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# Microbial Biofortification: A Sustainable Solution to Feed 10 Billion People

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# MICROBIAL BIOFORTIFICATION: A SUSTAINABLE METHOD TO FEED 10 BILLION PEOPLE

By

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# Introduction

Given the projected growth of the human population, the future of food security is an increasing concern. The global population is expected to exceed 9 billion people by 2050 (Wang, 2022). In order to meet food demands, worldwide food production as it currently exists will need to increase by 70% (Wang, 2022). In the context of human health, feeding a growing population is more comprehensive than simply increasing the quantity of food produced. The ultimate goal is to establish food security, or reliable access to a sufficient quantity of nutritious food (El Mujtar et al., 2019). Food security includes four pillars: availability, stability, access, and utilization (El Mujtar et al., 2019).

Availability and stability refer to the total food production for a given population. Currently, availability and stability of food security are largely dependent on external factors such as climate change and agricultural practices. One characteristic that these environmental stressors have in common is their influence on soil health. Soil degradation is characterized by a decline in soil quality and can be caused by physical, chemical, or ecological factors (Lal, 2015). Lal (2015) reported that nearly 33% of earth’s land surface is affected by soil degradation which places stress on agronomic activity in affected areas. In relation to food production, current agriculture practices have severely diminished the quantity of usable soil for crop production (Lal, 2015). Additionally, agrochemical application, or the use of fertilizers and pesticides to control pathogens, has also been found to decrease soil quality by selecting for antimicrobial resistant bacteria (Zhang et al., 2021). Due to the widespread overuse of chemical fertilizers and pesticides, soil biodiversity has decreased, and long-term plant growth has been limited

(Zhang et al., 2021). Given that soil is a nonrenewable resource, the preservation of its quality is essential for sustaining long-term crop productivity and maintaining balance within ecosystems.

Likewise, climate change has been found to decrease soil fertility. In a climate change experiment, Tripathi and contributors (2022) analyzed bacterial and fungal soil microbes that were exposed to one of three experimental conditions: increased carbon dioxide levels, warming, and precipitation. Researchers reported that each experimental group yielded decreased microbial diversity and abundance, suggesting that climate change conditions may reduce the quantity of soil microbes (Tripathi et al., 2022). At the soil level, climate change and agrochemical application hinder plant growth which decreases crop productivity and food production. In other words, drastically increasing global food production through traditional agricultural practices will only place more stress on already-dwindling natural resources. Mitigating abiotic and biotic stressors must be considered in the effort to achieve the availability and stability pillars of food security.

The access and utilization pillars of food security refer to equity within food distribution and the nutritional quality of food, respectively. Thus, food security is not only dependent on increasing the quantity of food produced, but also its nutritional quality. Human nutrition depends on the availability and balance of nutrients in one’s diet. In the context of human health, nutrients are defined as organic materials that are required for growth and sustenance of life. Whereas macronutrients refer to large organic materials (proteins, lipids, and carbohydrates), micronutrients refer to vitamins, minerals, and other trace elements that are essential for proper bodily function (Bertola et al., 2021). Micronutrients are involved in several biochemical processes. Zinc, for example, is a cofactor for over 100 enzymes as well as acts as a transcription factor that regulates DNA binding (Shenkin, 2006). Many micronutrients also have antioxidant properties. Antioxidants are important mitigators of reactive oxygen species, or byproducts of oxidative metabolism that are harmful to cells in excessive quantities (Shenkin, 2006).

In the context of plant health, nutrients refer to inorganic materials in the soil such as phosphorus and nitrogen that are required for growth and acquisition of nutrients (Bertola et al., 2021). Human health is dependent on plant uptake of inorganic nutrients from the soil (Bertola et al., 2021). Thus, the micronutrient density of crops for human health is dependent on the soil composition. Over time, micronutrient deficiency is associated with biochemical or physiological consequences. For example, iron deficiency results in inadequate synthesis of hemoglobin and myoglobin, resulting in poor oxygen transport (Musallam & Taher, 2018). The physiological side effects of iron deficiency are fatigue, lethargy, and dyspnea (Musallam & Taher, 2018). While micronutrient deficiencies like iron deficiency are common worldwide, they are most prevalent in low-income countries (Sharma et al, 2017).

It is estimated that over 820 million people suffer from hunger and malnutrition, but the scope of food insecurity extends far beyond this number (Wang, 2022). In addition to hunger and malnutrition, over two billion people experience micronutrient malnutrition (Sharma et al., 2017). Otherwise known as “hidden hunger,” micronutrient malnutrition is a severe manifestation of micronutrient deficiency. Hidden hunger is defined as overall poor quality of nutrition despite intaking a sufficient number of calories (Sharma et al., 2017). Micronutrient malnutrition is associated with poor health conditions, including cancer, cardiovascular conditions, osteoporosis, and neurogenerative disorders (Bertola et al., 2021). To fulfill the pillars of access and utilization, attaining food security not only includes supplying the global population with enough daily calories, but also with sufficient nutrients. In other words, to meet the nutritional needs of the growing global population, food production efforts need to focus on producing high-yield, nutritionally dense crops. Given the role of soil for each of the pillars of food security, maximizing current resources to feed a growing population needs to occur at the soil level.

