



Review article

Microalgae, soil and plants: A critical review of microalgae as renewable resources for agriculture

Adriana L. Alvarez^{a,*}, Sharon L. Weyers^b, Hannah M. Goemann^c, Brent M. Peyton^d,
Robert D. Gardner^{a,1}

^a Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN 55108, USA

^b USDA-ARS, North Central Soil Conservation Research Laboratory, Morris, MN 56267, USA

^c Department of Microbiology and Immunology, Montana State University, Bozeman, MT 59717, USA

^d Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT 59717, USA

ARTICLE INFO

Keywords:

Microalgae
Cyanobacteria
Soil fertility
Plant growth
Sustainable agriculture

ABSTRACT

The tension between a growing global demand for food, rising environmental impacts of agricultural intensification, and the uncertainty of future changes in our global climate highlight a crucial need for sustainable alternatives to maintain and increase agricultural productivity. Microalgae, including cyanobacteria, are renewable resources with a broad range of options for applications in agricultural settings. This review incorporates fundamental and applied aspects of microalgae impacting critical agricultural needs such as biological nitrogen fixation, soil phosphorus cycling, effects on soil microorganisms, plant growth promotion either by soil nutrient cycling and/or phytohormones or root associations, biocontrol, and soil stabilization. In this context, the review summarizes progress on microalgal biofilms and consortia as platforms for technological improvement. To complete the review, research needs for future advances are outlined, including evaluations on different types of soil and agroecological regions, scaled production of inoculum and methods of deployment, and further developments on applications such as biostimulants and soil reclamation and restoration.

1. Introduction

Global food security in the coming decades relies on achieving agricultural sustainability. Intensification of agriculture has increased productivities over the last decades, but has also caused global detrimental impacts on the environment [1,2]. This situation is aggravated by a rapidly growing population that will demand a 60% increase in agricultural output in the next 30 years [3], and by a changing climate that threatens current and future agricultural production [4]. As a consequence, maintaining high agricultural productivities while simultaneously mitigating environmental impacts and promoting environmental regeneration is an urgent challenge [1,5].

Microalgae exhibit a pool of traits with unique value for addressing this challenging agricultural scenario. Microalgae are a highly diverse group of primarily photosynthetic microorganisms that includes cyanobacteria (prokaryotic organisms) and eukaryotic organisms (e.g., green algae, euglenoids and diatoms) [6–8]. In agricultural settings, microalgae improve soil fertility and contribute to plant growth and

protection and offer an alternative to reduce our dependence on chemical fertilizers and pesticides [9–11]. Microalgae are beneficial for soil nutrient cycling and can promote plant growth by improving nutrient availability [12–14], producing bioactive substances such as phytohormones [15,16], forming root associations [17,18], or by protecting plants against phytopathogens and pests [9,19,20]. Microalgae also fix carbon dioxide (CO₂) through photosynthesis for carbon capture and some produce exopolysaccharides (EPS) that improve soil structure [21–23]. Cyanobacteria, in particular, are considered biofertilizers due to their long-known ability to fix atmospheric nitrogen (N₂) [14,24,25], and more recently, for solubilizing immobilized phosphorus (P) [26,27]. Furthermore, microalgae can be grown on nutrient-rich waste effluents, capturing excess nutrients that can be recycled for plant growth with a slower nutrient release rate than chemical fertilizers [28–31].

In this context, microalgae are platforms for the potential development of products for soil improvement and crop production and protection, such as biofertilizers, organic fertilizers, biostimulants, biocontrol agents and soil conditioners. However, dispersed knowledge

* Corresponding author.

E-mail address: alvar353@umn.edu (A.L. Alvarez).

¹ Deceased.

and lack of understanding of the effects and mechanisms of microalgae on soil and plants under a broad range of conditions still limit their widespread use in agricultural settings. This review synthesizes insights from laboratory and greenhouse research as well as field studies that describe and evaluate a variety of microalgal strains and plants in the context of these applications to increase our understanding of the potential of microalgae in agricultural settings. This review also outlines research needs for future progress and aims to contribute to increasing the value of microalgae as key renewable resources in the development of innovative bio-economies critically needed for the sustainability of agricultural systems.

2. Microalgae and relevant traits for use in agriculture

The term “algae” often does not refer to a formal taxonomic group but to an assemblage of O₂-producing, photosynthetic organisms with the pigment chlorophyll *a* (with exceptions) that do not necessarily share a common ancestor. Algae contribute to approximately 50% of the photosynthetic productivity on Earth and range from microscopic single cells to macroscopic aggregates and complex leafy structures of seaweeds that may grow up to lengths of 60 m [7].

Algae are commonly divided into macroalgae and microalgae. Macroalgae are large algae, commonly known as seaweeds, although the group includes freshwater and marine species [32,33]. In agriculture, seaweed extracts stimulate seed germination, plant growth and plant defense [34,35]. Microalgae are microscopic algae, found as part of phytoplankton and in nearly all aquatic, terrestrial and sub-aerial surfaces, including all types of soil [36–38]. Microalgae are either

prokaryotic such as cyanobacteria (divisions Cyanophyta and Prochlorophyta), which are gram-negative bacteria, or eukaryotic, for instance green algae (division Chlorophyta), diatoms (class Bacillariophyceae), euglenoids (class Euglenophyceae) and dinoflagellates (division Dinophyta) [7,8,38,39]. Cyanobacteria (class Cyanophyceae), traditionally known as blue-green algae, are found as unicells or filaments, solitary or aggregated in colonies, as free-living organisms, or in symbiotic associations with diatoms, ferns, lichens, cycads, sponges, plants and other organisms [7,40–42].

Microalgae are versatile potential resources in agriculture. Unlike conventional chemical fertilizers, microalgae are an input of organic carbon (C) when applied to soil [22,43]. This is an increasingly relevant aspect considering that depletion of soil organic C is a major type of degradation in croplands that leads to decreased soil quality and fertility [44]. Microalgae incorporate organic C into their biomass through photosynthesis, and many strains release EPS, which functions as C source and sink and improves soil aggregation and stabilization [45,46]. Microalgae also influence soil microbial populations (biomass, activity, community composition and diversity) [47–49], produce phytohormones and other bioactive substances that influence plant growth and control pests and pathogens [50–54], and the biomass can be decomposed and converted into plant available nutrients [55–58]. Cyanobacteria are able to fix atmospheric N₂ [59,60], solubilize P (described at a smaller extent) [26,27], and associate with plant roots, providing nutrients and eliciting plant-defense responses [19,61,62] (Fig. 1).

Most of the beneficial effects for soil and plants have been described for both groups of microalgae (cyanobacteria and eukaryotic microalgae), however, as explained later in this review, it is important to note

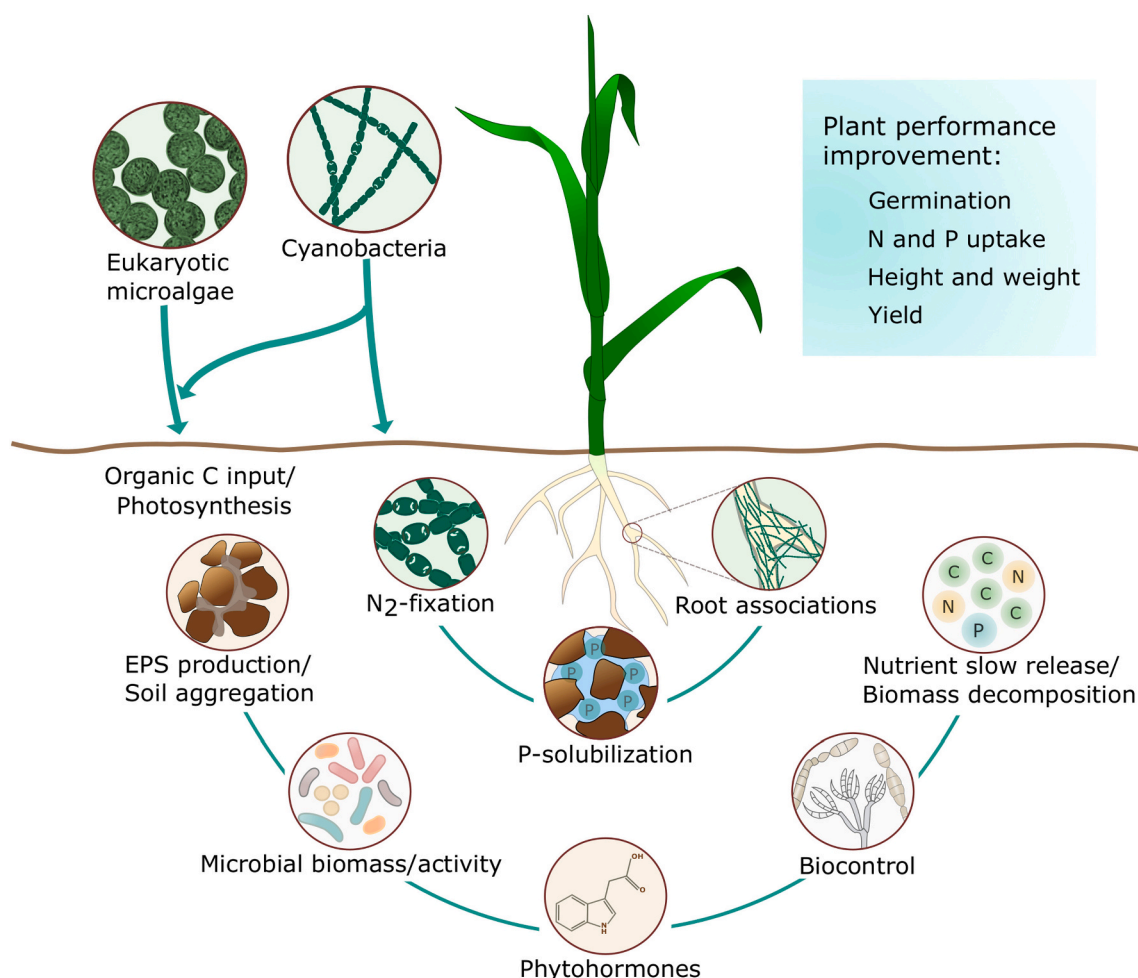


Fig. 1. Selected beneficial effects of microalgae on soil and plants.

from Fig. 1 that N_2 -fixation only occurs in cyanobacteria and, similarly, root associations have only been reported with cyanobacterial groups. Solubilization of inorganic P has been reported for cyanobacteria while both, cyanobacteria and eukaryotic microalgae, could have a role in soil P-cycling through mineralization of organic P (nutrient release). To date, although studies on beneficial traits for use in agriculture have been more focused on cyanobacteria than eukaryotic microalgae, reports with eukaryotic microalgae are emerging for overall plant growth promotion [63–65], detection of phytohormones in extracts improving plant growth [66], and potential applications in biocontrol [67,68].

The diverse effects that microalgal biomass (or microalgal compounds) have on soils and plants, and the different mechanisms of action, offer the opportunity to potentially derive multiple agricultural products from microalgae with applications for soil improvement and crop production and protection (Fig. 2). When applied to soil (microalgal soil amendment), the microalgal biomass can improve physical properties such as soil structure and water retention, and therefore one of the potential applications is as soil conditioners [43,69,70]. According to evolving definitions, microalgae can also be used as biofertilizers, that is, microbial inoculants that improve plant growth when applied to soil, seeds, or plant surfaces by enhancing the supply or availability of nutrients to the plant through the activity of living microorganisms [71,72]. These activities include for example nitrogen (N_2)-fixation and P-solubilization [71,72]. In general, microalgal biomass as soil amendments that supply nutrients could be considered as organic amendments or organic fertilizers [73–75], even when applying non-living microalgal

biomass, for example oven-dry biomass [55,56]. In addition, microalgae are evaluated as new sources of plant biostimulants [47,66,76], understanding plant biostimulants as substances, mixtures or microorganisms that improve plant growth, nutrient use efficiency, tolerance to abiotic stress, quality traits and availability of confined nutrients in soil or rhizosphere, independently of their nutrient content [50,77]. Growing evidence also supports the potential of microalgae and microalgae-derived compounds as biopesticides and biocontrol agents [11,78]. This section summarizes studies that describe the fundamentals for the potential development of microalgae-based biofertilizers, organic fertilizers, plant growth promoters (through different mechanisms), biocontrol agents, and soil conditioners.

