Plant Biostimulants: A Categorical Review, Their Implications for Row Crop Production, and Relation to Soil Health Indicators

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Abstract: Plant biostimulants are specialty products used to increase crop production and are quickly becoming common in the agricultural seed and chemical marketplace. Unlike traditional crop inputs, such as fertilizers or pesticides, biostimulants are unique in that a single product may have multiple avenues for influencing crop growth and development based on both the timing and the placement of application. This review presents a summary of the current status and descriptions of plant biostimulants with available literature on their uses in the row crop production of maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), wheat (*Triticum aestivum*), and other major crop species. Biostimulants have much potential to improve crop production through enhanced yields, grain quality, and increased sustainability of agronomic production systems, particularly in relation to nutrient management. However, there is great variability in the efficacy of biostimulants and a limited understanding of the mechanisms responsible in field-tested scenarios where differences are observed. These unknown mechanisms may align with the recognized soil health indicators, providing opportunities for unrealized biostimulant potential beyond crop growth and development. This review aims to identify the predominant types of crop biostimulants, the known understandings of their modes of action, and examples of their current field efficacy with an outlook for their future.

**Keywords:** biostimulants; biologicals; seaweed extracts; humic acids; BNF; PSB; AMF; soil health

1. Introduction

Plant biostimulants are the most common term for certain specialty products used for the benefit of crop production, but other categorical names to describe these products include biofertilizers, plant probiotics, biostimulators, and metabolic enhancers [1–5]. Although they are not defined by any U.S. federal agency, the 2018 U.S. Farm Bill described biostimulants as, "a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield" [6]. With this current description, along with an updated comprehensive outline for what distinguishes biostimulants and plant growth regulators, a public comment period was opened by the EPA in November 2020 and, to date, is still under draft review [7]. In contrast to the US, the European Union has defined biostimulants and includes them as a distinct product type under the regulation of fertilizer products. Their approach is claims-based, where the product regulation is determined by how the product works and not defined by its composition [8]. Biostimulants have been used in commercial agriculture for decades, but in more recent years the number of these products available to and used by growers has markedly increased. The global market for these products was estimated at USD 2.6 billion in 2019 with a projected value in 2025 at over USD 4 billion [9]. Commonly listed biostimulant products include seaweed extracts, organic acids, beneficial microbes (bacteria and fungi), protein hydrolysates or amino acids, and chitosan [10–12] and less common but growing categories include microbial extracts, biochar, and concentrated enzymes.

With the exception of the concentrated enzymes, the remaining biostimulants vary greatly in the composition of individual products, which, more often than not, is not entirely known. This knowledge gap is due to the biological origin of these products containing multiple constituents, where it is hypothesized that the beneficial activity of the product is the result of synergies among the components as opposed to separate components individually [13,14]. Yakhin et al. [14] report that synergy among constituents within a product makes it difficult to ascertain the exact mechanisms responsible for inducing a crop response and, as such, a better approach for describing biostimulant action should be based on the efficacy in their application. Thus, the remainder of this review will focus on applied biostimulants for their agronomic impacts in row crop production and their potential relationship with soil health and its recognized indicators.

2. Common Uses and Application Methods

Commercial biostimulants are typically first utilized in specialty crops, which often have higher profit per acre potential compared to row crops [15]. Specialty crops are typically more susceptible to environmental stressors [16]; for this reason, the potential return on investment of an applied biostimulant can be greater for those crops that are more susceptible to climate-induced stress. However, the total area harvested of maize, soybean, and wheat in 2018 was 84.8 and 53.0 million hectares for the United States and Brazil, respectively. The total area of all other harvested crops in these two countries was, correspondingly, 25.6 and 17.2 million hectares [17]. Thus, the area under row crop production of maize, soybean, and wheat in the United States and Brazil is 3.2 times greater than all other cultivated crops, providing a larger market for biostimulants, which are usually labeled and sold on a unit of volume per area basis. The opportunity for the expanded use of these products in row crop production is to apply them in combination with already established agronomic management practices, such as with the planter pass and/or an herbicide or fungicide application (Figure 1).
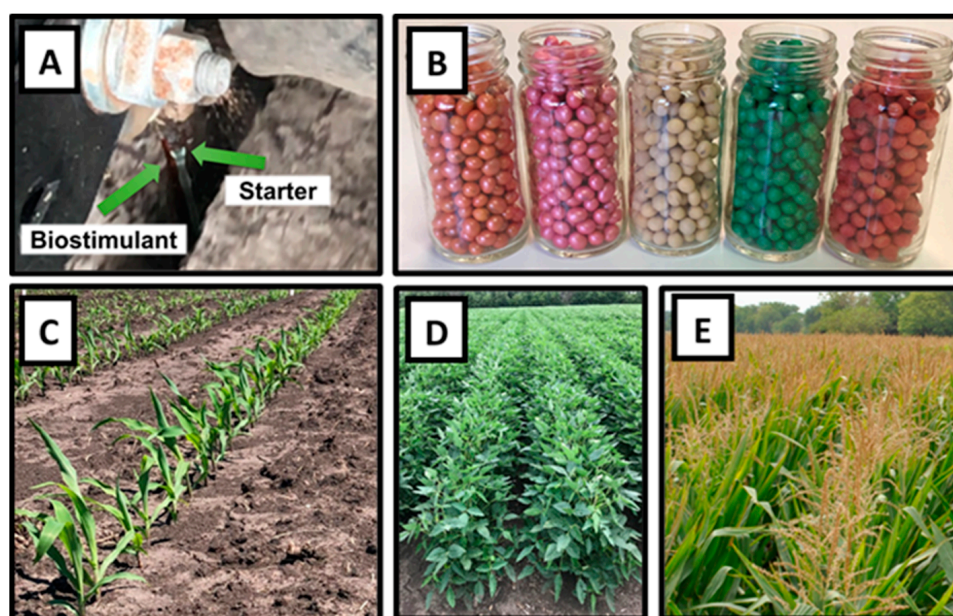
Figure 1. Crop growth stages representing the potential "free-ride" opportunities for applications of biostimulants with other management practices in row crop systems. (**A**) At planting as a liquid in-furrow. (**B**) On the seed as a seed treatment. (**C**) Early postemergence herbicide timing (~V5 growth stage maize and soybean). (**D**) Fungicide timing in soybean (~R3 growth stage). (**E**) Fungicide timing in maize (VT/R1 growth stage).