Microbial biofortification works at the soil level by tailoring the rhizosphere to meet food production needs. Soil is characterized by its microbiome, or the presence of bacteria, fungi, pathogens, and nutrients within its environment (Bertola et al., 2021). Within the rhizosphere, soil microbes work symbiotically with the plant roots to facilitate processes like carbon cycling, nitrogen fixation, and phosphorus solubilization, each of which is crucial for plant growth and development (Neemisha et al., 2022). Likewise, microbes involved in these metabolic processes have been found to increase the nutritional content of the plant for human consumption. For example, Yadav and associates (2022) reported significantly increased concentrations of iron, zinc, and copper in wheat cultivars that were treated with microbial biofortification.

There are several approaches to microbial biofortification, including application of plant growth promoting bacteria (PGPB), arbuscular mycorrhizal fungi (AMF), plant probiotics, and pasture management. While its application varies, microbial biofortification is broadly defined as a technique that increases plant growth and nutritional density. Microbial biofortification can be accomplished through the management of soil microbiome composition or manipulation of microbial metabolic processes. In addition, microbial biofortification often increases soil biodiversity, or the variety of microorganisms found within the soil (El Mujtar et al., 2019).

Generally, increases in soil biodiversity are associated with higher resiliency to environmental change (El Mujtar et al., 2019). In relation to the pillars of food security, microbial biofortification works to mitigate stressors that influence plant growth, which supports the availability and stability of food production. Microbial biofortification also increases the nutritional value of the plant for human consumption, which works to expand access and utilization to food production. In an effort to establish food security for a growing population, microbial biofortification offers a sustainable approach to maximize current resources while enhancing the yield and nutritional density of crops for human consumption.

# Presentation of Research

## Post-Harvest Fortification

Micronutrient malnutrition became a prominent issue in the 1930s and 1940s when severe diseases such as anemia were linked to micronutrient deficiencies (Olson et al., 2021). In order to combat the rising death rates due to these diseases, various fortification methods were implemented and aimed to add micronutrients to processed foods. Throughout history, the majority of food fortification has occurred after harvesting the crops, otherwise known as post-harvest fortification (Olson et al., 2021). Currently, this type of fortification is primarily achieved by large-scale and point-of-use food fortification.

Large-scale fortification is a process that adds micronutrients to commonly consumed foods such as salt, flour, and sugar during processing (Olson et al., 2021). Although widely used, large-scale fortification has proven only to be practical for foods that are milled into flours or ground into salts or sugars (Olson et al., 2021). Additionally, the intense machinery needed to reinfuse micronutrients into already processed foods is expensive. These expenses create barriers for low-income countries that currently lack economic and technological advancement needed to reap the benefits of large-scale fortification (Olson et al., 2021). Limited practicality and considerable expenses make large-scale fortification ineffective at distributing adequate nutrients to the global population.

Unlike large-scale fortification, point-of-use food fortification involves adding micronutrients to food that has been cooked (Teshome et al., 2017). In this method, micronutrients such as sodium, iron, and zinc are ground into a powder and packaged into single-serving packets. These packets are distributed to individuals who add the micronutrient powder on top of their meals. Historically, point-of use fortification has been used to provide impoverished schools, refugee camps, and pregnant women with equitable access to micronutrients critical for child development (Teshome et al., 2017). Although point-of-use fortification has proven effective at targeting individual populations, human error in dosage delivery has made the large-scale use of these products impractical and difficult to regulate (Teshome et al., 2017).

### Pre-Harvest Fortification

In contrast to post-harvest fortification methods, pre-harvest fortification, also known as biofortification, works at the cellular level to increase the nutritional density of crops before they are harvested (Teshome et al., 2017). Originally developed as a food-based strategy to address widespread micronutrient deficiencies, biofortification has since been considered an innovative approach to improving nutritional density in crops (Teshome et al., 2017). There are many supported advantages to biofortification, including its applicability to large-scale fortification, practicality for crops that are not milled into flours or salts, and low cost of sustained use after initial development (Hall & King, 2022). Over time, three main approaches to biofortification have emerged: transgenic breeding, conventional breeding, and agronomic fortification.

Transgenic breeding involves transferring desirable genes from one plant species to another (Garg et al., 2018). Specifically, Garg and contributors (2018) concluded that transgenic methods are the most successful type of pre-harvest fortification when there is limited genetic variation in a population of crops. Inadequate genetic variation often inhibits the uptake of micronutrients into the plant and transferring desirable uptake genes from one plant to another has shown to drastically improve nutritional density in those crops (Garg et al., 2018). Although promising results have been gained from transgenic breeding, the release of these successfully bred cultivars is dependent on the approval from national biosafety and regulatory processes (Bouis & Saltzman, 2017). In other words, transgenic breeding has not been implemented on a large-scale due to the lack of knowledge about the effects of consuming artificially bred genetic specimens and the strict regulations imposed because of it.