2.1. Soil fertility

2.1.1. Biological nitrogen fixation by cyanobacteria

Nitrogen (N) is often the most limiting nutrient in agriculture and the largest and most costly input for crop production [79]. From 2002 to 2018, the world agricultural use of nitrogen fertilizers increased from 84 to 109 megatonnes [80], however about half of the applied fertilizer (e. g., ammonium sulphate, urea, etc.) is not taken up by crops but lost to the environment [81]. For example, in rice fields, around 50–60% of N fertilizer is lost through ammonia volatilization, nitrification and denitrification, surface runoff and leaching, or it can be immobilized by microorganisms [82].

Dinitrogen (N_2) is abundant in the atmosphere (78.1% of the gas in

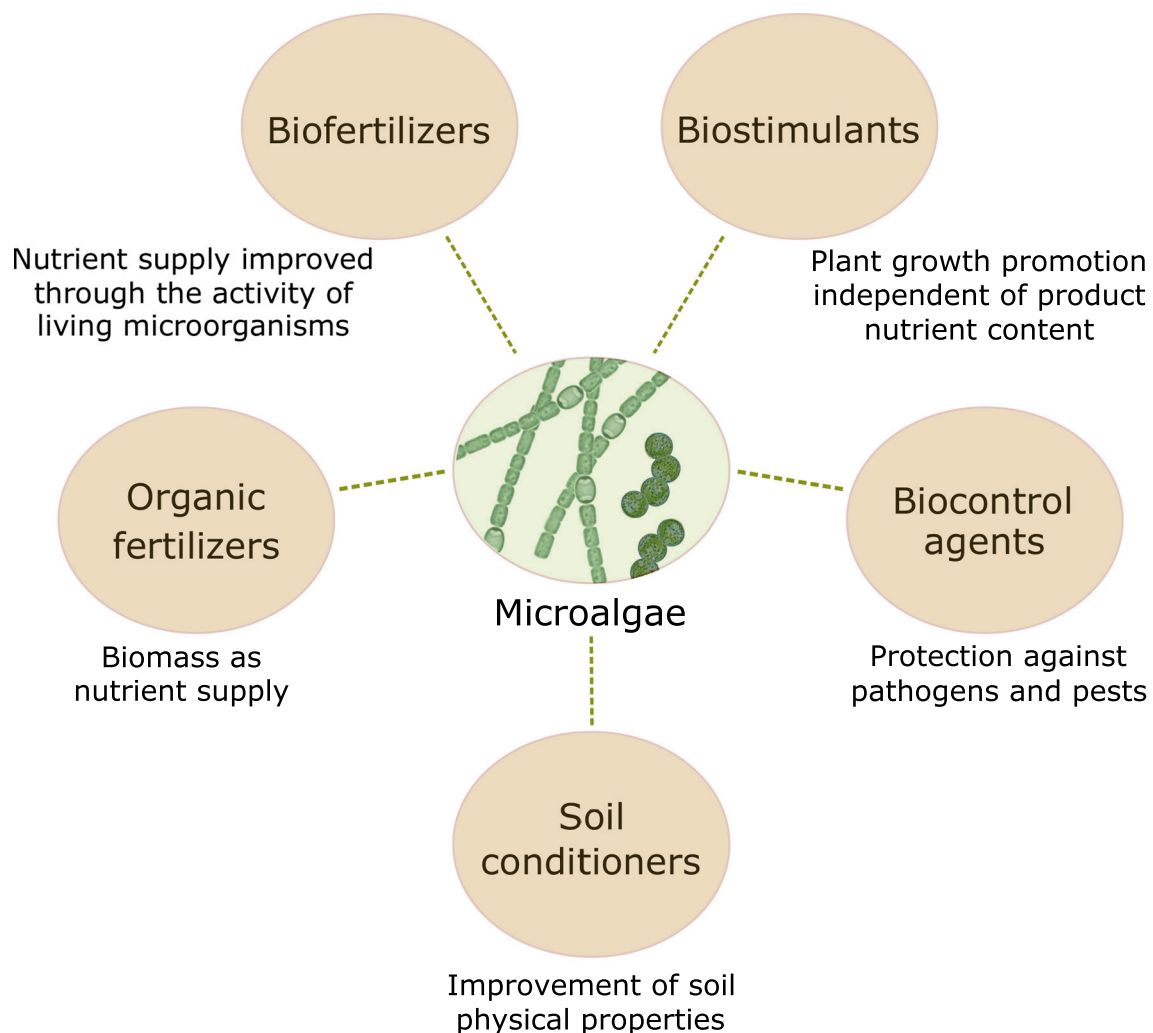


Fig. 2. Potential agricultural microalgae-derived products for soil improvement and crop production and protection.

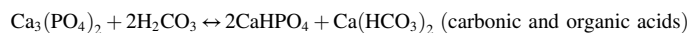
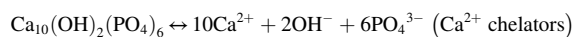
the air) but is not bioavailable to most organisms, including plants. It must be reduced to ammonia (NH_3) before its incorporation into biological molecules. The industrial production of synthetic NH_3 through the Haber-Bosch process was one of the most important discoveries of the past century but is largely dependent on non-renewable fossil fuels and consumes around 1% of the total annual energy supply [60,83]. The reduction of atmospheric N_2 to NH_3 occurs in nature through the biological nitrogen fixation (BNF) by microorganisms known as diazotrophs [84]. The BNF is catalyzed by the enzyme nitrogenase which is found in two of the three domains of life, Bacteria and Archaea, but not Eukarya [84]. Among microalgae, many (though not all) cyanobacteria are diazotrophs [85]. Some cyanobacteria fix N_2 in highly specialized cells within filaments known as heterocysts (e.g., *Anabaena* and *Nostoc*) [84]. Heterocysts are photosynthetically inactive, their formation is repressed by usable sources of N, such as ammonium (NH_4^+) or nitrate (NO_3^-), and up to 5–10% of vegetative cells in the filaments differentiate to heterocysts when deprived of these sources of combined nitrogen [85–87]. The N_2 -fixing ability of cyanobacteria is the basis of their economic importance as an N source for crop plants [59,60,79,88,89].

N_2 -fixation by cyanobacteria increases the soil N pool and this positive soil N balance has benefits for soil fertility [90,91]. Once atmospheric N_2 is fixed, one of the main mechanisms of N transference from cyanobacteria to plants is through mineralization of the cyanobacterial biomass after their death [59]. In 1950, N_2 -fixing cyanobacteria were suggested for the first time as “green manure” to increase the fertility of rice fields [58]; then, Venkataraman [92] proposed the term “algalization” to denote soil inoculations with specific N_2 -fixing cyanobacteria to increase crop production and soil fertility. Paddy (waterlogged rice fields) responded significantly to algalization due to the ideal environment for cyanobacterial growth [93]. The potential of algalization for a more sustainable rice production has been studied for decades [59,60,91] (see Section 3.1), although the interest expands to different crops such as wheat, corn, vegetables, ornamental plants and others [13,19,92,94,95]. The term algalization is mostly used for rice crop, but it is not exclusive to rice [10,92,93,96,97]. However, a clarification of the terminology is needed. Some authors have referred to algalization using cyanobacterial extracts [98], or soil inoculations with other algae different from cyanobacteria [99], while other authors recommend the alternative term “cyanobacterisation” when referring to soil inoculations with living cyanobacteria [70,100].

2.1.2. Phosphorus (P) solubilization, soil P cycling and micronutrients

Phosphorus (P) is the second most important limiting nutrient in agriculture after N [301], and achieving food security in the near future will greatly depend on a reliable P supply [101]. Globally, nearly half of agricultural soils have low available P [102], leading to an increase in the consumption of P fertilizers from 34 megatonnes in 2002 to 41 megatonnes in 2018 [80]. Phosphate fertilizers are produced from P-rich geological deposits (phosphate rock), a non-renewable source located in only a few countries of the world (mainly Morocco, China and the US) that may be depleted in the second half of this century [103]. In 2007, phosphate rock prices peaked when the cost rose 700% in one year, with another peak predicted around 2030 [103]. However, only a portion of the applied fertilizer is taken up by crops, such that a considerable amount of P is lost by erosion and leaching [104], contaminating groundwater and causing surface water eutrophication [105].

Phosphate solubilizing microorganisms (PSM) are common in soils [301]. Most soils contain P in organic or inorganic forms although it is usually unavailable for plant uptake due to its tendency to form complex organic molecules or insoluble inorganic salts with calcium (Ca), iron (Fe) or aluminum (Al). Cyanobacteria are able to solubilize immobilized inorganic phosphate (PO_4^{3-}) [106]. Mandal et al. [26] explained that cyanobacteria solubilize bound PO_4^{3-} with either the synthesis of chelators for Ca^{2+} that change the pH, or the release of carbonic and organic acids, or a combination of both, and described the reactions as:



Examples of this include the solubilization of tricalcium phosphate [107,193], of hydroxyapatite by *Anabaena* sp. and *Nostoc* sp. [108], and the release of phthalic acid as possible mechanism for P-solubilization of phosphate rock and tricalcium phosphate by *Westiellopsis prolifica* and *Anabaena variabilis* [27].

In addition to P-solubilization of insoluble inorganic P, microalgae could have a role in the release of inorganic P from organic P-compounds in soil. In aquatic environments, microalgae can utilize various forms of dissolved organic P (DOP) when the inorganic P is scarce [109]. Phosphoesters, a form of organic P, are used by microalgae for growth either directly or via enzymes like alkaline phosphatases, phosphodiesterases and 5'-nucleotidases present in eukaryotic microalgae and cyanobacteria [110,111]. However, most of the organic P in soil occurs as phytate, the main form of P storage in cereal grains and legume seeds, which is reported to be a major P load in aquatic environments that lead to eutrophication and to cause nutritional problems in monogastric animals [112,113]. Whitton et al. [114] tested 50 cyanobacterial strains, all of them could grow on phosphomonoesters as their sole source of P, 47 on a phosphodiester, and only 35 on phytic acid. In eukaryotic microalgae, phytase activity (hydrolytic enzyme that releases inorganic P from phytic acid) was recently described in a wild-type of *Euglena gracilis* [113] and phytase genes were characterized in the green alga *Chlamydomonas reinhardtii* [112], opening new opportunities for microalgal P utilization in environmental and agricultural applications. Overall, the microalgal enzymes that release inorganic P from organic P-compounds could contribute to the availability of P for plants (P-mineralization) and, therefore, to soil P-cycling. Plant available P can also be released from organic P-compounds or polyphosphates incorporated into the microalgal biomass during decomposition [26,57]. More research would help estimate potential contributions of microalgae to soil P-cycling through P-solubilization and P-mineralization for applications in agricultural settings.

Microalgae also play a role in micronutrient cycling. Micronutrients, such as Mg, Fe, Ca, Zn, Na, S, Cl, B, Mn, Mg and Co, are essential for microalgal growth [111]. Soil inoculations with cyanobacteria-based formulations have increased Fe, Zn, Mn and Cu soil content [115], as well as micronutrient concentration in plant parts, such as Zn in maize leaves [116] and Fe, Zn, Mn and Cu in wheat grains [117]. The mechanisms of plant micronutrient enrichment by soil microalgal inoculations are not well understood, but one proposed mechanism is the production of siderophores by cyanobacteria [117]. Siderophores are low-molecular-weight nitrogenous compounds with strong affinity for Fe^{3+} that contribute to Fe solubilization and mobilization, and potentially of other metals [118]. Micronutrient malnutrition affects almost half of the global population, and plant micronutrient deficiency leads to susceptibility to diseases in food crops, so elucidating the potential role of microalgae to address this issue is of critical value [116,117].

2.1.3. Soil microbial dynamics, activity and diversity

Soil microorganisms are responsible for fundamental soil processes that sustain soil fertility and impact global biogeochemical cycles, including aggregation, degradation of soil organic matter (SOM) and nutrient cycling [119,120]. The maintenance of robust, dynamic microbial communities is essential for ecosystem functioning and a significant priority for sustainable agriculture as agricultural intensification, with the overuse of synthetic fertilizers, has been linked to decreased soil microbial diversity, and soil quality [121–123]. Agricultural systems (including organic farming) can benefit from active and functionally diverse microbial communities that release plant-available nutrients from organic substrates and support high-yielding crops [124]. Hence, soil microorganisms are important for the preservation of soil

functions and considered indicators of soil quality [125,126]. Among the microbiological parameters for soil quality, soil microbial biomass, microbial activity (e.g., soil enzymes), and microbial community composition and diversity are commonly used [119,127–129]. And, in particular with microalgal amendments, most reports evaluate the effect of cyanobacterial biofertilizers on microbial biomass carbon (MBC) and enzyme activity, with fewer studies reporting on community composition and diversity or including eukaryotic microalgae in their treatments.