Including the biostimulant application with a current standard management practice provides a so-called "free-ride" for the product, as there is no additional cost of application.

For example, planting offers the opportunity for biostimulant application to all planted acres via in-furrow or seed treatment. This magnitude of potential use of biostimulants is followed by foliar application with herbicides, which were applied to 98 and 99 percent of US maize and soybean hectares in 2008, respectively, and foliar protection with fungicides or insecticides, which were estimated as applied to 10% of maize and soybean hectares during that same year [18]. An extensive review of biostimulants by Wozniak et al. [19] involving 126 trials and totaling 380 plant biostimulant treatments showed that 60% of the applications were applied as a foliar spray, 30% applied as soil and 10% as some form of seed treatment. While foliar applications are the most predominant, the question of when to spray and which application method is the best for a given biostimulant largely depends upon the product being used and the intended purpose of the application. One of the greatest challenges to the integration of biostimulants into a grower system is product compatibility with other agronomic inputs such as fertilizers and/or pesticides. With many unique chemistries and products on the market, there is limited knowledge and a requirement for research characterizing the potential interactions that may occur upon field application. Furthermore, biostimulants may differ in response based on the crop growth stage at time of application or the interactions with climate conditions where extremes of temperature and precipitation may influence crop response. Proper application requires knowledge of the different biostimulant categories, including, what they are, how they influence crop growth and development, and in which application scenarios are they likely to be most effective.

3. A Review of Biostimulant Categories

3.1. Seaweed Extracts

3.1.1. Composition and Proposed Mechanisms

The category of seaweed extracts represents a large group of biostimulants that come from the processing of various algae species, most often from the macroalgae (seaweeds). The species used vary in their composition and their utilization as biostimulant products (Table 1). Macroalgae is a renewable resource and those species that are used to produce the biostimulants are closely monitored to allow for continual harvest to maintain the supply [20]. The commercial products vary greatly in their components, which is dependent upon the species selected, stage harvested, and the proprietary extraction process utilized by individual companies [21]. The most common method of extraction is alkaline hydrolysis, while others include acid hydrolysis, water-based, microwave-assisted, ultrasound-assisted, enzyme-assisted, super-critical fluid, and pressurized liquid extractions [22].

The macroalgae differ from other organic-based products in their high abundance of specific carbohydrates, namely alginate, fucoidan, and laminarin, which are abundant in brown algae, carrageen in red algae, and ulvan derived from green algae [23]. Fucoidan, carrageen, and ulvan are heavily-sulfated compounds and the extraction process is key to minimizing the development of sulfhydryl compounds that can inhibit crop growth [24]. The positive effects of applying seaweed extract components to crops are largely related to stress relief since they are known to have antioxidant effects, which can reduce cell damage from reactive oxygen species (ROS) that occur during times of abiotic or biotic stress.

Table 1. Summary of the primary macroalgae species utilized in commercially available seaweed extract biostimulants. Sixty-nine individual products were identified and grouped by macroalgae species listed as the active ingredient. Data compiled from Khan et al. [25] (*n* = 25); Sharma et al. [26] (*n* = 28); and supplemented with additional products (*n* = 16) that were not previously included.

Species	Type	Percentage of Surveyed Products with Species Listed as the a.i. †
Ascophyllum nodosum	Brown	63.7
Laminaria spp.	Brown	8.6
Durvillaea spp.	Brown	7.2
Ecklonia spp.	Brown	4.3
Fucus spp.	Brown	2.9
Macrosystis pyrifera	Brown	2.9
Lithothamnium calcareum	Red	1.4
Unspecified	-	15.9

† Total percentages add up to greater than 100 percent as some products contain multiple species.

In addition to the carbohydrates, seaweed extracts contain plant hormones, brassinosteroids, polyamines, and betaines [27]. It is the complex combination of these compounds that induce beneficial plant responses such as improved plant growth, tolerance to abiotic and biotic stresses, and enhanced crop quality through greater nutrient uptake. While it is possible to evaluate a particular crop response of interest (plant growth, grain yield, nutrient uptake, etc.), the ability to identify the specific metabolite and mechanism responsible is very difficult in field studies due to the interactions of the environment and agronomic practices. Therefore, their application efficacy is best evaluated through crop growth and yield response.

3.1.2. Field Application and Efficacy

The use of seaweed extracts in agriculture has been documented during the first century where the Roman Columella utilized them as mulch and organic manure amendments to their fields [28]. The majority of current row crop applications of seaweed extracts consists of foliar and the targeted mode of action is the mitigation of abiotic stress related to environmental conditions, which predominately consists of drought tolerance. There has also been a renewed focus for soil applications to enhance root growth and microbial activity of the root zone. The use of seaweed extracts has been extensively reviewed and reported to increase plant growth, nutrient uptake, and grain yield as a result of phytohormones within the product, mitigation of stresses, and/or upregulation of plant metabolism [24,25,29,30]. The lack of published studies indicating reduced growth and yields, however, does not mean that seaweed extract products always improves crop performance, as most previous studies focused on differences between treated and non-treated and there exists limited research on seaweed extract interactions with other agronomic practices in the field.

The abundance of studies identifying such a diverse range of benefits highlights the flexibility of seaweed extracts and the responses they may elicit. For these reasons, the opportunities for the utilization of seaweed extracts in agronomic production systems are vast due to the numerous combinations of seaweed source, processing method, application timing and placement, crop of interest, and targeted response.