Alternatively, conventional breeding is regarded as the most trusted approach to biofortification and does not face the numerous regulatory hurdles that transgenic breeding encounters (Garg et al., 2018). This approach relies on high genetic diversity within a crop species and involves crossing efficient nutrient sequestering parent lines with offspring lines containing suitable agronomic traits (Garg et al., 2018). Although this process is regarded as the "fastest" way for farmers to cultivate more nutritious crops, it takes several generations to produce cultivars with desirable traits (Bouis & Saltzman, 2017). In addition to the time-consuming nature of conventional breeding, it is well known that overall genetic diversity in crop species is decreasing (Garg et al., 2018). This means that, over time, there will be less desirable uptake traits present in a population of crops. Given these limitations, conventional breeding is not a sustainable option for feeding 10 billion people.

Lastly, agronomic fortification requires the physical application of plant micronutrients either to the soil or to the plant in order to temporarily improve the nutritional and health status of the crop (Jurkonienė et al., 2021). Currently, there are two commonly used methods of agronomic fortification: foliar fertilization and multi-micronutrient fertilization. Foliar fertilization includes applying micronutrient fertilizers directly to the plant's leaves (Aziz et al., 2019; Bana et al., 2022). In their experiment, Bana and contributors (2021) applied a fertilizer of zinc, copper, boron, iron, and manganese to the leaves of *Triticum aestivum*. The results showed that increasing the bioavailability of micronutrients through foliar fertilization significantly increased the concentration of those nutrients in the flour of the crop (Bana et al., 2021). In contrast, multi-micronutrient fertilizers (MMFs) are mixtures of multiple micronutrients such as copper, zinc, boron, and iron that are added to the soil in which the plant is growing (Aziz et al., 2019). In an experiment testing the effectiveness of MMFs, Aziz and contributors (2019) concluded that MMFs paired with the recommended dose of macronutrient fertilizers (RDF) had substantial benefits on the growth and nutrient content of *Solaum melongena* crops. Specifically, the correct pairing of MMF and RDF played a vital role in improving the growth, yield, micronutrient sequestration by the crop, and health of the soil microbiome around *S. melongena* plants (Aziz et al., 2019). Despite these promising results, both forms of agronomic fortification require a regular, physical application of micronutrients which creates the need for additional labor, time, and money that not all countries have access to.

Although biofortification methods have successfully enhanced the micronutrient levels in crops, these approaches are often expensive, require long-term monitoring, and act at unsustainable rates to meet the growing global food demand (Jaiswal et al., 2022). Given the similar limitations of currently utilized post- and pre-harvest fortification processes, another avenue of fortification needs to be explored to ensure food security for the increasing population.

## Plant Growth Promoting Bacteria (PGPB)

Microbial biofortification is another, yet newer, approach to biofortification and is proposed as a more sustainable option of establishing food security. Plant growth promoting bacteria (PGPB), also known as plant growth promoting rhizobacteria (PGPR), have the ability to enhance plant growth and are commonly found in the rhizosphere (Olanrewaju et al., 2017). Due to their ubiquity, wide-ranging abilities, and capacity to quickly adapt to changing conditions, the utilization of PGPB has recently emerged as a promising method of microbial biofortification. By applying beneficial bacteria, rhizobia, or endophytes to the soil, PGPB works alongside other microbes to facilitate metabolic processes related to plant growth and nutrient acquisition (Rolli et al., 2017). When allowed to colonize the roots of crops, PGPB have been shown to increase plant nutrient levels and preserve soil health (Dogra et al., 2019).

## PGPB & Micronutrients

There are many mechanisms that PGPB utilize to help sequester nutrients into plants. The most researched method PGPB employ is the use of siderophores. Siderophores are chemical compounds that are often deployed by PGPB to aid in micronutrient acquisition (Olanrewaju et al., 2017). Iron mobilization has been a large area of research pertaining to siderophores because iron derivatives found in the soil are only slightly soluble. Research has found that siderophores produced by some PGPB have a high affinity for iron which allows them to complex with the micronutrient (Olanrewaju et al., 2017). Complexing with the siderophore makes the iron more soluble and catalyzes the uptake process into the plant (Olanrewaju et al., 2017). Although the relationship between iron and siderophores has been exclusively researched, the type of siderophore produced is specific to the genotype of the PGPB (Rolli et al., 2017). In other words, siderophores can be generated to specially target zinc, manganese, and other micronutrients. However, research is limited in these areas.