The soil microbial biomass functions as both a source and sink of nutrients and an agent for transformation and cycling of SOM (e.g., organic C, N, and P mineralization) [120,129,130]. The microbial biomass is a sensitive indicator of early changes in soil conditions and management and is highly influenced by soil C availability (i.e., SOM) [120,131]. The quantity and quality of SOM declines with agricultural intensification and leads to a decrease in the microbial biomass, so agricultural practices that deliver C and restore the microbial biomass in cultivated soils are a relevant need [120]. Cyanobacterial biofertilization (live inoculants) has consistently improved MBC in soils under plant growth; some examples include: 1) individual cyanobacterial strains under wheat [12,132] and chickpea [133]; 2) mixtures of cyanobacteria under rice [134] and wheat [12,132]; and 3) in consortia/biofilms of cyanobacteria with other beneficial microorganisms under rice [134], wheat [132], cotton [11], chickpea [133], okra (*Abelmoschus esculentum*) [115] and chrysanthemum [94] (see also Section 4). An oven-dried mixture of green algae and cyanobacteria grown on sewage wastewater also increased MBC as compared to NPK chemical fertilizer under wheat growth [31]. These studies suggest that across various crop and soil types, microalgal soil amendments widely increase soil MBC and are thus beneficial for soil functions and soil quality. In addition, these studies described benefits for plant growth and yields as being the effect of microalgal amendments improving multiple soil properties besides MBC, including other biological, chemical and physical indicators of soil quality.

Soil nutrient cycling and the release of plant-available nutrients from

organic substrates relies on the activity of the microbial biomass, but the microbial biomass does not describe the microbial activity by itself [129,130]. The potential soil microbial activity can be measured with soil enzyme assays affecting specific nutrient cycles including C, N and P, or with other enzyme assays that indicate general microbial activity such as dehydrogenase and fluorescein diacetate (FDA) hydrolysis [119,135]. When added to soil, microalgae provide C, N and other micronutrients, so it is reasonably expected that these inputs should impact the activity of the soil microbial populations, although the timeframe of these impacts would likely be closely related to environmental conditions and inoculum establishment.

Most available studies examining the effects of microalgae on soil enzyme activity have used cyanobacteria-based formulations. Table 1 summarizes an overwhelmingly positive response in soil enzyme activities to cyanobacteria-based amendments, with most studies reporting significantly enhanced enzyme activities in amended samples as compared to non-amended samples across a variety of cyanobacterial species and crop plants. The selected examples include measurements of dehydrogenase and FDA hydrolysis for general microbial activity, invertase for C cycling, and phosphomonoesterase and alkaline phosphatase for P cycling. Dehydrogenases represent the oxidative metabolic activity of live intact cells and usually correlate with microbial biomass [119,136]. FDA hydrolysis provides a broad representation of soil microbial activity as FDA is hydrolyzed by a wide pool of nonspecific enzymes such as lipases, proteases and esterases [136,137]. Invertase catalyzes the hydrolysis of sucrose to glucose and fructose and can correlate with soil organic C [48,138,139]. Phosphomonoesterases (phosphatases) cleave the ester bond from organic P compounds releasing inorganic PO_4^{3-} available for plant uptake and soil microbes [119,136]. Alkaline phosphatase activity is related to SOM content and highly correlates with microbial biomass [115,132,138].

It is important to note that while some enzyme activities, such as dehydrogenase, are only associated with live cells, many enzymes of microbial origin can remain active in cell debris, soil solution or complexed in humic or clay colloids, as is the case for all the other enzymes

Table 1

A non-exhaustive collection of reported soil enzyme activities in response to cyanobacteria-based amendments. Inoculants and types of study are listed for the first occurrence of each study with 2nd occurrences indicated by “”. Crops are listed in alphabetical order. FDA: fluorescein diacetate, BF: biofertilizer, CA: cyanobacterial amendment (dried at 60 °C), (c): cyanobacteria, (b): bacteria.

Nutrient cycle	Enzyme	Inoculant	Crop	Effect	Reference
General activity	Dehydrogenase	<i>Tolypothrix tenuis</i> (c), <i>Microchaete tenera</i> (c)	Corn	Increase in BF ^a	[142]
		<i>Anabaena</i> sp. (c) + <i>Providencia</i> sp. (b) consortia, <i>Calothrix</i> sp. (c)	Okra	Increase in BF	[115]
		<i>Anabaena doliolum</i> (c), <i>Cylindrospermum sphaerica</i> (c), <i>Nostoc calcicola</i> (c)	Pearl millet, wheat	Increase in CA	[96]
		<i>Nostoc ellipsosporum</i> (c), <i>N. punctiforme</i>	Pearl millet, wheat	No difference	[48]
		<i>Anabaena laxa</i> (c), <i>Anabaena</i> sp., <i>A. oscillarioides</i> , <i>Providencia</i> sp. (b), <i>Brevundimonas diminuta</i> (b), <i>Ochrobactrum anthropi</i> (b)	Rice	5-Fold increase in BF	[143]
		<i>Anabaena torulosa</i> (c), <i>A. doliolum</i> , <i>Nostoc carneum</i> (c), <i>N. piscinale</i>	Rice	Increase in BF	[134]
		<i>Anabaena laxa</i> (c), <i>A. azollae</i> , <i>A. oscillarioides</i> , <i>Calothrix crustacea</i> (c) + other bacterial co-inoculants	Rice, wheat	Increase in BF	[141]
		Co-cultures of cyanobacteria and bacteria	Wheat	51.21% increase in BF	[132]
		“”	Rice, wheat	Increased in BF	[141]
		“”	Wheat	7.26% increase in BF	[132]
C	Invertase	“”	Pearl millet, wheat	Increase in CA	[48]
P	Phosphomonoesterase	“”	Pearl millet, wheat	Increase in CA	[48]
		“”	Okra	Increase in BF	[115]
	Alkaline phosphatase	“”	Rice	6-Fold increase in BF	[143]
		“”	Rice	Decrease in BF	[134]
		“”	Wheat	51.11% increase in BF	[132]

^a Increase/decrease in BF or CA indicates the effect on soil enzyme activity observed with cyanobacterial biofertilizer (BF) or dried cyanobacterial amendment (CA) treatments as compared to non-amended controls.

in Table 1. Regardless, an increase in live soil microbial populations is expected to increase overall enzyme production. At the same time, enzyme activities start to change sooner than other soil properties (e.g., organic C) in response to soil management, so management practices that promote soil quality result in higher enzyme activity [119]. The enhanced soil enzyme activities observed with cyanobacteria-based amendments therefore indicate increased activity of soil microbial populations and suggest potential benefits for long-term soil quality if the practice continues over time. On the other hand, enzyme assays can be sensitive to soil temperature, moisture, pH, and testing methodology which can affect the accuracy of enzyme assays and decrease the ability for cross-study comparisons [140]. Also, most reports in Table 1, except for Rana et al. [141] and Prasanna et al. [134], were studies conducted in pots (under either controlled or natural conditions), which are necessary for recommending inoculants but are not expected to translate accurately to field conditions.

Microbial biomass or microbial activity do not capture changes in soil microbial community composition or diversity, but a comprehensive knowledge of these aspects could assist in identifying key beneficial microbial components for plant productivity in agricultural systems [144,145]. Several studies have described changes in the microbial population after cyanobacterial inoculations. Ibrahim et al. [91] reported increased counts of *Azotobacter* spp. and nitrifiers after inoculations with the cyanobacterium *Tolypothrix tenuis*, while Rogers & Burns [146] found increases of some bacterial groups, actinomycetes and fungi after inoculating soils with *Nostoc muscorum*. In burned soils, inoculation with a cyanobacterial consortium increased cell counts of cellulolytic microbes (cellulose-mineralizers), amylolytics (starch-mineralizers), ammonifiers (NH_4^+ -producers), and nitrifiers (NO_2^- and NO_3^- -producers) [147]. In an okra crop, soil inoculations with *Calothrix* sp. and a consortium of *Anabaena* sp. and *Providencia* sp. (bacteria) revealed shifts in soil bacterial communities as compared to non-inoculated controls, using polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) analysis [115]. Recently, inoculations with biofilms of *Anabaena torulosa* with either *Trichoderma viride* (fungus) or the bacterium *Azotobacter* sp., increased bacterial and cyanobacterial-specific gene counts but decreased counts of archaea in soil with chrysanthemum plants [94]. In addition, some studies have reported changes in microbial communities of specific plant organs after inoculations. Priya et al. [144] found 10-fold increases in bacterial densities (represented in species of *Bacillus*) in roots and shoots of rice seedlings after cyanobacterial inoculations, while Prasanna et al. [148] detected distinct PCR-DGGE profiles in archaeal and bacterial populations as well as in the native cyanobacterial community in root nodules of chickpeas after inoculations with *Anabaena laxa* and a biofilm formulation with *A. laxa* and bacterium *Mesorhizobium cicero*. The wide array of cyanobacterial species used for inoculations as well as variable environmental conditions and fertilizer treatments enhances the difficulty in determining its core effects on the microbial community composition. However, trends in changes in community diversity may be more indicative of important shifts in ecosystem functioning.

Defining the relationship between soil biodiversity and ecosystem functioning is a current challenge in microbial ecology [149]. In light of this challenge, recent work has suggested that microbial diversity can be a feasible predictor of ecosystem multifunctionality and that management practices designed to foster soil microbial alpha diversity (i.e., species richness) may have the potential to improve overall functioning in agroecosystems [150,151]. Advances in molecular biology technology such as high-throughput sequencing have increased our ability to detect, with high-resolution, changes in microbial communities, although few studies have applied this technology to application of microalgal amendments. In a greenhouse study, Lv et al. [152] improved cucumber growth with inoculations of *Anabaena circinalis* or *Scenedesmus quadricauda* (eukaryotic microalga) compared to unamended plants and sequenced the 16S and ITS1 rRNA gene regions to analyze the bacterial and fungal rhizosphere communities respectively. While no

changes in bacterial richness (number of species) were detected, some changes in composition including an increase in Chloroflexi and loss of Gemmatimonadetes taxa were reported. Fungal richness increased over the 45-day greenhouse experiment, with increases in *Fusarium*, *Humicola*, *Metarhizium*, *Penicillium*, *Phoma* and *Trichoderma* genera in biofertilizer-treated pots compared to unamended pots, and an overall decrease in Zygomycota in biofertilizer-treated pots. Similar responses have been observed in organic farming practices where organic fertilizers had little long-term effect on bacterial community diversity but resulted in increased fungal diversity [124]. It is important to note that DNA-based methods do not necessarily reflect the active microbial community as the majority of community members are dormant at any given time [153], and therefore detecting changes in community diversity may be de-coupled from observed changes in function as observed by enzyme assays. However, coupling enzyme assays with RNA-based, high-throughput transcriptomics could capture the potential and realized responses of the active microbial community.

The effects of microalgal soil amendments on soil microbiological parameters such as MBC and enzyme activities are reported as consistently positive, although there remains a need for an increase in field studies to better represent changes in soil nutrients and to investigate long-term effects on soil quality. Also, most available studies used cyanobacteria-based formulations and basic knowledge regarding the effects of eukaryotic microalgae on soil microbiological parameters is still lacking. Current challenges in the field of microbial ecology are centered around understanding functional relationships with community composition and diversity, and we are only beginning to understand the effects of fertilization practices on long-term soil ecosystem functioning [154]. A better understanding of the link between soil microbiological parameters and long-term crop productivity is also a general need [124]. Finally, few studies with microalgae have reported specific changes in soil microbial community composition or diversity, while those that have utilized next generation sequencing have yet to report congruent results. This is in part due to the difficulty in comparing effects between studies with different soil types, climates, inoculant species, and crops. Ideally, future studies would focus on connecting high-resolution community composition and diversity to ecosystem functioning in long-term field experiments.

2.2. Plant growth promotion

Growing evidence supports that both cyanobacteria and eukaryotic microalgae are effective plant growth promoters and have high potential for the development of biostimulants. Table 2 compiles studies conducted at laboratory and greenhouse scales that describe base effects of application of cell suspensions or extracts of single microalgal strains on growth of cereals of economic importance (wheat, corn, rice, sorghum), and some spice crops and vegetables. The table illustrates consistent plant growth and performance improvements with soil inoculations or extract applications, although specific plant responses vary with the microalgal strain, modes of application and experimental conditions [51,98,155–157].