3.2. Humic and Fulvic Acids
3.2.1. Composition and Proposed Mechanisms

The microbial decomposition of organic matter is a complex process that produces a multitude of various by-products along the pathway of degradation [31]. Traditional views of this pathway imply that soil organic matter (SOM) as an end product contains stable chemical compounds collectively called humus, which is composed of recalcitrant substances that are resistant to further degradation. These substances are often grouped as humic acids (HA) (alkali soluble but acid insoluble), fulvic acids (FA) (alkali and acid

soluble), and humin (alkali and acid insoluble) and, together, they compose up to 60% of the organic matter in soils [32]. While traditional views consider these compounds to be resistant to degradation, a more recent view considers the idea that organic matter decomposition is dynamic and those compounds previously thought to be stable undergo reversible reactions; thus, they possess potential to stimulate the soil microbiome [33]. Humic acids can be derived from various sources, such as composted organic material, peat soils, and leonardite [34,35], and the source and stage of decomposition of the HA or FA influences the structure, composition, and its effect on crop and soil systems [36]. While HA and FA differ in their alkali/acid extraction, they also differ in molecular size and elemental composition, which can provide insight into their potential differences in efficacy as biostimulants. Humic acids are generally larger in molecular size, while FA contain more functional carboxylic acids groups and are thus often viewed as having a greater number of biologically active sites per molecule (Table 2) [37].

Table 2. Chemical composition of humic and fulvic acids as summarized from the literature.

Characteristic	Humic Acids	Fulvic Acids
Molecular Weight	10,000–100,000 Daltons	1000–10,000 Daltons
	——-% of organic components——	
Carbon	50–60	40–50
Hydrogen	4–6	4–6
Nitrogen	2–6	1–3
Oxygen	30–35	44–50
Sulfur	0–2	0–2

The high carbon (C) level of these organic compounds can act as a direct energy source for various soil microbial species, thus stimulating their activity and potentially resulting in a more productive soil [38]. Humic acids have also been shown to induce auxin-like effects on plants due to their aromatic ring and N-containing structures [39–41]. These auxin-effects are most often associated with increased root elongation and root hair initiation, which can result in increased nutrient uptake. Humic acid applications can also increase nutrient uptake because of increased membrane permeability and better nutrient availability through cation chelation [42,43]. An improvement in soil structure through increased aggregate stability has also been reported from HA an FA applications [44].

Laboratory extractions of HA and FA are performed using alkali or acid solutions at pH ranges that rarely occur in the natural soil environment of agriculture fields. Thus, it is important to note that concentrated HA and FA extractions do not reflect their abundance in native soil systems; instead, it is the ability to concentrate them that separates commercial products from the natural acids and organics already present in soils and why they can be effective biostimulants.

3.2.2. Field Application and Efficacy

Humic and fulvic acids have been used as inputs in agriculture production for many years and have been extensively studied for their effects on plant growth, microbial communities, and nutrient availability [45–48]. How HA and FA are used varies greatly and it depends upon the intended goal. In row crops the two primary uses are as soil amendments or to enhance nutrient uptake.

These humic and fulvic acids come from many sources and the source can have a large influence on the activity of the commercial products. For example, compost sources were shown to exert a greater stimulation of microbial growth and subsequent vegetative growth of chicory (*Cichorium intybus* c.v. Catalogna) when compared to a lignite-based HA [49]. Sulfur-enriched leonardite (HA parent source) and HA (extracted) were found to mitigate both phosphorus (P) and drought-stress, resulting in higher yield when applied as pre-plant soil amendments compared to an untreated control [50]. Sarir et al. [51] applied varying rates of HA across different application methods and reported a 28% increase in maize

yield from a spray application on the soil surface, with nominally higher yields observed when HA was either broadcast incorporated or banded. They also reported increases in soil nitrogen (N) and P availability after HA application, with a subsequent increase in plant N uptake, which indicates that the soil-applied HA can stimulate soil biochemistry related to nutrient cycling. A meta-analysis of the effect of humic substances on root and shoot weights conducted by Rose et al. [52] summarized the most consistent practices for crop growth and yield benefits of applied humic substances. Of the 390 papers in their original review, they retained 81 and noted that while all studies evaluated root and shoot growth, only a few followed the crop through to maturity and yield. Their analysis concluded that, while the source and rate of the humic substances were significant factors in their activity on plant growth, the method of application was not a significant factor. Furthermore, humic substances from compost sources were more effective than peat and lignite source material, inferring that the higher N levels of those sources applied at higher rates may be responsible for the greater responses in plant growth. All data collected were related to short-term effects of the humic applications on plant growth, whereas the long-term effects of repeated applications on soil characteristics and health were not evaluated. In addition to individual applications, there is new interest in the use of HA and FA in combination with beneficial microbes to provide a synergistic plant growth response. Applications of HA have been reported to increase maize root exudate production, which then enriches the rhizosphere for enhanced microbial activity near the root [53]. Other examples of synergistic responses between HA, vesicular arbuscular mycorrhizal fungi (AMF), and plant-growth-promoting rhizobacteria (PGPR) were reported for wheat yields [54]. Similarly, a mixture of beneficial microbes with HA increased nutrient uptake and the yield of maize [55]. Leonardite-based HA have been shown to increase the concentrations of soil N and P, resulting in increased microbial biomass and greater root and shoot growth of maize [56]. An additional potential market for HA and FA is their application with fertilizers, particularly N. The negative charges of HA and FA provide the potential to chelate cations such as nickel (Ni), which is essential for urea hydrolysis by urease [57], and humic-coated nitrogen fertilizer, which has been reported to increase fertilizer use efficiency compared to uncoated fertilizer [58].

The use of HA and FA in field studies varies and it is often noted that the success of their applications for increasing grain yield is not consistent enough when compared to the application of adequate amounts of common commercial fertilizers. Additionally, there are many reports of no benefits or even adverse effects in response to applying HA or FA at the field-scale [59,60]. However, HA and FA can influence soil biochemistry related to N and P cycling, as well as retain soil ammonium and improve soil structure. Therefore, alternative perspectives for the HA and FA market may be best focused on nutrient management and the capacity for improving soil health. These perspectives are best evaluated on a long-term scale, which has the potential for improving soil productivity over time with increases in crop yields likely to follow.

