS to explore soil microorganisms presents an unprecedented opportunity to address global challenges by gaining insights into their effects on soil health and ecosystem resilience. Agriculture gains a tool for fostering sustainable practices that simultaneously enhance productivity and mitigate environmental impact from the lens of microbial diversity. The microbial perspective contributes directly to SDG 15 (Life on Land) by emphasizing terrestrial ecosystems' conservation and sustainable use (Kumawat et al., 2023; Li et al., 2023b). Besides that, climate change-induced abiotic stresses exacerbate agricultural challenges, particularly when compounded by chemical fertilizer application, which degrades soil health (Wijitkosum, 2018; Azahari & Sukarman, 2023). Nonetheless, leveraging beneficial microorganisms presents a sustainable solution, aligning with SDG 15 by fostering soil resilience and enhancing plant stress tolerance, thus promoting sustainable agricultural practices in the face of climate change.

Understanding and optimizing microbial communities through precision fertilization strategies aligns with SDG 12 (Responsible Consumption and Production), optimizing the application of fertilizers to enhance plant growth while minimizing environmental impact. Precision fertilization leverages microbial processes, such as nitrogen fixation, to make nutrients available to plants, thereby reducing the reliance on synthetic fertilizers (Shi et al., 2021; Lin et al., 2021). This aligns with responsible consumption and production, lowering the environmental footprint of chemical input production and use.

Moreover, a microbial perspective offers innovative solutions to combat climate change (SDG 13). Microorganisms contribute to combating climate change by enhancing soil fertility and structure through the breakdown of organic matter and storing carbon in the soil as a natural sink (Kozjek et al., 2023). Additionally, certain microbes, such as methanotrophic bacteria, play a crucial role in converting methane, a potent greenhouse gas, into less harmful byproducts, aiding in the reduction of overall greenhouse gas emissions (He et al., 2023). The understanding and harnessing microbial processes offer innovative solutions for managing greenhouse gas dynamics, contributing to global efforts to mitigate climate change (Hussain et al., 2022). Harnessing NGS technologies to decipher the microbial contributions to climate mitigation provides a pathway for agriculture to address global climate challenges proactively. By acknowledging the central role of microorganisms in the intricate balance of agroecosystems, the global community can forge a path toward achieving the broader aspirations of the Sustainable Development Agenda.

6. Challenges and Future Directions

Current Limitations in NGS-Based Studies

While NGS has revolutionized our understanding of microbial dynamics in response to fertilizers, several challenges persist in NGS-based studies. One notable limitation is the sheer volume and complexity of generated data (Larson et al., 2023). The extensive datasets produced by NGS technologies present storage, processing, and analysis challenges, demanding sophisticated computational infrastructure and expertise (Larson et al., 2023). This computational bottleneck poses challenges for researchers, particularly those with limited access to high-performance computing resources.

Additionally, the inherent biases and errors introduced during the sequencing process can impact the accuracy and reliability of results. Amplification biases, sequencing errors, and variations in bioinformatics pipelines may contribute to artifacts in the data, necessitating stringent quality control measures (Beckers et al., 2017; Notario et al., 2023). Addressing these technical challenges is vital to ensure the fidelity of NGS-generated information and to foster confidence in the conclusions drawn from such studies. The interpretability of NGS data remains a challenge. As our knowledge of microbial functions and interactions expands, so does the complexity of interpreting the functional implications of microbial diversity changes revealed by NGS (Toole et al., 2021). Integrating multi-omics data and advancing bioinformatics tools are essential for unraveling the functional significance of

microbial shifts, thereby bridging the gap between taxonomic identification and ecological understanding.

In addition to technical challenges, issues related to standardization and comparability across studies pose hurdles in NGS-based agricultural microbiome research. The lack of standardized protocols for sample collection, DNA extraction, and data analysis can lead to variations in results, reducing the comparability of findings across other related studies (Serrano-Silva & Calderon-Ezquerro, 2018; Malczynski et al., 2021). Establishing a set of best practices and guidelines for NGS-based studies in agricultural microbiome research would enhance the consistency and reliability of results (Forry et al., 2023; Hiergeist et al., 2023). Collaborative efforts within the scientific community to develop and adopt standardized methodologies will build a more robust foundation for interpreting and synthesizing insights from diverse studies (Biswas et al., 2023). By addressing technical and standardization challenges, NGS can more effectively contribute to advancing our understanding of microbial responses to fertilizers and supporting the development of sustainable agricultural practices.