Live cell suspensions or hydroponic co-cultures with cyanobacteria or green microalgae have shown positive results in cereals, legumes and vegetables. For example, soil applications of cell suspensions or fresh biomass of N_2 -fixing cyanobacteria improved plant N, plant weight, dry matter or grain yield in corn, wheat and rice plants [12,155,159,160,164]. Similar improvements were observed with hydroponic co-cultures with live N_2 -fixing cyanobacteria in bean and sugar beet [158]. In tomato, live N_2 -fixing cyanobacteria increased plant N, P and germination [19]. Soaking seeds (grains) in cell suspensions of N_2 -fixing cyanobacteria, non- N_2 -fixing cyanobacteria and green microalgae increased germination, and growth of corn seedlings [161]. Cell suspensions of green microalgae also improved germination of okra and tomato seeds [51,163], and plant length and yield of leafy vegetables (Chinese chives and spinach) [68]. Plant nutritional improvements with

Table 2

Examples of enhanced plant growth or performance under laboratory or greenhouse conditions after application of A: live cell suspensions or fresh biomass, B: dry microalgal biomass, C: cell extracts or hydrolysates of single microalgal strains. Plants are listed in alphabetical order. Improved plant parameters are indicated with an “x”. Other results of the 2nd occurrence of a study are indicated by “”. (c): cyanobacteria, (n): N₂-fixer, (g): green microalgae, (r): red microalga.

Plant	Microalgal strain (s)	Mode of application/type of study	Germination	Shoot/ root length	Plant fresh weight	Plant dry weight	Plant height	Other results	Reference
A. Live cell suspensions or fresh biomass									
Bean, sugar beet	<i>Nostoc</i> sp. (c, n)	Hydroponic co-culture (live cells co-cultured with plants)		x		x		Increased N in shoots and roots.	[158]
Chinese chives, spinach	<i>Chlorella fusca</i> (g)	Foliar spray and soil irrigation/Greenhouse			x			Increased yield and marketable value.	[68]
Corn	<i>Nostoc</i> sp. (c, n)	Applied to pot surface soon after germination/Tunnel house				x		Increases in dry matter yields up to 49%. Increased N uptake and N in tissue.	[159]
Corn	<i>Nostoc</i> spp. (c, n)	Applied to pot surface at two leaf stage/Glasshouse				x		Increased N uptake and N in tissue.	[160] ^a
Corn	<i>Anabaena</i> sp. (c, n) <i>Microcystis aeruginosa</i> (c) <i>Chlorella</i> sp. (g)	Grains soaked in cell suspension/Laboratory incubations	x	x	x	x		Increased leaf length and weight.	[161]
Corn, lentils, sorghum, wheat	<i>Nostoc muscorum</i> (c, n)	Applied to seeds/Laboratory incubations	x	x		x		Increased total N, free amino acids, soluble proteins. Enhanced plant N enzyme activity.	[155]
Coriander, Cumin	<i>Anabaena laxa</i> (c, n) <i>Calothrix elenkinii</i> (c, n)	Applied to potting mix/Phytotron		x	x			Increased peroxidase activity in shoots and roots.	[156]
Lettuce	<i>Chlorella vulgaris</i> (g)	Applied to soil in pots/Greenhouse			x	x		Increased total proteins and pigments in seedlings. Decrease in soluble carbohydrates, soluble proteins, and total free amino acids.	[162]
Okra	<i>Chlorella vulgaris</i> (g)	Applied to seeds and soil in pots/Not reported	x				x	Increased germination and yield, faster maturity.	[163]
Rice (in arsenic contaminated flooded soil)	<i>Anabaena azotica</i> (c, n)	Applied to pots/Greenhouse				x		Increased grain, straw, husk and root dry weight. Enhanced N, P, K, and S uptake in grains. Decreased total arsenic accumulation in grains.	[164]
Tomato	<i>Anabaena laxa</i> (c, n) <i>Anabaena variabilis</i> (c, n)	Applied to carrier, used in potting mix/Phytotron	x					Increased plant P. Increased shoot N in plants challenged with <i>Fusarium oxysporum</i> .	[19]
Tomato	<i>Acutodesmus dimorphus</i> (g)	Applied on seeds and leaves/Greenhouse	x	x	x		x	Increased number of flowers and branches.	[51]
Wheat	<i>Calothrix ghosei</i> (c, n) <i>Hapalosiphon intricatus</i> (c, n) <i>Nostoc</i> sp. (c, n)	Applied to soil in pots near the root region 15 days after sowing/Glasshouse					x	Early fruit development. Increased grain yield.	[12]
Wheat	<i>Chlorella vulgaris</i> (g)	Mixed with nutrient-poor soil/Greenhouse				x		2/3 of chemical N fertilizer replaced.	
								Increased root and shoot dry weight of 8-week old plants, comparable to mineral fertilization. Enhanced N and P content in plant tissues.	[65]
B. Dry microalgal biomass									
Corn	<i>Arthrospira (Spirulina) platensis</i> (c) <i>Chlorella vulgaris</i> (g)	Air-dried biomass applied to soil in pots/Greenhouse		x	x		x	Increased grain yield as compared to non-inoculated controls.	[165]
Lettuce	<i>Chlorella vulgaris</i> (g)	Oven-dried applied to soil in pots/Greenhouse			x	x		“”	[162]
Tomato	<i>Nannochloropsis oculata</i> (g)	Oven-dried biomass applied to substrate/Greenhouse			x	x	x	Increased N, P in leaves, and sugars/carotenoids in fruits as compared to fertilizer controls.	[56]

(continued on next page)

Table 2 (continued)

Plant	Microalgal strain (s)	Mode of application/type of study	Germination	Shoot/root length	Plant fresh weight	Plant dry weight	Plant height	Other results	Reference
Wheat	<i>Chlorella vulgaris</i> (g)	Spray-dried biomass mixed with nutrient-poor soil/Greenhouse				x		""	[65]
C. Culture filtrates, cell extracts or hydrolysates									
Corn, lentils, sorghum, wheat	<i>Nostoc muscorum</i> (c, n)	Culture filtrate applied to seeds/Laboratory incubations	x	x		x		""	[155]
Cucumber, squash, tomato	<i>Anabaena vaginicola</i> (c, n)	Cell extract applied to seeds and on soil surface in pots/Not reported	x	x	x	x	x	Increased stem and leaf fresh weight.	[157]
Cucumber, pumpkin	<i>Westiellopsis prolifica</i> (c, n)	Cell extract applied to seeds/Laboratory incubations	x		x		x	Increased N in roots, shoots and leaves.	[98]
Lettuce	<i>Chlorella vulgaris</i> (g)	Culture filtrate/Greenhouse			x	x		""	[162]
Lettuce	<i>Scenedesmus quadricauda</i> (g)	Cell extract applied to substrate of seedlings in irrigation solution/Growth chamber		x	x	x		Increased protein, chlorophyll and carotenoid content, and enzyme activities in leaves.	[63]
Tomato	<i>Arthrospira (Spirulina) platensis</i> (c) <i>Dunaliella salina</i> (g) <i>Porphyridium</i> sp. (r)	Polysaccharides extracts used for irrigation/Phytotron		x	x	x		Increased number of nodes, carotenoid content and plant N enzyme activities.	[64]
Wheat (under salt stress)	<i>Dunaliella salina</i> (g)	Hydrolysate or exopolysaccharides extract applied to seeds/Laboratory incubations	x	x				Exopolysaccharides had a protective effect under salt stress.	[166]

^a Corn plants had a negative effect on the establishment of cyanobacteria in the soil, possibly due to competition for nutrients.

live microalgal cells reflect biofertilizing properties of the strains used, especially of the N₂-fixing cyanobacteria for plant N, while improvements in germination and plant length reflect their biostimulating properties.

Whole microalgal biomass has also been applied in dry form. Air-dried or spray-dried biomass of the green microalga *Chlorella vulgaris* enhanced plant parameters in corn and wheat [65,165]. In lettuce, oven-dried *C. vulgaris* increased total and insoluble protein, although other plant parameters decreased [162]. Oven-dried *Nannochloropsis oculata* also showed improvements in tomato plants [56]. With the application of non-living dry biomass (oven-dry or spray-dried) plants benefit from the nutrient released through mineralization processes [56]. In this scenario, the dry microalgal biomass is an organic (slow-release) fertilizer rather than a biofertilizer as no living microorganisms are present [56,65,72]. Plant growth promotion with non-living biomass could also be the result of biostimulation due to biologically active microalgal compounds [76]. Schreiber et al. [65] and A. Faheed and Abd-El Fattah [162] compared the use of *C. vulgaris* fresh and dry biomass, with improvements in both treatments as compared to unfertilized controls; however, for lettuce seedlings, dry biomass was more promising for plant weight [162]. For wheat, results in shoot and root parameters from fresh and dry biomass varied depending on the substrate (sand vs. artificial nutrient-deficient mix) [65]. These observations highlight the need for further understanding of the factors impacting plant growth promotion across different plants and soils or substrates when using whole microalgal biomass.

Plant growth improvements have also been observed with cell filtrates, extracts or hydrolysates with biostimulant properties. Water extracts of N₂-fixing cyanobacteria enhanced seed germination, plant weight and plant N in pumpkin, cucumber and squash [98,157]. A water extract of the green microalga *Scenedesmus quadricauda* lysed with methanol improved growth and pigments of lettuce seedlings and increased enzyme activities in leaves that suggested activation of N and C metabolism and plant secondary metabolism [63]. A chemical

hydrolysate (with sulfuric acid) of the green microalga *Dunaliella salina*, and the extracted EPS, increased wheat germination and seedling growth, and the EPS had an additional protective effect against salt-induced stress [166]. Similarly, the EPS from *Arthrospira platensis*, *Dunaliella salina* and the red alga *Porphyridium* sp. improved growth and performance of tomato plants [64], demonstrating that microalgal EPS (or their associated constituents) have plant biostimulant properties.

Plant-growth promotion effects of microalgal suspensions and extracts have also been assessed in field experiments. For example, in winter wheat, Michalak et al. [167] sprayed different rates of supercritical extracts of *Arthrospira (Spirulina) platensis*, with biologically active compounds including polyphenols, and achieved grain yields comparable to commercial plant biostimulants. Similarly, foliar applications of a *Spirulina*-based product increased number of fruits and yield of eggplants (*Solanum melongena*) [168], and foliar spraying of *Chlorella vulgaris* suspensions increased leaf greenness, bunch weight, size of berries and yield of grapes [169]. With another application method, one-minute immersions of onion seedlings in mixtures of humic acids and suspensions of lyophilized *Scenedesmus subspicatus* improved bulb caliber and yield, which was not related to nutrient uptake but rather to C metabolism and accumulation of sugars and water [170]. More research is still needed but these field studies strongly support that microalgal suspensions or extracts can improve plant-growth and performance in field conditions and are promising alternatives for more sustainable and eco-friendly agricultural practices.

Different factors could trigger the observed plant growth promoting effects of microalgae, including (1) enhanced soil fertility (increased C, N, P, organic matter and microbial activity) [12,15,142]; (2) macro/micronutrient mobilization and uptake [19,116,164]; (3) production of phytohormones [54,66], or other bioactive compounds such as amino-acids, polyamines or free volatile fatty acids [171,172]; (4) stress-tolerance metabolites [48,166,173]; (5) reduction of heavy metal translocation to plant tissue [164,174]; and (6) direct plant root-associations [175]. The production of phytohormones and the

associations with plant roots are robust mechanisms identified in other plant-growth promoting microorganisms (bacteria and fungi) [176,177], and have been important research targets for microalgal strains, especially cyanobacteria. However, a deeper understanding of all the above-mentioned factors would advance microalgae-based plant-

growth promotion applications and development of microalgal bio-fertilizers and biostimulants.

2.2.1. Phytohormones

Phytohormones are organic compounds produced at very low

Table 3

Examples of plant growth promotion in phytohormone-producing cyanobacteria by method of application. IAA: auxin indole-3-acetic acid, IBA: auxin indole-3-butyric acid, IPA: auxin indole-3-propionic acid.