3.3. Nitrogen-Fixing Bacteria

3.3.1. Common Species and Known Mechanisms

Nitrogen is essential for all living microorganisms and is key for the biosynthesis of key compounds such as proteins and nucleic acids. Dinitrogen gas (N_2) in the atmosphere is the largest reservoir of readily accessible N; however, only a few microorganisms (diazotrophs) are able to convert N_2 into a bioactive form (NH_3) by conversion with nitrogenase (Equation (1)).

$$N_2 + 8e^{-1} + 8H^+ + 16\ ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i \tag{1}$$
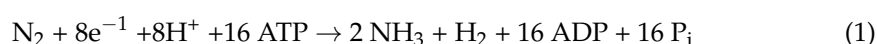
Equation (1). Redox reaction of the reduction in atmospheric N to ammonia that is catalyzed by the enzyme nitrogenase.

There are three types of nitrogenase enzyme complexes that differ in their metal cofactor: iron-iron (Fe-Fe), molybdenum-iron (Mo-Fe), or vanadium-iron (V-Fe) [61]. While the Mo-Fe cofactor is the most common, not all microbes utilize all three nitrogenases and,

as such, cofactor mineral availability may limit N fixation [62]. The activity of nitrogenase is irreversibly inhibited by oxygen, which requires that the bacteria develop methods to protect the enzyme from oxygen when they are in aerobic environments. There are multiple mechanisms by which bacteria can isolate themselves from oxygen, with the predominant mechanisms being the formation of a heterocyst (common to cyanobacteria in aquatic systems) or a nodule (associated with bacteria-plant symbiosis, e.g., *Rhizobia–Legume*). Understanding how the diazotroph of interest protects itself from high oxygen concentrations is key to the placement of that species into an agronomic system to ensure adequate biological N fixation (BNF) and product efficacy. There are many species from various biomes that mediate the above reaction and these have been well reviewed [63]. The more common N-fixing bacteria used as agricultural biostimulants are outlined in Table 3.

Table 3. Common bacteria known to fix nitrogen and that are often cultivated and utilized as biostimulants and some example corresponding crops that they are used with.

Bacteria Species	Bacteria Type	Crop Family	Example Crops ‡
Bradyrhizobium japonicum	Endosymbiotic	Fabaceae	Soybean
Rhizobium leguminosarum	Endosymbiotic	Fabaceae	Soybean
Azospirillum brasilense †	Free-living	Poaceae	Maize, Rice, and Wheat
Azotobacter vinelandii	Free-living	Poaceae	Maize, Rice, and Wheat
Gluconacetobacter diazotrophicus	Endophytic	Poaceae	Sugarcane and Maize
Klebsiella variicola	Free-living	Poaceae	Maize, Rice, and Wheat

† *Azospirillum brasilense* is often referred to as a member of the plant growth promoting rhizobacteria (PGPR) as it is known to produce plant hormones, while nitrogen-fixation by this species is mediated by native N levels. ‡ Rice, *Oryza sativa*; Sugarcane, *Saccharum officinarum*.

It was estimated that biological N fixation (BNF) accounts for 139 to 175 million tonnes annually of terrestrial N inputs and that up to 60 percent of these inputs come from symbiotic associations with plants found in arable land and pastures [63,64]. A more recent review places these numbers at 50–70 million tonnes for agricultural systems [65]. Estimates of a legume (*Fabaceae*) crops' total N acquisition derived from BNF range from 23% to 66% [66–68].

An important use of N-fixing bacteria in agricultural systems is the potential for symbiosis with grass (*Poaceae*) crops that traditionally have not been known to associate with BNF microorganisms for supplemental N. *Azospirillum brasilense* is one of the most common bacteria being utilized commercially to provide BNF and it is well studied regarding its production of plant hormones and its classification as a plant growth promoting rhizobacteria (PGPR). The production of hormones is understood to occur for this species when associating with plants in most environments, whereas in areas of excess N it will not fix N [69]. New tools are being utilized to genetically edit N-fixing microbes to circumvent the various mechanisms controlling N-fixation gene regulation. The presence of N can inhibit BNF, whereas editing the microorganism to ignore this inhibition allows continual N fixation, thereby developing an additional N source for agronomic cropping systems. More recent discoveries include symbiotic endophytes, which associates with plants by colonizing roots and/or shoots intracellularly. It is not known how endophytes infect the host without elucidating a defense response, but the proposed symbiotic relationship enables N fixation by the bacteria in exchange for carbohydrates from the plant. While the exact relationship of the host crop and the endophytic bacteria is not fully understood, most known endophytes colonize intercellular spaces, dead plant cells, vascular tissue, and aerenchyma of the host as opposed to living cells [70]. The N fixed by these microorganisms may not be released until microbial death and degradation and, therefore, the supply of N to the crop is delayed through this slow-release mechanism rather than the symbiosis of active partnership between *Rhizobia* and legumes [70].

The challenges of utilizing N-fixing bacteria as an applied biostimulant is related to the variety of species available, their plant associations, and the strategies they utilize to optimize BNF in aerobic environments. The advances in genomic tools adds even greater

potential to optimizing the utilization of BNF microorganisms as agronomic biostimulants. Once a species of interest has been identified, it is vital to know the limits of its N production for crop supply potential, its known crop symbionts, and the various specifics related to its N-fixation genes and opportunities for genetic manipulation to develop a more efficient strain.

3.3.2. Field Application and Efficacy

The obvious agronomic advantage for the use of N-fixing microbes is the potential supplementation of N to the growing crop, which partially alleviates the need for N to be supplied as fertilizer. The key to optimizing the efficacy of these bacteria is to place them near the root of the growing crop through in-furrow applications or seed treatment. The bacteria–crop relationship has been well studied for the *Fabaceae* (*Leguminosae*) plant family, containing the beans, peas, alfalfa, and clovers [71]. Soybean inoculation has been the predominant role of N-fixing microbes as biostimulants, with *Bradyrhizobium japonicum* being the leading bacterium in this market. A meta-analysis of 339 *Bradyrhizobium* trials across the United States and Argentina showed that, on average, a 1.67% and 6.39% yield increase in soybean in the US and Argentina, respectively, as a result of inoculation [72]. More recent attention has been given to co-inoculation, often with *Azospirillum* spp. Co-inoculation can increase soybean nodule number and size, root biomass, and shoot growth when compared to *Bradyrhizobium* alone, although co-inoculation did not increase grain N content or yield [73].