Due to the diversity of PGPB and the variable plant growth promoting factors they produce, researchers have started experimenting to determine which PGPB species, or combination of species, can be paired with specific crops to best promote plant growth and nutrient sequestration. For example, Nishanth and contributors (2021) applied biofilms of various combinations of *Anabaena torulosa* (a nitrogen-fixing bacterium), *Trichoderma viride* (a fungi), and *Providencia* sp. (a nitrogen-fixing bacterium) to maize kernels in an effort to determine the best combination of microbes that would promote plant growth. The results showed that the *A. torulosa* and *T. viride* biofilm as well as the *A. torulosa* and *Providencia* sp. biofilm displayed the greatest increase in leaf pigments and total chlorophyll in the maize crops (Nishanth et al., 2021). These statistics directly correlated with higher overall nitrogenase activity and greater nutrient mobilization. The results of this study give insight into the vastness of PGPB characteristics and their effects on plant growth and nutrient sequestration.

To further display the importance of investigating growth promoting characteristics in PGPB and how they pair with the plant, Dogra and others (2019) set out to determine the effects that several isolates of PGPB had on the growth and nutrient content of two chickpea cultivars. Their results indicated that the application of the local isolate, *Pseudomonas citronellis,* to chickpea seeds gave the best enhancement of overall growth (Dogra et al., 2019). Alternatively, the treatments containing *P. citronellis*, *Pseudomonas* sp., *Frateuria aurantia*, and *Serratia marcesens* all displayed an increase in iron accumulation in the first cultivar, whereas the second cultivar only showed an increase in iron when treated with *S. marcesens* (Dogra et al., 2019). In other words, the combination of PGPB that worked best at improving iron uptake was different for each cultivar. The results gained by Dogra and contributors (2019) as well as Nishanth and others (2021) indicate the importance of experimentation when pairing plants with PGPB. These experiments show that one PGPB that enhances micronutrient uptake in a certain cultivar may not work as effectively in another. Before wide-ranging implementation of microbial biofortification can occur, further research must be done on ideal plant-PGPB pairings and the factors that influence their efficacy.

## Other Benefits of PGPB

The issue of feeding 10 billion extends much further than purely addressing micronutrient malnutrition. For that reason, it is important to consider the overall effects PGPB have on plants as well as on soil health in order to conclude on the sustainability of this method of microbial biofortification. In addition to sequestering micronutrients, PGPB possess other plant growth promoting techniques such as improving soil enzyme activities, phosphorus solubilization, and heavy metal immobilization (Ju et al., 2020). All of these actions play important roles in maintaining the integrity of the rhizosphere, the soil, and the environment.

PGPB are known to improve soil enzyme activities and enrich microbial diversity. In copper-contaminated soil planted with *Medicago sativa*, Ju and coworkers (2020) aimed to determine the effects of *Paenibacillus mucilangiosus* and *Sinorhizobium meliloti* on soil enzyme activities—specifically that of ACC deaminase. Produced by some PGPB, ACC deaminase has been shown to break down the “stress ethylene” produced by the plant under taxing conditions such as pathogenic attacks or heavy metal contamination (Olanrewaju et al., 2017). The researchers found that the co-inoculation of these PGPB overall increased the concentrations of available carbon and nitrogen in the soil as well as the microbial biomass (Ju et al., 2020). High microbial biomass is generally associated with plant-pathogen suppression, pollutant degradation, and improved nutrient cycling which the authors attributed to the higher concentrations of plant growth promoting biomolecules such as ACC deaminase that the treatment groups produced (Ju et al., 2020). These results suggest that co-inoculation of PGPB offers a sustainable method to improve plant growth and nutrient cycling under stressful environmental conditions.

Other stressful conditions may include limited macronutrients that are critical for plant health. Phosphorus is the second most limiting nutrient in plant growth and nutrient uptake (Wan et al., 2020). In other words, increasing available phosphorus in the soil has the potential to increase plant growth. Currently, phosphorus is most commonly introduced into soil via traditional fertilization methods. Yet, studies have shown that these methods tend to worsen soil quality and lessen total phosphorus availability with long-term use (Wan et al., 2020). As a more sustainable alternative, Wan and contributors (2020) applied phosphorus along with phosphorus solubilizing bacteria (PSB), a type of PGPB, with the aim of improving soil quality and plant growth. Eighteen different PSB were found to be capable of integrating themselves into the preexisting soil microbiome which resulted in an increase in total available phosphorus for the plants (Wan et al., 2020). Unexpectedly, Wan and others (2020) also found that some PSB implored heavy metal immobilization mechanisms, which offered benefits to the plant by reducing the severity of adverse environmental impacts.

There is no doubt that promising results have supported the use of PGPB as a form of microbial biofortification. However, gaps in knowledge still exist concerning the various mechanisms PGPB utilize, the genes involved in the regulation of those mechanisms, and which PGPB provide the most support to certain cultivars. Additionally, detailed experiments have not been performed to determine the lasting impacts of introducing non-native microbes into fields such as whether the introduced microbes will colonize that area or if they will disperse elsewhere.