Plant	Cyanobacterial strains	Detected phytohormones	Improved growth/performance parameters	Reference
A - Seed incubation with live cell suspensions				
Wheat	<i>Phormidium</i> spp.	Auxins	<ul style="list-style-type: none"> Seedling root and shoot length Seedling number of roots and leaves Seedling weight 	[193]
Wheat	<i>Anabaena</i> sp. <i>Oscillatoria</i> sp. <i>Phormidium</i> sp. <i>Chroococcidiopsis</i> sp. <i>Synechocystis</i> sp.	IAA Cytokinins	<ul style="list-style-type: none"> Seedling shoot length Lateral roots Fresh and dry weights Shoot length, spike length and weight of 100 seeds in mature plants 	[199]
B - Seedling root incubations with live cell suspensions				
Wheat	<i>Leptolyngbya</i> sp. <i>Phormidium</i> sp. <i>Chroococcidiopsis</i> sp. <i>Synechocystis</i> sp.	IAA	<ul style="list-style-type: none"> Seedling shoot length Shoot and root weight Root and leaves number Shoot and root auxin content 	[16]
C - Culture supernatants applied to seeds in vitro				
<i>Lupinus termis</i>	<i>Anabaena flos-aquae</i> <i>Nostoc muscorum</i>	IAA Cytokinin Gibberellin	<ul style="list-style-type: none"> Germination percentage Carbohydrates in seedling shoots Phytohormones in shoots 	[198]
Pea	<i>Chroococcidiopsis</i> sp. <i>Synechocystis</i> sp.	IAA	<ul style="list-style-type: none"> Lateral roots Root length^a 	[190]
D - Biomass extracts applied to seeds in vitro				
Pea	<i>Scytonema bohneri</i>	IAA IBA	<ul style="list-style-type: none"> Seedling root and shoot length 	[171]
E - Extracts or hydrolysates applied on leaves				
<i>Petunia x hybrida</i>	<i>Arthrospira platensis</i>	IAA Cytokinins Gibberellins Ethylene Absciscic acid Other hormones	<ul style="list-style-type: none"> Root and flower weight Flower number Enhanced N and other nutrients foliar concentrations^b 	[66]
<i>Petunia x hybrida</i> (salinity conditions)	<i>Arthrospira platensis</i>	IAA Cytokinins Gibberellins Salicylic acid Jasmonic acid	<ul style="list-style-type: none"> Salt stress tolerance Leaf number and plant dry weight at high salinity Flowering in spring season 	[196]
F - Biomass water extracts sprayed on pot surface				
Cucumber, squash, tomato	<i>Anabaena vaginicola</i> <i>Nostoc calcicola</i>	IBA (predominant) IAA	<ul style="list-style-type: none"> Plant height Plant root length 	[200]
Peppermint	<i>Anabaena vaginicola</i> <i>Cylindrospermum michailovskoense</i> <i>Nostoc calcicola</i>	IAA IPA	<ul style="list-style-type: none"> Essential oil percentage Plant root/shoot length Stem and leaf dry weight Leaf number and ramification 	[202]
G - Soil inoculations with fresh biomass or cell suspensions on pot surface				
Chamomile	<i>Nostoc carneum</i> <i>Nostoc punctiforme</i> <i>Wolleea vaginicola</i>	IAA IPA IBA	<ul style="list-style-type: none"> Root length and weight Weight of essential oil 	[203]
Pea	<i>Nostoc entophyllum</i> <i>Oscillatoria angustissima</i>	IAA Cytokinin Gibberellic acid Absciscic acid	<ul style="list-style-type: none"> Germination percentage Seedling root and shoot length, dry weight and leaf area Seedling pigments, carbohydrate, total N and P, and protease and amylase activities Carbohydrate and protein in seeds at fruiting stage 	[54]
<i>Plantago major</i> L.	<i>Anabaena vaginicola</i> <i>Cylindrospermum michailovskoense</i> <i>Nostoc kihlmani</i> <i>Anabaena cylindrica</i>	IAA IPA IAA Cytokinins Gibberellins	<ul style="list-style-type: none"> Phenolic and flavonoid content Leaf number, root length, leaf and root weight, length of inflorescence 	[201]
Wheat	<i>Nostoc kihlmani</i> <i>Anabaena cylindrica</i>	IAA Cytokinins Gibberellins	<ul style="list-style-type: none"> Germination percentage Seedling root length and dry weight Seedling shoot length and fresh weight 	[15]
Rice	<i>Nostoc carneum</i> <i>Nostoc commune</i>	IAA	<ul style="list-style-type: none"> Seedling growth promotion (root, shoot) Biofertilizer plus 50% of chemical fertilizer showed similar yield parameters as full dose fertilizer (spikes and grains) 	[192]

^a Root length increase was dependent on filtrate concentrations; a decrease was observed at lower filtrate dilutions.

^b Decreased leaves, stems and petioles dry weight.

concentrations that regulate physiological processes in plants [52,178]. Plants produce hormones endogenously and a wide range of microorganisms (bacteria and fungi) produce and release phytohormones that influence plant growth [179,180]. Auxins, cytokinins, gibberellins, ethylene and abscisic acid (ABA) are known as the “classical” phytohormones, and others include brassinosteroids, jasmonic acid, salicylic acid, nitric oxide, strigolactones and polyamines [178]. Phytohormones interact in synergistic or antagonistic ways during plant growth and development and stress responses [181]. Auxins and cytokinins are critical for modulating almost every aspect of plant physiology, including root and shoot architecture and growth, and development of vascular networks and organs [182,183]. Gibberellins are involved in numerous processes such as stem elongation, leaf expansion, early flowering, sex expression, fruit maturation, and inhibition of seed dormancy [184,185]. Ethylene affects a broad spectrum of processes, such as germination, flowering, senescence and abscission, acceleration of fruit ripening, and responses to biotic and abiotic stress; while ABA is a positive regulator of seed dormancy and a major stress hormone, especially related to drought stress and dehydration [181,184]. Ethylene promotes seed germination, but ethylene and ABA inhibit seedling growth [181].

In algae, the production of phytohormones is better recognized in seaweeds, as they produce at least 10 groups of hormones, including auxins, cytokinins and gibberellins [186,187]. The evidence of microalgal phytohormone production and function is increasing but is fragmentary [52], and suggests similarities but also differences to metabolic pathways and regulation strategies described for plants [188,189]. One of the most studied auxins, indole-3-acetic acid (IAA), is produced by cyanobacteria as a tryptophan-dependent product [190–194]. Another auxin, indole-3-butyric acid (IBA), was predominant in *Anabaena vaginicola* and *Nostoc calcicola* [195] and was observed in other species of cyanobacteria like *Scytonema bohnieri* [171]. Cytokinin and gibberellin production has been documented in numerous cyanobacteria, for example, in enzymatic hydrolysates of *Arthrospira (Spirulina) platensis* [15,54,196–198]. Other reports indicate the presence of ethylene and abscisic acid in some strains including *Arthrospira platensis*, *Synechococcus* sp., *Anabaena* sp. and *Nostoc* sp. [52,66].

Table 3 summarizes a body of literature of effects on plant growth and performance with the utilization of cyanobacterial strains with identified phytohormone production. Different modes of application include direct incubations of seeds or roots with cyanobacterial cells [16,199], culture supernatants [190,198], intracellular extracts or enzymatic hydrolysates applied on seeds, leaves or substrate [66,196,200], and fresh biomass applied on soil or substrate [54]. Most reported effects include improvements in germination, plant root and shoot length and weight, leaf number, or flower parameters, and some reports relate to increased carbohydrates, proteins, pigments, nutrient content, essential oils or phytohormones in plant tissue, as well as enhanced tolerance to abiotic stress [16,54,66,196,198,201] (Table 3). Although most effects are beneficial for plant growth and support the application of microalgae as biostimulants, attention should be paid to potentially negative effects. Ahmed et al. [190] reported that extracellular IAA concentrations from two cyanobacterial strains were positively correlated to culture age and tryptophan concentration, among other factors. When supernatants of older cultures with higher IAA concentrations were applied to pea seeds, root number of seedlings was increased but root length was negatively affected. Similarly, Plaza et al. [66] reported that the enzymatic hydrolysate of *Arthrospira (Spirulina) platensis*, with different detected phytohormones, decreased the dry weight of leaves, stems and petioles of *Petunia x hybrida*. A deeper understanding is needed on dose-dependent effects of microalgal phytohormones and their synergistic or antagonistic interactions to avoid undesired outcomes in plant performance.

The five “classical” phytohormones have also been identified in eukaryotic microalgae [52,189], although studies of their influence on plant growth are still scarce. Biomass extracts of *Neochloris* sp. [204],

Chlorella sp., *Coenochloris* sp., *Tetracystis* sp., and *Chlamydomonas* sp. [205] exhibited auxin-like activity that increased number of roots in cucumber cotyledon bioassays. Whereas extracts of *Chlorella* sp., *Coenochloris* sp., *Tetracystis* sp., *Chlamydomonas* sp., *Scenedesmus quadricauda* [205], *Chlorella minutissima* and *Protococcus viridis* [53] showed cytokinin-like activity that increased cotyledon weight. Plaza et al. [66] identified IAA, cytokinins, gibberellins, ethylene, abscisic acid, salicylic acid, and jasmonic acid in protease-hydrolysates of *Scenedesmus almeriensis*. The foliar application of these extracts on *Petunia x hybrida* increased plant dry weight, flower weight and number, shoot and leaves numbers, and P, K, Ca and Mg foliar concentrations, and provide evidence of plant growth promotion with extracts of phytohormone-containing eukaryotic microalgae.

The role of phytohormones in microalgal physiology and their biochemical pathways are not well understood, some functions parallel to those in plants have been hypothesized, including growth, development, and stress tolerance [52,184,206]. Besides promoting plant growth, these phytohormones represent an opportunity for modulating microalgal growth and improving yields in large-scale microalgal cultivation for inocula preparation for applications in agriculture or other fields of commercial interest [207–209]. As the effects of microalgal phytohormones on plant growth continue to be described, elucidating how microalgal phytohormone endogenous production and excretion is affected by multiple factors such as environmental stresses, culture age or co-cultivation will gain importance for further applications.

2.2.2. Root associations with cyanobacteria

A few genera of cyanobacteria, including *Nostoc* and *Anabaena*, are known to form natural symbioses with plants, like liverworts, hornworts, the fern *Azolla*, cycads (gymnosperms), and the angiosperm *Gunnera* [41,210]. *Nostoc* is the most common genus in natural symbioses with the widest host range [41]. In symbioses between plants and N₂-fixing cyanobacteria, 40–90% of the fixed N is released as ammonium to the plant, or as organic nitrogen in the case of cycads [211]. Non-symbiotic plant root associations might also provide N; for instance, N₂-fixing cyanobacteria were visualized on the roots of epiphytic orchids [212]. Natural symbioses do not occur in plants of agricultural importance, however, artificially induced associations between N₂-fixing cyanobacteria and crop plants have been explored as a tool to advance N-independent cereals or the reduction of chemical N-fertilizer use [213–215]. One advantage is that the fixed N, as well as other metabolites, could be transferred to the plant and other soil organisms during cyanobacterial growth rather than after death and decay [216], and also, cyanobacteria can fix N₂ in oxygenic environments, in contrast to other N₂-fixing microbial technologies such as rhizobia [215,217].

Numerous successful artificial associations between cyanobacteria and crop plants have been established at the laboratory scale with effects on plant growth parameters. In Table 4, most studies are in hydroponic systems (co-cultivation of plant seedlings with cyanobacterial strains), except for Priya et al. [144] in water-agar and Prasanna et al. [218] with amended compost. Some studies have established artificial associations using naturally symbiotic strains of *Gunnera* or cycads (cyanobionts) in non-natural hosts such as rice and wheat [175,216,219], while others have used free-living strains [17,19,62,158,220]. Symbiotic strains could have potential advantages including the ability of cyanobionts to transfer most of the fixed N to the plant and their growth in heterotrophic conditions or microaerobic dark conditions [175], although free-living strains have also shown benefits for plant N content [158,215] as well as plant growth, defense and salt-stress tolerance [17–19,173]. Also, most experiments have used heterocystous filamentous strains of the Nostocaceae family since the naturally symbiotic strains belong to this group, but non-heterocystous strains have also successfully associated with roots [62]. Other experimental approaches to induce artificial associations with plants have been summarized by Gusev et al. [214]

Table 4

Examples of artificial root associations by N₂-fixing cyanobacteria in crop plants. Most are hydroponic experiments except for Priya et al. [144] and Prasanna et al. [218]. (h): heterocystous strain, (nh): non-heterocystous strain.