While *Azospirillum* spp. have been field tested over the past two decades, studies evaluating other N-fixing bacteria on grass crops are largely limited to preliminary greenhouse screening trials [74,75]. Utilizing seed inoculation of *Azotobacter chroococcum* increased N uptake of maize across multiple N rates, although there were no data reported on any grain yield changes [76]. *Azospirillum brasilense* has been used in Brazil to improve N use and yield via a seed treatment and resulted in increased maize plant growth and yield when combined with only half of the optimal rate of fertilizer N [77,78]. A meta-analysis of *Azospirillum* spp. indicated that yield increases were achieved in maize when the bacteria was applied without additional N and only nominal increases when applied with N [79]. For both maize and wheat, applying *Klebsiella* increased yields when plants were grown under either low or high levels of N and P fertilization [80]. Most current studies are focused on improving plant parameters with the addition of BNF microorganisms, while the interactions with other agronomic practices are relatively unknown in a field setting. It has been reported that the addition of phosphate fertilizer inhibited the activity of *Azospirillum brasilense* compared to inoculation without phosphate fertilizer [81]. The ability to fix N and to store it in the soil via these BNF microorganisms also possess the potential to improve soil health and C:N ratio by stimulating the native soil microbiome. The use of these bacteria will require recommendations specific to each individual farm to determine the appropriate microorganism with the proper application method and with the right agronomic management to assure a positive crop response.

3.4. Phosphorus Solubilizing Microorganisms (PSM)

3.4.1. Common Species and Known Mechanisms

While P accounts for 0.05% of soil content (w/w), only 0.1% of that P is plant-available in the water-soluble fraction and this low availability dictates the need for fertilizer P to meet plant nutrient requirements for a desired crop yield [82]. Unfortunately, up to 90% of applied fertilizer P can become fixed in soils, rendering it unavailable for plant uptake, but still part of the soil reservoir of P. Unavailable soil P can be made plant available through the action of soil microorganisms by solubilization of inorganic phosphates or mineralization of organic-P pools. The solubilization of inorganic phosphates by microorganisms is predominantly accomplished through the secretion of organic acids [83], which can increase P availability by one of two methods: (1) through chelation by cations, e.g., $Fe^{2+/3+}$ (Ferrous/Ferric Iron) and Ca^{2+} (Calcium), which prevents them from fixing available P; and,

(2) by decreasing the soil pH, which releases mineral P-complexes, particularly Ca [84]. The production of specific organic acids varies among species but gluconic and 2-ketogluconic acids tend to be the most common [84]. Since all organisms require P as an essential element for the synthesis of enzymes, nucleic acids, nucleotides, and phospholipids, all microorganisms possess strategies for P acquisition, although some are more efficient than others. Several microorganisms are known to solubilize P, and current studies indicate that *Aspergillus* and *Penicillium* fungi as well as *Bacillus*, *Pseudomonas*, and *Rhizobium* bacteria are the most efficient [79]. Among the bacteria, Gram negative types are able to solubilize P to a greater extent than Gram positive ones [85]. In the search for potential PSM candidates, many studies use tricalcium phosphate as the insoluble P source even though many microorganisms can readily solubilize it. Thus, the simultaneous use of multiple P sources has been recommended as a better option for selection of PSM [86]. These sources could include Ca-phosphates for alkaline soils, Fe- or Al (Aluminum)-phosphates for acidic soils, and phytates for soils with large pools of organic P. Another method for increasing the level of soil-available P is through hydrolysis of organic phosphates via production of extracellular enzymes [87]. While mechanisms of P solubilization are well understood, the efficacy of enhancing those mechanisms through management or inoculation to increase crop production is much less known.

3.4.2. Field Application and Efficacy

Phosphorus solubilizing microorganisms have been studied for decades and there is an abundance of research conducted in both natural ecosystems and laboratory conditions [88–90]. However, PSM have only recently been used as an agronomic input and the full potential of their commercialization is yet to be realized [83]. As both the level and the source of soil P varies among soil types and agronomic practices (fertilization, tillage, and rotation), the use of the correct microorganism to optimize P solubilization for the given system is critical. Applications of three PSM strains were found to individually increase yield of wheat by 19–24%, while their co-inoculation resulted in a 33% yield increase when compared to an un-inoculated control [91]. *Bacillus megaterium M3* was identified as the most valuable inoculant (of five studied) because it increased all three parameters of soil P cycling: soil microbial biomass P, soil phosphatase activities, and crop P uptake [91]. Different strains of *Bacillus megaterium* have also been reported to increase soil P solubilization and soil-available P and most notably increased sugarcane yield by 12.6% over an uninoculated control, while allowing for a 25% reduction in P fertilizer application without reducing yields compared to a full P application rate [92]. Less understood, however, are the interactions that applied PSM have on native microbial communities and how the environment in which they are applied can influence efficacy. A greenhouse study using multiple bacterial strains with and without P added to maize reported that *Bacillus megaterium* enhanced plant growth in non-sterile pots, whereas other strains that were effective under sterile conditions were unable to influence plant growth in non-sterile soils [93]. Collectively, these findings indicate that *Bacillus megaterium* is not only effective as a PSM through direct P solubilization but also through an ability to enhance activity of the native soil PSM populations.

Additional studies in both greenhouse and field scenarios have identified *Aspergillus awamori*, *Bacillus subtilis*, and *Pseudomonas putida* as potential organisms that can be applied in accordance with other agronomic inputs, such as irrigation and P fertilizer for greater efficiency compared to individual practices alone [94–96]. While the use of PSM in agronomic systems clearly has the potential to increase soil-available P, crop P uptake, and crop grain yield when used in concurrence with other agronomic practices, the challenge is to understand the species by environment interactions to optimize their use. Additionally, the ability to use PSM to make P available for crop growth, instead of fertilizing with external P, minimizes P pollution of waterways, and can support growth of other soil microorganisms.