As NGS continues to be at the forefront of agricultural microbiome research, addressing these limitations will be pivotal for maximizing the technology's potential and ensuring that insights gained contribute meaningfully to developing sustainable agricultural practices. The ongoing refinement of methodologies, improvement in computational tools, and collaborative efforts across scientific disciplines will shape the future directions of NGS-based studies in unraveling the intricate relationships between fertilizers and microbial communities.

Potential Technological Advancements and Methodological Improvements

The future of NGS studies in agricultural microbiome research holds promise with anticipated technological advancements and methodological innovations. One area ripe for improvement is the enhancement of sequencing technologies themselves. Ongoing efforts to increase read lengths, reduce error rates, and elevate sequencing throughput will alleviate existing challenges in data generation (Callahan et al., 2021; Zhou et al., 2021), making NGS studies more accessible and cost-effective for a broader range of research endeavors. Long-read sequencing platforms like Oxford Nanopore and PacBio offer longer read lengths than traditional short-read platforms (Cook et al., 2024). Error correction tools such as Nanocorr and Canu help improve the accuracy of long-read data (Hu et al., 2020; Marić et al., 2024). For hybrid assembly pipelines, tools such as Unicycler and St. Petersburg Genome Assembler (SPAdes) combine long-read and short-read data for more accurate assemblies (Latorre-Pérez et al., 2020; Xu et al., 2022).

Moreover, the integration of multi-omics approaches is poised to refine our understanding of microbial responses to fertilizers. Coupling metagenomics with meta-transcriptomics, meta-proteomics, and metabolomics can provide a comprehensive view of microbial functions, shedding light on the dynamic interplay between microbial communities and their environment

(Yamazaki et al., 2021; Qiu et al., 2023; Yang et al., 2023b). Moreover, integrating metagenomic and metatranscriptomic analyses using NGS has allowed researchers to move beyond taxonomic identification and understand the functional consequences of chemical fertilizer applications at the molecular level. Metagenomic studies reveal the complete genetic makeup of microbial communities, exposing the genes and pathways involved in important ecological processes. In contrast, metatranscriptomics captures actively expressed genes, offering insights into the functional dynamics of microbial communities (Qian et al., 2020). These advanced analyses have revealed shifts in gene expression associated with nutrient uptake, stress response, and the synthesis of secondary metabolites (Chevrette et al., 2022; Law et al., 2022; Tartaglia et al., 2023). Identifying specific functional genes involved in these processes enhances our ability to predict and manage the ecological outcomes of chemical fertilizer use.

The integration between metagenomics and metabolomics also provides a comprehensive understanding of microbial functionality by revealing both the taxonomic composition of microbial communities and insights into the actual biochemical processes and metabolites. Zhao et al. (2023) demonstrates that long-term fertilization enhances soil quality by promoting the growth of beneficial bacteria and fungi and releasing essential compounds to support plant growth and maintain overall productivity. Additionally, Tian et al. (2023) found that mulching reduces NO₃–N content and influences specific genes (*nrxA* and *nasA*), accelerating microbial metabolism and stimulating the production of various metabolites linked to key biological processes. The P-fertilizer application resulted in a decrease in the diversity of bacterial and fungal genes in the soil. The fertilizer also affected the soil metabolite spectrum, notably influencing seven primary metabolic pathways related to amino acids, plant hormones, and secondary metabolites (Cheng et al., 2022). Integrating metagenomic and metabolomic approaches is crucial for developing sustainable fertilization strategies that optimize nutrient use, minimize environmental impact, and foster a resilient soil microbiome, translating microbial diversity data into actionable insights for sustainable agriculture.

Advancements in bioinformatics tools will play a pivotal role in overcoming current data analysis and interpretation limitations. Machine learning algorithms, for instance, have the potential to discern complex patterns within large and intricate datasets, facilitating more accurate taxonomic assignments and functional predictions (Lee et al., 2022; Zhang et al., 2022b; Cheng et al., 2023). Additionally, user-friendly pipelines that streamline data processing and enhance accessibility will democratize the use of NGS technologies, enabling researchers with diverse backgrounds to leverage these powerful tools.