## Arbuscular Mycorrhizal Fungi (AMF)

In contrast to PGPB, the application of arbuscular mycorrhizal fungi (AMF) is a more well-researched method of microbial biofortification. AMF are known to colonize the roots of over 90% of plant species (Balsam et al., 2011). Once applied to the soil, AMF form a symbiotic relationship with the plant roots. AMF secrete lipochito-oligosaccharides which are recognized by the plant and activate a signal transduction pathway used for exchange of nutrients like phosphorus and nitrogen through the hyphae and arbuscules (Chen, et al., 2018). AMF also increase the absorption surface of inoculated plants, thus improving plant access to nutrients (Diagne et al., 2020). Furthermore, the plant provides fixed carbon for the fungi while the fungi assist the plant with essential processes like nutrient uptake and water retention (Chen et al., 2018). This results in a symbiotic relationship between the roots of the plant and the AMF which has the ability to enhance plant nutrient uptake and soil health.

## AMF & Environmental Stress

Drought is one stressor that impacts plant growth. Diagne and contributors (2020) report that plant response to stressors is mitigated in two ways by AMF inoculation. First, AMF hyphae increase the surface area of the plant root system which increases its capacity for water retention (Diagne et al., 2020). For example, Subramanian and associates (2006) inoculated tomato plants with *Glomus intradaices* AMF and allowed them to grow in drought-like conditions in two growing seasons. Compared to the control, tomato fruit yield was 24.7% greater than the experimental group under severe drought conditions (Subramanian et al., 2006). Upon analysis, researchers found that inoculated plants had significantly higher relative water content than control groups. Results suggest that AMF assist in the plant's capacity to store water in drought conditions leading to higher fruit yield (Subramanian et al., 2006). AMF may be one solution to maintain adequate crop yield and food quality despite worsening climate conditions.

Second, AMF produce phytohormones which regulate plant tolerance to abiotic stressors (Diagne et al., 2020). Abscisic acid (ABA) is an essential phytohormone that controls stomata behavior in plants (Diagne et al., 2020). Under stress conditions like drought, ABA is produced by plant roots and transported upward through the plant to promote stomatal closure and reduce cellular water loss. AMF inoculation has been found to regulate ABA concentrations (Diagne et al., 2020). For example, lettuce plants that were inoculated with AMF were found to have 1.5 times higher ABA content than the control when exposed to the same drought conditions (Ouledali et al., 2019). Additionally, the AMF-inoculated plants lost less water than the control, suggesting that AMF offers protective mechanisms against drought (Ouledali et al., 2019). Much like its capacity to increase water retention under drought conditions, AMF regulation of ABA production is a promising tool in tailoring plant stress responses to correspond with environmental change.

## Effects of AMF on Nutrition for Plants and Humans

Given that phosphorus is a limiting macronutrient for plant growth, another crucial benefit of AMF symbiosis is its role in phosphate transfer from the fungus to the plant (Chen, et al., 2018). In an experiment conducted by Balsam and contributors (2011), the effects of AMF application on three types of lettuce were assessed. Lettuce seeds were sterilized, allowed to develop leaves, and transferred to pots where they were inoculated with *Glomus fasciculatam* AMF. Lettuce was grown in a greenhouse for seven weeks and then analyzed for height and biomass as well as nutrient density. Researchers found that phosphorus concentrations were significantly higher in AMF-treated plants (Balsam et al., 2011). It was concluded that enhanced phosphorus solubilization likely contributed to increased height and mass of the inoculated lettuce (Balsam et al., 2011).

Likewise, Hart and associates (2015) investigated the role of AMF in improving the nutritional quality of tomatoes. Researchers inoculated tomato seeds with *Funneliformis mosseae* AMF and allowed the plant to fruit. Upon harvest, tomatoes were analyzed for nutritional density. Researchers found that phosphorus, nitrogen, and copper concentrations were significantly higher for the experimental groups (Hart et al., 2015). Plant biomass and fruit yield were also significantly higher for AMF-treated plants (Hart et al., 2015). In agreement with the conclusions of Balsam and contributors (2011), this experiment acknowledged the beneficial effects AMF had on plant growth while also recognizing its potential for improving food quality for human consumption (Hart et al., 2015).

Increasing nutritional density of plants is not only beneficial for plant growth, but also in producing nutritious food for humans. Baslam and contributors (2011) also found that concentrations of copper and anthocyanins were significantly higher in AMF-treated plants. In terms of human health, copper is considered an essential micronutrient for immunological function and organ formation in developing fetuses (Karim, 2018). Anthocyanins are another micronutrient linked to antioxidant protection and chronic and degenerative disease prevention (Hart et al., 2015). In agreement, Poulton and others (2001) reported that cherry tomatoes inoculated with AMF had significantly higher anthocyanin concentration in their leaves and fruits than the control. While the exact mechanism of AMF and anthocyanin production is poorly understood, researchers propose that higher nitrogen production in mycorrhizal plants might contribute to production of enzymes that are involved in synthesizing phenolic compounds like anthocyanin (Toussaint et al., 2007). The significance of copper and anthocyanins for human health relates to the overarching goal of establishing food security for a growing population. Food security not only involves increasing crop yield, but also crop nutrient density. Implementing AMF as a strategy to increase micronutrient density of plants offers one approach to achieve this goal.