3.5. Arbuscular Mycorrhizal Fungi (AMF)

3.5.1. General Morphology and Known Mechanisms

It is well known that soil fungi coexist in symbiotic associations with crop plants and these interactions have been reviewed for many crops including maize, wheat, soybean, and rice [97–100]. The most common fungal/plant association is endophytic and known as vesicular arbuscular mycorrhizal fungi (AMF), due to the morphological structures of arbuscules and vesicles that these organisms produce. These fungi are believed to have co-evolved with plant roots to allow for the adaptation to growth on dry land [101]. While bacteria can be isolated and differentiated at the species level through genetic analysis, AMF taxonomy is often based on the morphological characteristics of the asexual resting spores. Over time, AMF have developed unique genetic mutations through asexual reproduction that has resulted in microbial diversity within the species, rendering it difficult to separate between individuals and populations and introducing complexity in the understanding of their interactions with plants [102]. The symbiotic association that benefits crop growth can be attributed to the colonization of the AMF in the root cortex [103]. The fungi grow hyphae, which branch into the arbuscule and vesicle structures that associate with the root allowing for plant nutrient acquisition, while the plant supplies the fungi with carbohydrates.

This hyphae extension provides greater soil contact for the root, which is essential for uptake of non-mobile nutrients such as P. Many AMF have been found to be efficient solubilizers of P by using mechanisms of chelation and acid hydrolysis, which are similar to the phosphorus solubilizing microorganisms. In addition to the relationship of AMF and plants is their synergistic relationship with soil bacteria, in which a better understanding due to advancements in microbiological research have resulted in the concept of potential co-inoculation [104]. The discovery that AMF can act together with soil bacteria and thereby affect the formation or action of biostimulants to promote plant growth and soil health will be significant for future crop production.

3.5.2. Field Application and Efficacy

Several studies and reviews have evaluated the potential of AMF as applied biostimulants, with the predominant roles as mitigating stress of saline conditions, protection against plant pathogens, and the ability to increase nutrient availability and uptake, particularly related to P [105]. Salt stress was mitigated via increased uptake of N, P, K, Ca, and Mg in maize under saline conditions when AMF were present [106]. Additionally, salt stress was eased more with native AMF present than from inoculation with foreign species through greater upregulation of photosynthesis and a reduction in the production of and in response to reactive oxygen species [107]. A meta-analysis of AMF inoculation of wheat reported increased N, P, and zinc (Zn) uptake in addition to grain yield [108]. Often overlooked, however, are the interactions that applied inoculants can have with the crop genotype, where inoculation with AMF increased grain yield in one wheat variety but not in another [109]. Greenhouse studies using *Penicillium bilaiae* (or *bilaii*) coated on hybrid maize seed indicated an effectiveness for increasing P uptake and maize growth when adequate fertility was supplied, but no response was observed when other macro-nutrients such as N were limiting [110]. This finding shows that adequate availability of other nutrients, such as N, is key to AMF inoculation success, as the fungi also require nutrients and under N-limited conditions they may compete with the crop and actually reduce growth and yields [111]. While these studies were performed under artificial conditions with low N supply, it is an important reminder that the beneficial microorganisms used as biostimulants also require resources of nutrients and water and, as such, their efficacy depends upon climate and fertility, which is similar to crop production.

3.6. Other Beneficial Microorganisms and Their Application

While the primary use of living microbes in agricultural production systems is focused on nutrient acquisition through biological nitrogen fixation (BNF), phosphorus solubilizing microorganisms (PSM), and arbuscular mycorrhizal fungi (AMF), there are other oppor-

tunities for different microbial biostimulants to be used in row crop production. Farm management practices of tillage, rotation, and intercropping are changing with increases in minimal-till/no-till management, increased maize–maize rotations, or the intercropping of maize–soybean/soybean–wheat and cover crops. These changes are enabling increases in plant residue accumulation in grower fields. Soybean stover returned to the soil at harvest can average 6 tons per hectare, with two times as much maize stover remaining at 12 tons per hectare [66,112]. These crop residues contain organic forms of nutrients such as N, P, and S that can be mineralized for further crop uptake. Crop residues are abundant in cellulose, hemicellulose, and lignin, which are structured polymers that require high energy input for degradation via hydrolysis by enzymes. The decomposition of lignin is predominantly mediated by fungi [113]. Bacteria and fungi readily act upon the simple soluble C compounds, while the complex insoluble compounds are degraded by oligotrophic fungi [114]. These microorganisms applied as biostimulants may also improve the health of soil as they can be utilized to catalyze residue decomposition, resulting in faster mineralization of the organic nutrients and increasing the fertility of the soil for the following crop, thereby providing an alternative biostimulant use in addition to BNF and P solubilization.

3.7. Emerging Biostimulant Categories

3.7.1. Enzymes

The application of phosphatases as a commercial product to be used in crop fields has introduced a new category of biostimulants, which are purified enzymes. Soils contain extracellular enzymes produced by organisms and this is notably observed in plants and microbes [115–117]. These enzymes act as biological catalysts to increase the rate of biochemical reactions, which in soils can be dependent upon organic N or P compounds. In recent years, industrial production of enzymes through microbial fermentation processes have resulted in the production and purification of enzymes that can be applied to soils in cropping systems [118]. In addition to phosphatases, enzymes related to the C cycle are of interest, as they can catalyze residue decomposition and provide a potential tool for optimized management in high organic matter systems such as no-till or cover cropping. These enzymes include cellulase and hemicellulase to degrade the polymers of plant tissues. Degradation of these larger polymers into smaller polymers or monomers renders them more hydrolysable by microbial communities. This degradation can create a chain reaction that than accelerates the mineralization of additional nutrients for future crop uptake. In theory, a perfect mixture could be developed that contains multiple enzymes following a pathway of degradation targeted for specific organic components and the release of certain nutrients. However, despite their expanding commercial use, there is currently little to no research on the effects of enzyme products applied to the root zone at the field-scale, which creates a need for more research on the understanding of these products and their agronomic influences.