Crop plant	Cyanobacterial strain	Selected outcome	Reference
Symbiotic heterocystous strains			
Rice	<i>Nostoc</i> spp.	<ul style="list-style-type: none"> • 23 of 57 strains colonized roots of seedlings • Nitrogenase activity was higher in associated cyanobacteria than in free-living cyanobacteria • Hormogonia induced by roots or shoots extracts 	[216,219]
Wheat	<i>Nostoc</i> sp. and cyanobiont from fern <i>Azolla pinnata</i> (auxin-producers)	<ul style="list-style-type: none"> • Root endogenous colonization • Higher number of leaves, root length, shoot biomass and chlorophyll • Hormogonia observed 	[175]
Free-living strains			
Chrysanthemum	<i>Anabaena torulosa</i> , <i>Anabaena doliolum</i> , or <i>Anabaena laxa</i> (all h)	<ul style="list-style-type: none"> • Biofilm formation on roots, colonization around root tissues • Increased shoot and root biomass and root protein • Increased IAA and phosphoenol pyruvate carboxylase in plant tissue 	[17]
Corn	<i>Nostoc</i> sp. (h)	<ul style="list-style-type: none"> • Tight associations with roots • Increased N in roots and shoots as compared to plants grown without nitrate 	[158]
Mung-bean and pea	<i>Chroococcidiopsis</i> sp. (nh)	<ul style="list-style-type: none"> • Root exogenous and endogenous colonization • Cyanobacterial auxin production after co-inoculation with seedlings 	[62,190]
Rice	<i>Oscillatoria acuta</i> (nh)	<ul style="list-style-type: none"> • Increased shoot and root length and fresh weight • Metabolic stress tolerance in leaves and rhizosphere 	[173]
Rice	<i>Anabaena</i> spp., <i>Nostoc</i> spp., <i>Calothrix</i> spp., <i>Cylindrospermum</i> sp., or <i>Mastigocladus</i> sp. (all h)	<ul style="list-style-type: none"> • 21 of 45 strains from rice fields showed significant associations. • One <i>Anabaena</i> strain showed the highest association under all conditions. 	[221]
Rice	<i>Anabaena laxa</i> (h) or <i>Calothrix</i> sp. (h)	<ul style="list-style-type: none"> • Colonization in root epidermis and cortex. • Higher plant weight and N₂-fixing potential • Increased hydrolytic and plant defense enzyme activities in roots 	[18]
Rice	<i>Calothrix elenkinii</i> (h)		[144]

Table 4 (continued)

Crop plant	Cyanobacterial strain	Selected outcome	Reference
Sugar beet	<i>Nostoc</i> sp. (h)	<ul style="list-style-type: none"> • Increased shoot and root length, plant fresh and dry weight, auxin production, chlorophyll and N₂-fixation potential • Higher hydrolytic and plant defense enzyme activities in roots and shoots. • Potential changes in microbiome 	[158]
		<ul style="list-style-type: none"> • Tight associations with roots • Increased N and dry weight of roots and shoots but reduced root length as compared to plants grown without nitrate 	
Tomato	<i>Anabaena</i> spp. (h)	<ul style="list-style-type: none"> • Colonization on root surface • Hyphae of the phytopathogen <i>Fusarium oxysporum</i> were reduced 	[19]
Wheat	<i>Nostoc</i> sp. (h)	<ul style="list-style-type: none"> • Colonization of root tissues and migration to stems and surfaces of leaves • Colonization increased nitrogenase activity and N content in roots 	[(213); (217)]
Wheat	<i>Nostoc muscorum</i> (h)	<ul style="list-style-type: none"> • Increased length and fresh weight of roots and shoots • Colonization and nitrogenase activity increased without nitrate 	[222]
Wheat	<i>Anabaena</i> sp. (h)	<ul style="list-style-type: none"> • Loose associations with roots • Increased N in roots and shoots 	[158]
Wheat	<i>Anabaena</i> sp. (h) or <i>Nostoc</i> sp. (h)	<ul style="list-style-type: none"> • Enhanced root and/or shoot length and N content depending on wheat cultivars • Loose associations with <i>Anabaena</i> and one strain of <i>Nostoc</i> 	[215]
Wheat	<i>Anabaena</i> spp. (h) or <i>Calothrix</i> sp. (h)	<ul style="list-style-type: none"> • Plant dry weight was up to 40% higher after 2 weeks of inoculations. • Increased N₂-fixation potential • Increased hydrolytic and plant defense enzyme activities in roots 	[223]
Wheat	<i>Leptolyngbya</i> sp. (nh)	<ul style="list-style-type: none"> • Root exogenous and endogenous colonization (enhanced by nitrate). • Cyanobacterial auxin production after co-inoculation with seedlings 	[62]

and Rai et al. [41], including exposing plant protoplasts (e.g., calli or plant cuttings) to the cyanobacterial strains and the use of chemical and biological agents [41,214].

In root association studies, symbiotic and free-living strains of *Nostoc* have been reported to form hormogonia, a stage of short motile filaments required in natural symbioses between cyanobacteria and plants, which are thought to facilitate associations [219]. Some reports with observed formation of hormogonia described tight associations with penetration of root tissue and the presence of cyanobacterial cells in intracellular and/or intercellular spaces (endogenous colonization) [158,175,213,216,217], while others have reported tight associations without plant tissue penetration (exogenous colonization) [39]. In contrast, penetration of root epidermis has also been reported without observed hormogonia with strains such as *Leptolyngbya* and *Chroococcidiopsis* [62,190] and *Calothrix ghosei* [224]. Phytohormones and plant metabolites could also play a role in artificial associations. For example, IAA facilitated the endogenous colonization by auxin-producing cyanobacteria strains of root epidermal cells in mung-bean, pea and wheat [62,175,224]. In addition, lower cytokinin biosynthesis in a mutant *Nostoc* strain resulted in a decreased ability to colonize rice and wheat roots and stimulate seedling growth in the laboratory [225]. The wild type strain from this study was reported to be endophytic as it was found naturally growing inside root tissue of rice plants [225]. Regarding plant metabolites, it has been proposed that they regulate the stability of natural symbioses with cyanobacteria through factors like hormogonia-inducers and repressors, chemoattractants, and regulators of cyanobacterial growth and metabolism, including heterocyst development [211,226]. In artificial associations, hydrolytic and plant defense enzymes, like polyphenol oxidase, phenylalanine ammonia lyase and β -1,4 endoglucanase had a significant role in facilitating colonization of wheat and rice roots [18,223], and plant exudates and extracts from natural hosts and nonhosts chemoattracted symbiotic *Nostoc* strains, with some sugars having a role, especially arabinose [39].

Other factors might affect the successful establishment of artificial root associations including the presence of nitrate, light, cyanobacterial extracellular polymers, and other bacteria in colonization complexes [214,221,222,227]. For example, nitrate in the medium enhanced colonization of wheat roots by *Leptolyngbya* sp. but inhibited endogenous colonization by *Chroococcidiopsis* sp. [62]. On the other hand, nitrate and light improved colonization of rice roots by free-living strains [221] and symbiotic strains [216] as compared to treatments without nitrate and in the dark. Another factor is the extracellular matrix. Gantar et al. [227] evaluated the extracellular polymers in associations with wheat roots of a *Nostoc* strain with a thick mucillagenous shell and a strain of *Anabaena* with a much less compact shell, and concluded that protein components in the extracellular matrix of *Nostoc* might be involved in developing firm associations with the root surface. Finally, Gusev et al. [214] co-cultured *Anabaena variabilis* and “satellite bacteria” obtained from natural symbiotic complexes in *Azolla* ferns, inoculated whole tobacco plants, and described colonization of plant upper parts and root nodule formation with the co-culture that were not observed with *A. variabilis* alone. Further evidence of the extent of these factors on stable associations is required for advancing artificial root associations in crops of agricultural interest.

In general, the underlying biochemical and molecular mechanisms for these associations are not understood and there is a lack of information on the effects of this induction technology in field experiments [228,229]. There is also little information on how plants influence the success of stable associations, therefore, revealing the genetic and physiological mechanisms of plant responses could open a path to engineer artificial associations through regulating mechanisms of the plant partner on the cyanobacteria [226,228].

2.3. Nutrient capture and recycling

Microalgae can be used simultaneously for wastewater treatment

and crop production. Microalgae grown on nutrient-rich waste streams are considered an alternative for recycling and supplying nutrients for crop growth [29,230]. The case of P is particularly critical as global supplies of mineral P are depleting and about 80% of all used P is lost in wastewater or surface waters [103,230]. Animal waste accounts for around 40% of the mined P annually and is suitable for microalgal growth with N and P removal from 60% to over 90% [230–232]. Microalgal biomass grown on several types of animal waste has been evaluated for plant growth. For example, biomass of *Arthrospira* (*Spirulina*) *platensis* grown on fish wastewater improved growth parameters and germination of herbaceous plants [233]. Also, microalgal biomass grown on anaerobically digested dairy wastewater resulted in plant dry weight and nutrient content comparable to a commercial fertilizer in corn seedlings, leading to estimate that at least 4 ha of corn could be fertilized with the biomass grown on waste from 100 animals [29]. Animal waste as organic fertilizer carries the risk of soil contamination with toxic metals and metalloids (i.e., Cd, Cu, Zn, As), pathogens (virus, bacteria and parasites), and antibiotics that can be translocated to grains [234]. Whether microalgal biomass grown on animal waste poses these risks remains to be determined. However, Franchino et al. [235] showed that microalgae reduced the ecotoxicity of piggy digestate at the lab scale. And, Mulbry et al. [29] reported that the amount of algal biomass grown on digested dairy manure needed to fertilize corn would have heavy metal concentrations well below the limits allowed by the US Environmental Protection Agency (EPA).

Domestic sewage wastewater is also suitable for microalgal nutrient recycling for crops [236,237]. For example, microalgae grown on sewage wastewater (primary treatment) replaced 25% of chemical fertilizer for wheat plants, improving growth parameters and yield [31]. In a different approach, microalgal biomass (mostly *Chlorella* sp. and diatoms) from a municipal sewage treatment plant was anaerobically co-digested with primary sludge and 1% and 0.1% dilutions of co-digestate had no phytotoxic effect on germination or biomass of cress (*Lepidium sativum* L), and 0.1% dilutions showed plant growth stimulant effects. Additionally, heavy metal concentrations and presence of *Escherichia coli* in these dilutions were below limits suggested by the sludge European Directive and EU Directive draft [237]. Another strategy is the use of residual microalgal biomass left after lipid extraction for biodiesel production [238,239]. Sewage-grown *Scenedesmus* sp. was de-oiled and the residual biomass could replace 50% of chemical fertilizers and improve rice plant parameters, including grain yield [239]. This strategy can also generate unknown components toxic to plant growth, so additional testing of the biomass is recommended [238].

Microalgae can accumulate P, particularly from P-rich and store it in the form of long chains of inorganic phosphate or polyphosphate (poly-P) [28,230]. This poly-P enriched microalgal biomass has potential as a slow release P fertilizer [230,240]. Ray et al. [240] reported that microalgal biomass released P at lower amounts than super phosphate but still enough for rice seedlings, therefore reducing P excess in the soil. Also, Mukherjee et al. [28] used rice mill effluent with high soluble P concentrations to grow a microalgal consortium that showed the accumulation of poly-P granules and reached higher densities than monocultures. The consortium effectively removed nutrients to limits acceptable for crop irrigation, and the dried biomass showed gradual release of available P with higher shoot height and leaf width of rice seedlings than chemical P fertilizers. Overall, these approaches for nutrient recycling in agriculture would support sustainable microalgal production while reducing nutrient losses to the environment.

2.4. Biocontrol

Chemical pesticides have harmful effects on the environment and health of humans and animals but are widely used to prevent losses in plant crops due to pests and pathogens. The biological control of plant diseases are a safer alternative, but costs are not competitive and the knowledge on compounds with biocidal activity and mechanisms of

action is still limited [78]. Cyanobacteria and eukaryotic microalgae produce allelopathic chemicals - secondary metabolites that affect individuals other than the ones producing them - that can be used as algicides, fungicides, herbicides, insecticides and nematocides [9,241,242]. Some microalgal compounds also have antimicrobial, antiviral and antiprotozoal activities [243]. Comprehensive reviews on microalgal metabolites (mostly of cyanobacterial origin), chemical structures and known biocidal activities are available [78,243,244]. However, the effect of these metabolites specifically on phytopathogens and pests of agricultural importance has been less explored [78,245].

Kulik [246] highlighted the in vitro inhibition by cyanobacteria of the phytopathogenic fungi *Rhizoctonia solani* and *Sclerotinia sclerotiorum* as well as the saprophytes *Chaetomium globosum*, *Cunninghamella blakesleeana* and *Aspergillus oryzae* of potential interest. Table 5 summarizes examples of microalgae or microalgal extracts tested on some phytopathogens (fungi or bacteria) and pests (nematodes or insects). The biocontrol potential has been evaluated with culture filtrates, intracellular extracts, or soil/compost amendments. The mechanisms of action remain poorly understood, although progress with cyanobacteria indicate that in addition to secondary metabolites such as phenolic compounds and alkaloids [247], hydrolytic enzymes (e.g., chitinase, xylanase, endoglucanase) might directly contribute to fungicidal activity by fungal cell wall break down [245,248]. Also, components of cyanobacterial amendments or extracts such as poly- and oligosaccharides might elicit plant responses, as revealed by increased defense

enzymes (e.g., phenylalanine ammonia lyase, polyphenol oxidase, peroxidase) and pathogenesis-related enzymes (chitinase, chitosanase, β -1,3-glucanase) in plant tissues [19,20,156]. In addition, Holajjer et al. [9] especially indicated the potential of cyanobacteria to induce immobility, inhibition of hatching or alter reproduction of plant parasitic nematodes through the action of cyanotoxins, and explained the dependence of these effects on cyanobacterial strains, age of culture, the use of exudates (water soluble secondary metabolites), or different extracts (aqueous, methanolic, acetone extracts, sonicated cell extracts). Overall, these studies not only evidence the potential of microalgae for the development of effective biocontrol agents but also call for urgent research efforts in light of the need for safer alternatives in the field of pesticides.