## AMF & PGPB

More recently, AMF has been used in conjunction with PGPB for biofortification of crops. In an experiment conducted by Lingua and associates (2013), strawberry plants were co-inoculated with *Glomus* AMF and *Pseudomonas fluorescens* PGPB and allowed to grow in a greenhouse for 22 weeks (Lingua et al., 2013). The goal of this study was to analyze the effects that species of AMF and PGPB had on anthocyanin concentration of strawberry fruit grown in under-fertilized conditions (Lingua et al., 2013). Along with their antioxidant properties, anthocyanins are pigments responsible for red, blue, and purple colors in fruits and vegetables and often are indicators of overall plant health (Lingua et al., 2013). Results showed that the experimental group had two times higher concentrations of anthocyanins compared to the control group (Lingua et al., 2013). In addition, the experimental group grew sufficiently under low-fertilization conditions (Lingua et al., 2013). Researchers concluded that AMF and PGPB co-inoculation could be a sustainable alternative to produce nutritionally dense crops in environments with low fertilization (Lingua et al., 2013). Considering the dramatic decline in the quality of soil worldwide, implementing a method to improve crop yield and quality in unideal agricultural conditions is of environmental importance (Lal, 2015).

In an experiment conducted by Bona and contributors (2014), similar results were obtained when strawberry plants treated with a mixture of AMF and PGPB were grown in under-fertilized conditions. Co-inoculation yielded increased flower and fruit production, larger fruit size, and higher concentrations of sugars compared to the control (Bona et al., 2014). Researchers noted that regulation of abscisic acid, a known function of AMF, was also associated with bacterial modulation of photosynthesis and sugar concentration (Diagne et al., 2020; Bona et al., 2014). Bona and associates (2014) proposed that AMF and PGPB work synergistically to enhance photosynthetic activity and sugar production by the plant. As a result, improvements were seen not only in the amount of edible fruit produced, but also in its flavor and texture profile (Bona et al., 2014).

In addition to its benefits in low-fertilization areas, co-inoculation of AMF and PGPB has been found to improve plant growth in other unfavorable environmental conditions as well. Laranjeira and contributors (2014) conducted a study on chickpea performance in water scarcity conditions, with and without inoculation of AMF and PGPB. Results indicated that co-inoculation increased chickpea yield by 6% and 24% compared to single-inoculated (PGPB only) and non-inoculated plants, respectively (Laranjeira et al., 2014). This study demonstrates that while AMF and PGPB have shown to be effective at improving crop yield on their own, combining treatments has greater potential to improve plant productivity.

To further test the effects of co-inoculation of AMF and PGPB, Yadav and associates (2020) aimed to identify the best combination of PGPB and AMF to enhance crop yield and soil health. Wheat cultivars were inoculated with AMF and one of two PGPB isolates—*Bacillus subtilis* or *Pseudomonas chlororaphis*—allowed to grow, and then analyzed for growth and nutrient concentrations (Yadav et al., 2020). Growth results were such that wheat inoculated with AMF and *B. subtilis* exhibited a 24% increase in root length along with a 40% increase in plant biomass (Yadav et al., 2020). Nutrient analysis revealed a 45% and 48% enhancement in plant nitrogen and phosphorus content, respectively (Yadav et al., 2020). Researchers proposed that *B. subtilis* assists in mycorrhizal colonization by synthesizing lipopeptides that aid associations of the root to the fungus (Yadav et al., 2020). As a result of high mycorrhizal colonization, macro- and micronutrient uptake increased. Based on these results, researchers concluded that an application of *B. subtilis* and AMF was the most effective combination for improving plant growth and nutrient acquisition in two wheat cultivars (Yadav et al., 2020). While the exact mechanism of PGPB and AMF co-inoculation is not fully understood, current research yields promising results that the co-inoculation of AMF and PGPB can improve plant growth and nutritional density. In the context of food security, AMF and PGPB applications offer a sustainable solution for feeding a growing population.

While current research regarding the use of AMF in combination with PGPB shows positive results, more research is needed on the application of these methods in large-scale, long-term farming. Most experiments discuss the successfulness of AMF and PGPB co-inoculation under greenhouse conditions but these methods of microbial biofortification have not been applied to large-scale farming environments. There is also little research on the effects of AMF and PGPB on the surrounding ecosystems. It is generally unknown how these methods impact native microbes or the surrounding microbiomes. Furthermore, identification of the best combination of PGPB and AMF requires more research. While Yadav and contributors (2020) concluded that a combination of *B. subtilis* and AMF offered the most beneficial results for wheat growth, factors such as cultivar type, soil quality, climate conditions, and accessibility to certain microbes may change what combination of AMF and PGPB is most effective for a given crop cultivar. Additional research needs to be performed on the exact mechanisms AMF and PGPB utilize in order to determine how to apply microbial biofortification most efficiently as a method of establishing food security.