3.7.2. Biochar

Biochar is formed through high heat processes, resulting in the thermochemical decomposition of a fuel source without the addition of oxygen, known as pyrolysis [119]. The end product is a highly carbonaceous material with varying properties dependent upon the source, the temperature range, and the length of time for processing [120]. One of the most common forms of biochar is charcoal, which is sourced from woody biomass. The predominant sources of biomass contain hemicellulose, cellulose, and lignin and each increases in temperature required to decompose the structures, resulting in varying stability and activity of the biochar [121]. Biochar is used in many different industries as a source of fuel, building material, filtration, and, recently, as an agricultural soil amendment. Biochar is highly resistant to degradation and acts as a stable C source when applied to agricultural field. Since it is porous and has a large surface area, it has chelating capabilities with ions in the soil. Agricultural benefits attributed to biochar include increased plant productivity,

greater water holding capacity of treated soils, and the ability to retain nutrients [122]. As stable biochar is itself a highly carbonaceous compound that is resistant to degradation, its process and application to agronomic fields also serves as a source of C sequestration and, thus, it has been considered a "win-win-win" for agricultural use [123]. A comprehensive meta-analysis regarding the use of biochar in agronomic systems found an overall 10% increase in yield, but with a large range from −28% to +39% [124]. The authors made an important note that most of the included studies were responses after one to two years of biochar applications and that the long-term implications are not yet known. While increasing the C content of the soil is generally viewed as a positive outcome, it can also enable increased microbial activity and immobilization of soil N, thereby limiting potential crop-available N. However, the novelty of biochar as a sustainable solution for long-term soil productivity, increased crop production, and improved soil health is relatively unknown and continued research into its use when paired with proper agronomic management may result in a promising biostimulant with the immediate target being the soil as opposed to most direct biostimulant applications being focused on increasing plant growth and yield within a growing season.

4. Biostimulants and Soil Health

4.1. Soil Health Indicators as Simultaneous Mechanisms of Biostimulant Action

4.1.1. Soil Enzymes

While biostimulants can increase nutrient uptake by crops, the specific mechanisms regarding the increased nutrient availability and uptake are not always known. One potential mechanism of biostimulant action on nutrient availability is through changes in enzymatic activities because soil enzymes catalyze the hydrolytic and oxidative degradation of organic matter [125–127]. These transformations occur in multi-step mechanisms with specific enzymes catalyzing at each step, rendering it difficult to know precisely where the biostimulant influence may occur. Although these processes involve multiple steps that are individually catalyzed by a specific enzyme, the sequence of enzyme-mediated decomposition steps are bounded by the initial and terminal steps. Terminal-step enzymes can be highly sensitive to treatment effects because they catalyze the transformation of a nutrient to its bioavailable form. Therefore, the terminal step in nutrient release is an indirect measure of nutrient availability and provides insight as to how a given biostimulant may be making nutrients more available. Soil organic matter of nutrient rich soils primarily consists of C, N, P, and S (stoichiometric ratio of 61:7:1:1 [128]) and, as such, enzymes related to the mineralization of those nutrients are potential indicators for elucidating biostimulant mechanisms related to nutrient availability. It is their role in the cycling of organic matter that render soil enzyme activities potential indicators of soil health, providing a potential use of biostimulants as soil health products. The primary terminal-step enzymes include the following.

β-Glucosidase: Catalyzes hydrolysis of glycosidic bonds of polysaccharides to release glucose [129]. These bonds are key to cell wall structure and their breakdown is mediated by bacteria and fungi. Variability in the potential activity of β-glucosidase indicates differences in residue decomposition potential and soil C accrual and is being used by the USDA NRCS Soil Health Division as an official indicator of soil biological health [130].

Protease: An enzyme that hydrolyzes proteins, the major constituent of soil organic N reserves, and, thus, is thought to be the rate-limiting step in the mineralization of N from soil organic matter [131]. Proteases catalyze the hydrolysis of proteins by non-specifically cleaving the peptide bonds between amino acids, yielding shorter fragments of the protein (peptides) that are then acted upon by aminopeptidases to produce amino acids and crop-available N. Variability in protease activity is an indication of increased microbial or root activity for residue decomposition and nutrient cycling [132].

Leucine aminopeptidase: This enzyme cleaves amino acid residues from the N-terminus of peptides and proteins. There are many enzymes involved in protein degradation specific to the peptide bond type (i.e., the two amino acids linked); however, leucine

aminopeptidase is common across diverse soils and is therefore a good indicator of protein degradation [133]. As with other aminopeptidases, leucine aminopeptidase plays a key role in organic N release as the final step of protein degradation into microbial-available amino acids. Greater levels of leucine aminopeptidase activity indicate enhanced mineralization of organic N and thus N release from soil organic matter after the activity of deaminase.

Phosphatases (phosphomonoesterase and phosphodiesterase): Phosphatase enzymes are involved in the release of crop-available orthophosphate from organic P forms [134]. Organic phosphates can be present as either a monoester or diester bond that is cleaved by a specific enzyme which are either phosphomonoesterase or phosphodiesterase, respectively. Biological microorganisms evolve to fill various ecological niches and some may specialize in the phosphomonoesterase pathway and others the phosphodiesterase pathway [135]. Thus, evaluating activity potential of both phosphate-producing enzymes provides a comprehensive assessment of total P potential. Analogous to N mineralization from soil organic matter, these two P enzymes work together to release crop-available P from the organic pool. Additionally, variability in the activities of these two P enzymes may indicate which biological species were affected by product application.

Arylsulfatase: This enzyme is the catalyst for the release of inorganic S (SO_4^{2-}) by cleaving the ester bond tying S to the organic matter fraction [136]. Up to 98% of total soil S may be tied up in organic matter and 30–75% of that is attributed to the sulfate esters [137]. Activity levels of arylsulfatase can infer to changes in available soil S for potential crop uptake.

Thus, the evaluation of soil enzyme potential activities as influenced by biostimulants may allude to how the biostimulant is influencing nutrient cycling of the root zone and, in the case of β-glucosidase, is acting simultaneously as an indication of the consequences on soil health.