2.5. Soil structure, erosion control and water retention

Declines in soil structure lead to land degradation through erosion, crusting and compaction [249]. Soil structure is determined by soil aggregation and soils with stable aggregates have improved aeration and hydraulic properties and less susceptibility to erosive forces like wind and water [250,251]. Soil microorganisms play a critical role in the formation and stabilization of these aggregates as their EPS acts as binding agents of soil particles [159,252]. Microalgae produce EPS at variable amounts and compositions [253,254]. And, the EPS forms a complex extracellular matrix containing proteins, lipids, nucleic acids,

Table 5

Selected examples of microalgal strains or extracts with biocidal activity on plant pathogens and pests.

Microalgal strains (application)	Phytopathogen/pest	Plant/disease	Selected result	Potential biocontrol mechanisms	Reference
Cyanobacteria <i>Anabaena</i> spp. (Culture filtrates)	Fungi: <i>Fusarium moniliforme</i> , <i>Alternaria solani</i> , <i>Aspergillus candidus</i> , <i>Drechslera oryzae</i> and <i>Pythium aphanidermatum</i>	No tests on plants/ Phytopathogenic	Growth inhibition	Hydrolytic enzymes, chitinase and xylanase with fungicidal activity.	[248]
<i>Anabaena laxa</i> and <i>Calothrix elenkinii</i> (Soil amendment/pots)	Fungus: <i>Fusarium oxysporum</i>	Coriander, cumin, fennel/ <i>Fusarium</i> wilt	Fungicidal activity of plant extracts	Peroxidase and endoglucanase activity in roots and shoots.	[156]
<i>Anabaena laxa</i> or <i>A. variabilis</i> (Amended compost/pots)	Fungus: <i>Fusarium oxysporum</i> f. sp. <i>Lycopersici</i>	Tomato/Wilt disease	Mortality reduction of 2-week old seedlings	Chitinase and endoglucanase in culture filtrates. Defense enzymes (phenylalanine ammonia lyase, polyphenol oxidase) and pathogenesis related enzymes (chitinase, β -1,3-glucanase) in root tissues.	[19]
<i>Anabaena</i> sp. (Water extract sprayed on leaves)	Fungus: <i>Podosphaera xanthii</i>	Zucchini (<i>Cucurbita pepo</i>)/Powdery mildew	Reduction of sporulation and infected area in leaves	Direct antifungal activity from the extract. Systemic accumulation of chitinase, peroxidase and β -1,3-glucanase activities, possibly triggered by poly- and oligosaccharides in the extract.	[20]
<i>Nostoc muscorum</i> and <i>Oscillatoria</i> sp. (Culture filtrates)	Fungus: <i>Alternaria porri</i>	Onion/Purple blotch	55.1–66.5% reduction of disease severity in greenhouse conditions	High concentrations of phenolic compounds and alkaloids with fungicidal activity.	[247]
<i>Anabaena flos aquae</i> (Intracellular extract)	Insect: <i>Spodoptera littoralis</i>	Cotton/Leaf worm	Larvae were susceptible, and eggs fertility and adult emergence was decreased	Bioactive secondary metabolites.	[61]
Eukaryotic microalgae (g: green alga, d: diatom) <i>Scenedesmus</i> sp. (g, intracellular extract)	Fungus: <i>Alternaria</i> sp.	No tests on plants/ Phytopathogenic	Inhibition of fungal growth	Secondary metabolites.	[99]
<i>Desmococcus olivaceus</i> (g, intracellular extract)	Bacteria: <i>Pseudomonas syringae</i>	No tests on plants/ Phytopathogenic	Inhibition of bacterial growth	Secondary metabolites.	[99]
<i>Chlorella fusca</i> (g, culture dilution)	Fungus: <i>Botrytis squamosa</i>	Chinese chives/Gray mold	24.2% reduction of disease occurrence on leaves	Not reported.	[68]
<i>Amphora coffeaeformis</i> (d, extract of fermented biomass sprayed on leaves and applied in drench)	Nematode: <i>Meloidogyne incognita</i>	Cucumber/Root-knot nematode	2.5–2.69 times increase in marketable yield	Secondary metabolites.	[67]

and other substances, that provides protection and an optimal environment with increased moisture and nutrients [46,252].

The effect of EPS on soil physical properties such as aggregation, aggregate stability and water retention, has been broadly studied in the field of biological soil crusts (BSCs) in arid and semiarid ecosystems, where cyanobacteria and eukaryotic microalgae are essential components. These studies are either in natural crusts or, more recently, in artificial crusts induced with cyanobacterial inoculations for restoration of degraded soils [46,70,255–257]. However, EPS-producing microalgae have also improved soil physical properties in agricultural settings, supporting potential as soil conditioners [48,69,258,259]. The EPS in BSCs facilitates initial soil aggregation and its amphiphilic nature grants cyanobacterial filaments the ability to interweave in networks that stabilize soil and form additional pores [252,257]. Improved soil aggregation and stability have also been reflected in experiments with arable soils. Aggregate size and stability in water were increased by inoculating low organic C soils with EPS-producing *Nostoc* sp. in a pot experiment with corn, although this effect was dependent on the strain used and the presence of plants [160,258]. Other examples include the increased water stability of soil aggregates observed with other cyanobacterial strains such as *Nostoc muscorum* [146], *Tolypothrix tenuis* [260] and a combination of *Aulosira fertilissima*, *Tolypothrix tenuis*, *Anabaena*, *Nostoc* and *Plectonema* [261]. In addition, the application of the EPS alone, isolated from *Nostoc muscorum*, improved aggregate stability in a saline-sodic soil [262]. Eukaryotic microalgae *Chlamydomonas mexicana* and *C. sajabo* also increased aggregate stability in temperate agricultural soils [69,263].

An improved aggregate stability is expected to translate into resistance to erosion by wind and water. In BSCs, Hu et al. [264] demonstrated improved resistance of fine sand to wind erosion, while Chamizo et al. [265] described lower sediment erosion after rain simulations in field experiments. With cyanobacterial inoculations, Sadeghi et al. [266] reported a reduction of up to 36% in soil loss by runoff after natural rainfall in field plots inoculated with *Nostoc* sp. and *Oscillatoria* sp. in abandoned agricultural lands, showing promising results to counteract erosion. On the other hand, EPS-producing microalgae can improve soil water retention and hydraulic behavior. The EPS is hygroscopic and captures water from rainfall and from non-rainfall sources such as fog, dew and water vapor [46,256]. It also reduces evaporative losses and retains water for longer periods, as demonstrated by experiments comparing intact BSCs with EPS-extracted BSCs [256,267]. However, at very low soil moisture (6–8%) in dry warm periods, water losses are similar in soils with and without BSCs [255,268] or in crusts with and without EPS [267]. In agricultural soils, microalgal amendments have increased soil water holding capacity (WHC) [26]. For example, an oven-dried mixture of the cyanobacteria *Anabaena doliolum*, *Cylindrocapsa sphaerica* and *Nostoc calcicola* (with EPS at around 35% of the total biomass) improved WHC and hydraulic conductivity in a semiarid soil under pearl millet-wheat growth [96]. In a similar experiment but with salt-affected soil and a mixture of two strains of *Nostoc*, WHC increased (although not significantly) and hydraulic conductivity was up to 58% higher [48]. In general, EPS confers a positive water balance by delaying water movements through soil, creating waterways, improving water uptake and WHC, and reducing evaporation losses [252].

3. Field applications of microalgal soil amendments

3.1. Cyanobacteria and rice

Cyanobacteria are accountable for most of the BNF and natural fertility in rice fields [269]. In rice fields without applied N-fertilizer, BNF by cyanobacteria was reportedly between 20 and 30 kg N crop⁻¹ [59,270]. Cyanobacteria in this ecosystem are favored by low-light conditions (when the rice canopy is dense or seasons cloudy), high P availability, neutral to slight alkaline pH values, and 30–35 °C

temperatures. But their density is affected by alternating dry and wet periods and grazing by invertebrates [14,90]. In rice fields, 50% of the common cyanobacterial genera are heterocystous, including *Anabaena*, *Nostoc* and *Gloeotrichia*, other common cyanobacteria found include non-heterocystous *Microcystis*, *Chroococcus*, *Oscillatoria*, *Lyngbya* and *Phormidium* [90,271].

Field trials on rice fields showed variable impacts on grain yield. A review of more than 300 studies pointed out that inoculation was not always effective, although some experiments in India resulted in a 14% relative increase in rice yield (about 450 kg grain ha⁻¹ crop⁻¹) [14]. Results compiled from India, Japan, USSR, Burma, Egypt, China and the Philippines highlighted the successful trials where yields increased by 10–24% [60]. On the upside, this report demonstrated that yield increases were progressive in time due to permanent establishment of inoculated cyanobacteria in soils after 3–4 consecutive cropping seasons, thus reducing the need for continuous re-inoculation [60]. However, a more recent compilation of 634 field experiments continued to show very large variability in yields between inoculated and non-inoculated crops, such that only 17% of the individual experiments had statistically significant differences between the two treatments [59].

One of the numerous factors for variable outcomes in rice fields is the density of natural or indigenous soil populations of cyanobacteria. Inoculated cyanobacteria, either indigenous or non-indigenous, do not become dominant in soils where cyanobacterial densities are already high [270,272]. In fact, in 102 samples of rice soils from the Philippines, India, Malaysia and Portugal, indigenous cyanobacteria (*Nostoc* sp., *Anabaena* sp. and *Calothrix* sp.) increased in density after soils were inoculated with recommended doses of non-indigenous strains [273]. Rice fields in Spain were inoculated with indigenous strains but grain yields did not increase probably because the number of indigenous cyanobacteria was already high [274]. To overcome this limitation, Roger et al. [273] recommended that research should focus on agricultural practices that enhance the growth of indigenous strains in situ, for example, limiting grazer pressure.

Other important factors affecting the success of cyanobacterial inoculations in rice fields are the use of chemical N fertilizers [88], and soil conditions [270]. Increasing the rates of ammonium sulphate over three consecutive rice crop seasons decreased soil BNF and cyanobacterial densities [88]. Also, in a summary of 180 experimental studies, Roger [59] reported that the average BNF was 20 kg N ha⁻¹ crop⁻¹ in plots without N, 12 kg N ha⁻¹ crop⁻¹ in plots with deep-placed urea and 8 kg N ha⁻¹ crop⁻¹ in plots with broadcast urea. On the other hand, Hashem [270] combined different rates of urea with a mixture of cyanobacteria and determined that soil conditions affected the impact of fertilizer use. Yield performance and resulting soil fertility were better in acid, saline and red soils than calcareous and neutral soils. This was probably because the cyanobacteria modified the soil pH towards neutrality, reduced salinity and increased soil available P and S.

Cyanobacteria are important for rice crop sustainability despite the multiple factors affecting the success of inoculations in increasing rice yield. The significance of cyanobacterial contribution to N in the soil-plant system in rice fields was determined by Fernández-Valiente et al. [88] by applying ¹⁵N labeled ammonium sulphate fertilizer or ¹⁵N labeled *Nostoc* sp. to soil. At harvest, the soil-plant system recovered 46.6% of the ¹⁵N from cyanobacteria versus 26% from fertilizer in field experiments. While there were no differences in yield in this study, the soil N pool during the crop cycle was more readily replenished by cyanobacteria than by ammonium sulphate. Fernández-Valiente et al. [88] suggested that cyanobacteria use be combined with a restricted amount of chemical fertilizer (up to 70 kg N ha⁻¹) to achieve higher yields and an active BNF cyanobacterial population.