## Plant Probiotics

Although the co-inoculation of AMF and PGPB is perhaps the most promising strategy for sustainably increasing crop productivity and nutritional density, consideration of lesser researched methods of microbial biofortification is required to create a holistic approach towards establishing food security. Plant probiotics are one lesser researched method of microbial biofortification. Plant probiotics concern solutions of isolated microorganisms that were commercially developed for the control of plant disease and fertilization (Jurkonienė et al., 2021). In contrast to PGPB, plant probiotics do not consist entirely of bacteria. Other microorganisms and biochemical solutions known to stimulate plant growth and nutrient acquisition could be included in their formulation as well (Jurkonienė et al., 2021). Additionally, the application of plant probiotics and PGPB can differ. While PGPB are directly applied to the soil, plant probiotics can be applied either directly to the soil or the plant itself via spray application (Rahman et al., 2018). While the application of plant probiotics is similar to agronomic fortification, it is still considered a form of microbial biofortification because microorganisms are included in its formulation and mechanism of action. Common mechanisms of action include stimulation of phytohormone production, facilitation of nitrogen fixation, and general micronutrient acquisition (Jurkonienė et al., 2021). In short, plant probiotics work by altering the composition of the soil and plant microbiome.

Jurkonienė and contributors (2021) studied the effects of plant probiotic bacteria on blackcurrant berry yield and nutritional quality. Blackcurrant fields were inoculated with ProbioHumus and NaturGel probiotic applications. Each probiotic treatment contained several types of bacteria along with amino acids, vitamins, and specific micronutrients like phosphorus, zinc, and copper (Jurkonienė et al., 2021). Berries treated with ProbioHumus and NaturGel increased in size by 28% and 43%, respectively (Jurkonienė et al., 2021). In terms of berry quality, both treatment groups yielded higher concentrations of anthocyanins than the control, suggesting enhanced antioxidant qualities of the berries for human consumption (Jurkonienė et al., 2021).

In another study, two plant probiotic bacteria were applied to strawberry fields via spray application and the berries were later assessed for fruit yield and antioxidant composition (Rahman et al., 2018). The treatment group demonstrated an overall increase in fruit yield of up to 48% compared to the non-treated control (Rahman et al., 2018). Researchers proposed that the probiotic bacteria facilitated fruit growth by production of phytohormones (Rahman et al., 2018). In agreement with Jurkonienė and others (2021), anthocyanin concentrations were also found to be higher in the treatment group compared to the control (Rahman et al., 2018).

Although plant probiotics have been shown to improve plant growth and berry production, there are large gaps in knowledge that need to be addressed before the large-scale application of these products can occur. In addition to the lack of research regarding plant probiotic use for long-term, large-scale farming, the stability of plant probiotics has emerged as an issue for manufacturers and consumers. Given that probiotics consist of live microorganisms, the shelf-life of formulations is a concern for manufacturers as special accommodations may be required for proper storage of these products (Bharti et al., 2017). Due to their complicated application methods, plant probiotics may not be the best use of resources and or the most efficient technique, especially in areas that have limited or low economic resources.

Moreover, the long-term implications of probiotics on the soil microbiome have not been assessed. As with PGPB, there is a lack of research regarding how probiotics colonize their environment and whether or not consistent use of probiotics adversely impact the health of native microbes. Looking at microbial biofortification as a whole, current research on plant probiotics may assist in understanding the mechanisms behind more well-researched methods of fortification such as PGPB and AMF. For example, in an effort to determine which PGPB species work best to maximize crop yield for a given plant or climate, referencing studies on plant probiotics could offer insight on biological mechanisms and relevant organisms to include in experimentation.

## Pasture Management

In addition to improving the quality of plants, microbial biofortification has also been found to improve the biodiversity of the soil. Often influenced by the surrounding environment, soil biodiversity can shift depending on activity in the proximate ecosystem (El Mujtar et al., 2019). Pasture management, or the method by which livestock is grazed in an agricultural setting, has been suggested as an additional approach to microbial biofortification because it has been found to influence soil nutrient status (Yang et al., 2019). The primary mechanism for fluctuations in soil biodiversity by pasture management is related to manure composition (Yang et al., 2019).

As an alternative to agrochemical application, manure is most well-known as a method of providing essential nutrients like nitrogen, carbon, and phosphorus to the soil to promote plant growth (Koninger et al., 2021). However, its application may also benefit the health of microbial communities. The composition of the soil microbiome is dependent on the source of nutrients available in the soil (El Mujtar et al., 2019). In a study conducted by Cui and associates (2018), maize crops treated with organic manure were found to have a significantly higher total carbon pool than the control. The composition of the soil microbiome associated with the manure-treated maize also differed significantly from the control (Cui et al., 2018). Microbial variation within the soil is proportional to the total carbon concentration (Kacprzak et al., 2022). Based on this idea, Cui and contributors (2018) proposed that manure application changed microbial composition by selecting for bacteria that prefer to use carbon as their primary nutrient source. As an alternative to agrochemical fertilizers which often select for pathogen-resistant bacteria, manure offers a more microbiome-friendly approach to elevate the nutritional density of the soil for plant growth while maintaining the health of the soil microbiome (Zhang et al., 2021).