4.1.2. Microbial Biomass and Community Diversity

Unless a biostimulant is itself an enzyme, any influence on enzymatic activities as a result of biostimulant application would be driven by changes in microbial or plant activities. Greater enzyme activities can occur through increased enzyme production per unit of microbe or plant [138] or by increasing the number of microbes or crop growth, therefore resulting in overall greater enzyme production. Thus, the evaluation of microbial biomass and microbial community diversity can further decipher the potential mechanisms and modes of action for soil-applied biostimulants. Furthermore, the assessment of microbial communities of soils can allude to soil quality impacts of differing agronomic management practices, providing insight for the influences of said practices on the overall health of the soil [139]. Various methods for evaluation of microbial parameters include total microbial biomass and/or C respiration to obtain an indication of the amount of all microbes present and the total nutritional value immobilized as microbial biomass. However, while microbial biomass provides insight into the total community growth, methods such as 16S rRNA analysis or phospholipid fatty acid analysis (PLFA) are the ones that explain the diversity among those present microbes [140]. The pairing of microbial biomass parameters with their diversity simultaneously answers how microbial communities are growing and which microorganisms are driving that growth. These methods are also able to identify the persistence of an applied microorganism and, when compared to an untreated control, how an applied microorganism influences the native populations of the soil. Although costly to conduct, the ability of microbial diversity analysis to infer the biostimulant mechanisms as well as the ecological implications of the application provides a useful parameter for the evaluation of biostimulants.

4.2. The Biostimulant and Soil Health Potential

The concept of soil health is not new to the agriculture community and in the past it has been referred to as soil tilth and soil quality [141]. Recent attention to environmental consequences of improper management resulting in greater soil erosion and nutrient

pollution of waterways has prompted a resurgence in the concept of soil health. Soil health is attributed to multiple parameters, many of which are biologically mediated and, as such, may be influenced by biostimulant application. The USDA NRCS is actively working with academic researchers across the United States to establish uniform methodologies for the evaluation and testing of soil health indicators and a select few include soil enzyme activities, soil respiration rates, and total soil organic C [130]. As highlighted above, these parameters can also be indicators of biostimulant action in row crops, thus providing potential for their application to simultaneously influence crop growth and soil health. Many farmers approach biostimulants looking for a yield response within a growing season with less focus on the potential for long-term impacts on their soils coupled with repeat applications over time. While a biostimulant may not induce a short-term response, there is potential for increases in soil health over time resulting in increased yields in subsequent years. However, evaluations of biostimulants on soils using a long-term perspective are lacking. In addition to a direct impact on soil biological activities for improved soil health is the potential for increased C sequestration. Applications of biostimulants such as biochar are a direct addition of C to farmer fields and as biochar is resistant to degradation it is presumed that repeat applications can increase soil C levels and soil productivity resulting in greater seasonal crop biomass production and biological C sequestration. The long-term addition of C, however, can change the C:N ratios of soils, which may immobilize more N and therefore reduce crop performance; this necessitates the need for long-term research of a biostimulant's influence on soil nutrient cycling, soil health, and soil C. While it would appear to be a perfect solution, the full realization and understanding of the value of biostimulant utilization will take years and diverse approaches to optimize agronomic management for long-term increases in soil health and crop yields.

5. Conclusions

The principal challenges to the biostimulant market are the endless possibilities in their applications. While all agronomic inputs (seed genetics, pesticides, fertility, soil amendments, tillage, and rotation, for example) contain multiple options of product or method selection, the intended purpose of the application is often direct. For example, herbicides, fungicides, insecticides, and nematicides are four unique pesticide inputs, but each has a single intended purpose, which is the suppression of the corresponding pest of weeds, fungal pathogens, insects, or nematodes, respectively. The fertilizer market has a multitude of options available to growers. However, regardless of the fertilizer source and chosen application method, it is most often used to supply the nutrients needed to support a growing crop at a desired yield level. Where biostimulants initially differ from other agricultural inputs is in the versatility of individual products regarding the desired response. Applying the same biostimulant, seaweed extract for example, at planting may influence the microbial communities in the application zone, whereas a foliar application at vegetative growth stages is targeted to induce signal pathways for mitigation of abiotic stress. The second leading challenge for using biostimulants is that the full composition of all compounds within a product is often unknown and this is especially true for the seaweed extracts, HA, and FA. Often a response to a biostimulant is observed without understanding which component was responsible, which introduces the challenge of repeatability of results, and/or knowing the implications of interactions with specific agronomic management practices and environmental conditions. The third confounding factor to using biostimulants is the increasingly diverse product selection that a grower can choose from. While biostimulants are not necessarily new, the last two decades have seen a surge in the number of products available and with the evolution of social media, marketing campaigns targeting their use are intensifying. While the pesticide and fertilizer markets are also increasing product options, the key difference with biostimulants is in their vague regulatory guidelines. With no legal definition of these products, the labeling of new products can be simpler than those, for example, that are regulated by FIFRA and, as such, the rate of new products on the market appears to be much greater than any other

agronomic input sector. It is the three factors of product versatility, limited understanding of product composition and related specific mechanisms of action, and available options that render the agronomic biostimulant market chaotic and ever changing for row crops. Additionally, current variability in single-season yield results makes farmers cautious and successful implementation of biostimulants upon grower fields currently requires a prescription approach that will take multiple seasons of fine-tuning for the successful integration of a new practice.

While the data are variable and the need for greater field testing is evident, it is clear that there are situations in which biostimulants can actively influence the crop and soil system for increased productivity. The focus on fertilizer recovery potential is currently the leading research strategy for biostimulant use in row crop systems, with growing attention to increasing grain yield, which is often a result of more efficient nutrient use. While many biostimulants are targeted for application to row crops for increased productivity, many products achieve these responses through impacts on soils and the biology of the root zone. A closer evaluation of biostimulant effects on soil quality and biological indicators may reveal previously unknown benefits to their application. With greater government and public awareness of agronomic practices and their influence on water quality and nutrient management, the use of biostimulants as a solution to more sustainable practices and improved soil quality provides a viable option even in the absence of measurable yield increases.

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