As technology continues to evolve, the integration of advanced studies, like NGS commonly used in medicine, along with other emerging technologies such as high-resolution imaging and remote sensing (Wang et al., 2020; Zhang et al., 2020; Wei et al., 2023b), holds promise for gaining a more holistic understanding of agroecosystems. These multidisciplinary approaches will

enable researchers to explore microbial dynamics at various scales, from the microscopic to the field-level, fostering a more nuanced comprehension of the intricate relationships between fertilizers, microbial communities, and sustainable agricultural practices.

The Importance of Interdisciplinary Collaboration for Comprehensive Insights

Addressing the complex challenges and unlocking the full potential of NGS in agricultural microbiome research requires a concerted effort in interdisciplinary collaboration (Chiusano, 2015). Integrating expertise from diverse fields such as microbiology, agronomy, bioinformatics, and data science is imperative for advancing our understanding of the relationships between fertilizers and microbial communities.

Microbial responses to fertilizers unfold within the broader context of agroecosystems, encompassing soil health, plant-microbe interactions, and environmental sustainability. An interdisciplinary approach ensures that researchers can navigate this complexity, drawing on various disciplines' insights to interpret NGS-generated data comprehensively. Microbiologists contribute their knowledge of microbial ecology, agronomists provide insights into crop-specific needs, and bioinformaticians bring essential tools for processing and analyzing large-scale genomic datasets.

Furthermore, collaboration between academia, industry, and policy-making entities is essential to bridge the gap between research findings and real-world applications (John et al., 2023). Industry professionals can offer practical insights into the scalability and feasibility of implementing NGS-informed strategies in diverse agricultural settings. Policymakers play a crucial role in translating research into actionable guidelines that promote sustainable and microbiome-conscious agricultural practices on a broader scale.

The challenges NGS studies face in agricultural microbiome research are multifaceted, and their resolution demands a collective effort. By fostering a culture of interdisciplinary collaboration, researchers can harness the strengths of different fields, enriching the depth and applicability of insights gained from NGS technologies. As we look to the future, synergy between diverse disciplines will be pivotal for overcoming challenges and realizing the full potential of NGS in shaping the sustainable future of agriculture.

4. Conclusion

In the journey through the nexus of fertilizers, microbial communities, and sustainable agriculture, this review has unveiled findings elucidated by NGS. NGS studies have provided unprecedented insights into the dynamic relationships between chemical and non-chemical fertilizers, soil types, crop specificity, and microbial diversity. Through comprehensive analyses, researchers have deciphered fertilizer applications' taxonomic and functional implications, offering a deeper understanding of agroecosystem dynamics. These insights underscore the pivotal role of microbial diversity in shaping resilient and sustainable

agricultural systems.

The potential impact of NGS on reshaping agricultural practices cannot be overstated. The depth and precision of information provided by NGS technologies empower farmers, researchers, and policymakers to make informed decisions that balance productivity with environmental and social considerations. NGS lays the foundation for a paradigm shift in agriculture from precision fertilization strategies tailored to specific soil types and crops to the development of microbiome-conscious agricultural policies. The technology's capacity to uncover microbial functions, predict ecosystem responses, and guide sustainable practices positions NGS as a transformative force with far-reaching implications for the future of global agriculture.

As we stand at the cusp of agricultural innovation, a resounding call to action echoes from the insights gained through NGS studies. It is incumbent upon the global community, including farmers, researchers, policymakers, and industry stakeholders, to embrace sustainable and microbiome-conscious agricultural practices. Leveraging the knowledge derived from NGS, we can design and implement strategies that enhance crop yields and prioritize soil health, biodiversity conservation, and climate resilience. This call to action implores us to bridge the gap between scientific knowledge and practical application, fostering a holistic approach that aligns with the principles of the SDGs. Through collaborative efforts, informed decision-making, and a commitment to stewardship of our agroecosystems, we could shape a productive but also sustainable, resilient, and harmonious agricultural future with the microbial world beneath our feet.

5. References

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