Alterations in pasture management influence manure abundance in agricultural land. Yang and coworkers (2019) aimed to identify the relationship between soil biodiversity and pasture management practices. Over a span of 13-years, their results indicated that control soils (i.e., agricultural land without pastures) harbored significantly lower biodiversity than soils from farms that implemented manure application (Yang et al., 2019). Across pasture management practices, Yang and contributors (2019) also noted that continuously grazed systems exhibited the highest levels of microbial biodiversity.

While research on the long-term impacts pasture management has on soil quality is more widely available than other methods of microbial biofortification, pasture management has several challenges that limit its contributions to the establishment of food security. While some studies agree that pasture management has the potential to increase soil biodiversity by introducing microbes from animal excrement, other research concludes the opposite (Yang et al., 2019; Olivera et al., 2016). Additional comparative research on the impact of grazing systems on microbial biodiversity is needed to draw a firm conclusion regarding the benefits and limitations of pasture management. It is also of note that the use of manure as a fertilizer may put human health at risk as animal excrement is known to introduce pathogens, antibiotics, and heavy metals into the soil (Kacprzak et al., 2022). In short, the inconsistency of research on pasture management and its overarching environmental impact is problematic when making conclusions on its efficacy as a reliable method of microbial biofortification.

Given its current status of research in the context of establishing food security, pasture management may not be the most effective method of microbial biofortification. However, implementing grazing strategies that foster soil biodiversity remains important in an effort to improve soil quality for plant and microbial health. The implementation of pasture management should be considered alongside other methods of microbial biofortification, such as PGPB and AMF application, in the effort to holistically improve soil quality, increase plant yield, and enhance nutritional density of crops for human consumption.

# Conclusions & Recommendations

Present-day mechanisms for food fortification have proven inadequate when attempting to equitably distribute nutrients to the global population (Olson et al., 2021; Teshome et al., 2017; Bouis & Saltzman, 2017; Jaiswal et al., 2022). As climate change and current agriculture practices continue to degrade soil quality, proper nutrients remain deficient in crops which perpetuates wide-spread micronutrient malnutrition (Lal, 2015). With the knowledge that food security is dependent on availability, stability, access, and utilization, there must be a shift in the current food production techniques in order to meet the demands of a growing population.

In a biological context, improving soil biodiversity is one step towards a comprehensive solution for feeding 10 billion people. The ubiquity and importance of the soil microbiome to crop and human health makes it a likely target in the fight for food security. Microbial biofortification specifically aims to enhance the soil microbiome and has growing research to support its efficacy and sustainability. Most of the research focuses on inoculating crop seeds with PGPB or AMF species that utilize specific plant growth promoting characteristics to support certain cultivars. Both PGPB and AMF methods have shown to improve the growth and nutrient density of macro- and micronutrients in crops while also increasing soil biodiversity (El Mujtar et al., 2019). When comparing the literature, it can be concluded that the co-inoculation of PGPB and AMF provides a sustainable approach to biofortification, particularly in its potential to mitigate the effects of environmental stress on plant growth. While other methods of microbial biofortification such as plant probiotics and pasture management could potentially aid in the creation of a comprehensive approach to feeding 10 billion people, they are generally less accessible and affordable options. In other words, instead of using these lesser researched methods in isolation, they should instead be used to further understand and enhance the overall effects of PGPB and AMF approaches.

Additionally, in its societal context, establishing food security is pivotal for ensuring long-term human health. Managing human health does not only mean producing enough food to meet daily caloric needs, but also providing populations with adequate macro- and micronutrients for proper bodily function. Microbial biofortification offers several mechanisms to enhance nutrient sequestration. Not only does microbial biofortification promote plant growth, but it also produces a more nutritious product for human consumption (Balsam et al., 2011; Hart et al. 2015; Olanrewaju et al., 2017; Poulton et al., 2001). To feed 10 billion people, food production efforts must focus on yielding nutritiously dense crops—microbial biofortification offers a sustainable method to meet this societal demand.

While current microbial biofortification methods show promising results for improving food security on a small-scale, more research on its feasibility for large-scale, long-term farming is needed. Additionally, while the sheer abundance of biofortification methods is promising in terms of their capacity for improving food production, further standardization of research methods is required to promote replicability of the data. Replicating small-scale experiments will help guide research as investigations expand into large-scale farming. Furthermore, additional research is needed regarding the long-term effects of microbial biofortification on the environment as well as its duration of nutritional benefits for plant and human health. Given the ultimate goal of holistically establishing food security for a growing population, it is recommended that future research should focus on the co-inoculation of PGPB and AMF method of microbial biofortification.

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