3.1.1. Cyanobacterial symbiotic associations with aquatic macrophytes for rice crop

The fern *Azolla* sp. (Salvinaceae) has been used as green manure to increase productivity in rice fields. This technology is based on the

natural symbiotic association between *Azolla* sp. and the cyanobacterium *Anabaena azollae*. In this association, cyanobacteria are located in cavities in the dorsal lobes of *Azolla* sp. leaves, and their cells differentiate into heterocysts at a higher frequency (up to 20–30%) as compared to free-living cyanobacteria (5–10%) [275]. Most of the N in the fern originates from BNF as confirmed by ^{15}N methods, and becomes available to rice after decomposition and mineralization, constituting from 20 to 34% of the N recovered by the rice plant [59]. In field trials, *Azolla* sp. increased rice yields by 0.4–1.5 tons ha^{-1} [186]. Furthermore, the incorporation of one crop of *Azolla* sp. either before or after transplanting rice can be equivalent to the application of 30 kg of N fertilizer, and the incorporation of two crops of *Azolla* sp. (one before and another after transplantation) can be equivalent to 60 kg of N fertilizer [276]. However, *Azolla* is susceptible to pests and its use is labor intensive. This technology was traditional for centuries in China and Vietnam but its use decreased in the 80's (partially due to low price of N and P fertilizers at that time), and the technology was not transferred to different countries [59].

Cyanobacteria grow on other macrophytes in rice fields forming epiphytic associations. In rice fields of Valencia-Spain, epiphytic cyanobacteria are more associated with the macroalga *Chara* sp. than rice plants. These cyanobacteria are responsible for most of the N_2 -fixation in the ecosystem, representing more than 45% of the nitrogenase activity and fixing 27.5 kg of N ha^{-1} crop $^{-1}$ [90,277]. These findings show that biologically fertilized crop production is likely subject to location-specific factors, and further suggest that to optimize crop yields with biological alternatives to chemical fertilizers, attention should be focused on the analysis of local biotic and abiotic factors for particular agricultural systems.

3.2. Examples of field applications in crops other than rice

Most field studies of microalgal soil amendments have been with cyanobacterial inoculations on rice fields but reports with other crops are increasing, with special focus on microalgal consortia and biofilms (Section 4). Soil applications of microalgae at the field scale benefit plant growth [278], crop yield [11,133,279], soil fertility [11,133], crop protection [11], and soil structure [69,263].

In chickpeas (*Cicer arietinum* L.), Bidyarani et al. [133] demonstrated that inoculation with fresh biomass of the cyanobacteria *Anabaena laxa* improved dry weight, number of pods per plant and yield (50% higher). At mid-crop, inoculation improved soil parameters (polysaccharide, dehydrogenase activity, MBC, available N and P) and plants (N content, N_2 -fixation and leghaemoglobin in nodules). In cotton, Prasanna et al. [11] evaluated fresh biomass inoculations of individual strains of *Anabaena* and *Calothrix* combined with 50% of the recommended dose of N fertilizers. Inoculations resulted in higher soil available N and plant fresh weight and height. In separate plots without chemical fertilizer, inoculations increased soil MBC and the activity of hydrolytic enzymes, reducing mortality in plants infected by the fungus *Rhizoctonia* sp. and conferring a level of protection comparable to a commercial formulation.

With eukaryotic microalgae, Shaaban [278] applied dried biomass of *Chlorella vulgaris* at different rates before sowing corn. Forty-days old plants showed improved plant growth (root volume, plant height, and root, shoot and total dry weight), increased chlorophyll in leaves and higher micronutrient uptake (Mg, Fe, Mn and Zn). High EPS-producing *Chlamydomonas mexicana* and *C. sajabo* were also effective soil conditioners in field applications [69,263]. In a 3-year study in corn, the enhanced aggregate stability at a rate of 7.8 kg of dry microalgal biomass ha^{-1} supported the feasibility of using microalgae to reduce erosion in soils with poor structure [263]. Large inocula of 10^{11} cells ha^{-1} year $^{-1}$ could produce up to 500 kg of polysaccharide ha^{-1} but extending commercialization of this process was limited by biomass production and dry product viability [186,259].

If live inoculants are used in field applications, the ability of

microalgal strains to reproduce and establish in the soil would reduce the need for subsequent re-inoculations and is an important indicator that the strains would overcome environmental stress (e.g., desiccation), soil predators and grazers, and competition with native soil microalgae [10,60,218,275,280]. The lack of this information when selecting strains could lead to inconsistent performance in the field and is an important limitation for widespread use of microalgae [10,281]. So, suitable markers to evaluate the establishment in the soil are needed (e.g., chlorophyll, biochemical and molecular markers) [218,280,281]. Only few field studies have reported soil colonization of the applied microalgal strains. In irrigated temperate soils, Metting [280] described a population increase of *Chlamydomonas sajabo* over a 10-week period at high-rate applications (5×10^{11} cells ha^{-1}), and Reynaud and Metting [275] reported that a local *Nostoc* strain was able to proliferate and predominate in a soil cropped to winter wheat with an average growth rate of 0.12 g m^{-2} day $^{-1}$ over a 66-day period, however, the biomass decreased drastically after harvest and desiccation. Prasanna et al. [218] successfully used molecular markers to confirm the establishment of different consortia in a wheat-rice cropping sequence, but more advancements are needed with the development of feasible markers to evaluate colonization and also of inoculant formulations that can establish in the soil and improve agronomic efficiency [10].

4. Microalgal biofilms and consortia for crop growth

Associations or biofilms of microalgae with other organisms are important for adaptation and colonization in natural environments [282]. Applications of mixed cultures and biofilm-forming organisms could bring about improved performance over inoculations with single strains [95,283], allowing larger microalgal populations in soil or more diverse metabolic activities [282]. Rational approaches along these lines have been advanced at the Indian Agricultural Research Institute (IARI), with the application of artificial mixtures (consortia) or biofilms with microorganisms with agriculturally important traits like plant-growth promoting rhizobacteria (PGPR), including diazotrophs and P-solubilizers [95,242] or microorganisms with activity against phytopathogenic fungi [284] yielding improved performance as compared to single strains.

Table 6 summarizes examples of consortia and biofilm formulations with N_2 -fixing cyanobacteria as inoculants in greenhouse and field experiments, with promising results to establish in the soil [95,134], improve soil and plant parameters, and replace from 25% to 50% of the recommended dose of N chemical fertilizer for grain yield [13,218,134]. Despite the variety of treatments and crops used in these studies, inoculations showed beneficial effects on soil nutrient content (e.g., N, P, organic C) and soil microbial parameters such as MBC and microbial activity. Enhanced plant parameters including grain weight, plant weight, N and P uptake, and plant enzyme activities (e.g., defense enzymes) were also reported. Different carriers have been used to apply these inoculants. In greenhouse experiments, carriers included compost and vermiculite [94] and charcoal and soil [95,132,242]. In field studies, paddy straw compost [141,218] and paddy straw compost with vermiculite [11,13,116,134,148] were tested. In some of the studies, seeds were coated with the inoculant formulations in addition to the carrier inoculation [13,95,132,141].

Fewer reports include eukaryotic microalgae in the formulations. A consortium of *Chlorella vulgaris* and *Pseudomonas putida* increased plant parameters of rice plants, including shoot and root length and dry weight, and decreased arsenic (As) translocation in roots and shoots as compared to non-inoculated controls [174]. Another approach was to use wastewater-grown microalgal biomass to inoculate soils and allow for the formation of microalgal biofilms. A *Chlorella vulgaris*-dominated biofilm increased shoot and leaf dry mass in 60-days old millet plants after soil inoculation with microalgal biomass grown on a primary effluent from a meat processing facility [285].

In many natural systems, biofilms composed of cyanobacteria,

Table 6

Examples of cyanobacteria in consortia and biofilms as inoculants for crop growth in pot experiments and field studies. Since cyanobacterial strains were N₂-fixers, some studies used the inoculants to partially replace the recommended dose of N plus full dose of PK (+PK). Second occurrences are indicated by “”. (b): bacteria, (c): cyanobacteria, (f): fungus.

Selected inoculants	Crop	Selected outcomes	Reference
A. Consortia in greenhouse or pot experiments			
<i>Anabaena</i> sp. (c) + <i>Bacillus</i> sp. (b) + <i>Brevundimonas diminuta</i> (b) <i>Anabaena</i> sp. (c) + <i>Calothrix</i> sp. (c) + <i>Bacillus</i> sp. (b)	Wheat	Inoculum applied with 50% N + PK and compared to full dose of NPK: • Increased crop biomass, grain weight, and soil microbial activity (FDA hydrolysis).	[242]
B. Consortia in field studies			
<i>Anabaena</i> spp. (c) + <i>Nostoc</i> spp. (c)	Corn	Inoculum applied with 2/3 N + PK and compared to full dose of NPK: • High available N in soil with savings of 1/3 N (40 kg N ha ⁻¹). • Positive interactions between soil microbial activity and plant parameters. • Plant height increased and cob weight at par.	[13]
<i>Anabaena</i> spp. (c) + <i>Nostoc</i> spp. (c)	Rice	Inoculum applied with 50% N + PK and compared to full dose of NPK: • High soil MBC, nitrogenase activity and available N in soil. • Grain and straw yield at par with savings of 50% N fertilizer.	[134]
<i>Anabaena oscillarioides</i> (c) + <i>Brevundimonas diminuta</i> (b) + <i>Ochrobactrum anthropi</i> (b)	Rice	Inoculum applied with 2/3 N + PK and compared to full dose of NPK: • Improved grain yield with higher macro- and micronutrient (Fe, Zn, Cu, Mn) grain content. • Higher soil microbial activity.	[141]
<i>Anabaena</i> spp. (c) + <i>Nostoc</i> spp. (c)	Wheat-rice sequence	Inoculum applied with 75% N + PK and compared to full dose of NPK: • Increased microbiological parameters (N ₂ -fixation and MBC). • Savings of 25% N for grain yield.	[218]
C. Biofilms in greenhouse or pot experiments			
<i>Anabaena torulosa</i> (c) + <i>Trichoderma viride</i> (f) <i>A. torulosa</i> (c) + <i>Azotobacter</i> sp. (b)	Chrysanthemum	Compared to non-inoculated soil or carrier alone: • Enhanced plant weight, length, flower	[94]

Table 6 (continued)

Selected inoculants	Crop	Selected outcomes	Reference
<i>Anabaena torulosa</i> (c) + <i>Pseudomonas striata</i> or <i>Serratia marcescens</i> (b, P-solubilizers) <i>A. torulosa</i> + <i>Azotobacter chroococcum</i> or <i>Mesorhizobium ciceri</i> (b, N ₂ -fixers)	Wheat	diameter, and plant enzyme activities. • Increased soil macro- and micronutrients, and microbiological parameters (MBC and microbial activity). • Increased cyanobacterial and bacterial abundance in soil. Compared to 50% N + PK and 50% P + NK chemical fertilizer: • Soil N, P, N ₂ -fixation, plant dry weight, and plant N and P uptake at par or higher • Establishment of cyanobacteria in soil	[95]
D. Biofilms in field studies			
<i>Anabaena laxa</i> (c) + <i>Mesorhizobium ciceri</i> (b)	Chickpea	Compared to non-inoculated control: • Increased leghemoglobin content in nodules, fresh root weight, plant defense and antioxidant enzyme activities. • Changes in nodule microbiome (archaeal, bacterial and cyanobacterial populations).	[148]
<i>Anabaena torulosa</i> (c) + <i>Trichoderma viride</i> (f) <i>A. torulosa</i> (c) + <i>Azotobacter chroococcum</i> (b)	Corn	“”	[13]
<i>Anabaena torulosa</i> (c) + <i>Trichoderma viride</i> (f) <i>A. torulosa</i> (c) + <i>Azotobacter</i> sp. (b)	Corn	Inoculum applied with 50% N + PK and compared to full dose of NPK: • Savings of 50% N fertilizer (60 kg N ha ⁻¹) for grain yield. • Enhanced plant-defense enzyme activity and increased Zn accumulation in flag leaf.	[116]
<i>Anabaena torulosa</i> (c) + <i>Trichoderma viride</i> (f)	Cotton	Crop challenged with fungus <i>Rhizoctonia</i> sp. and compared to chemical fungicide and commercial <i>Trichoderma</i> formulation: • Mortality reduction at par to chemical fungicide and better performance than commercial <i>Trichoderma</i> . • Enhanced plant defense enzyme activities. • Higher soil MBC.	[11]
	Rice		[134]

(continued on next page)

Table 6 (continued)

Selected inoculants	Crop	Selected outcomes	Reference
<i>Anabaena torulosa</i> (c) + <i>Trichoderma viride</i> (f) <i>A. torulosa</i> (c) + <i>Pseudomonas</i> sp. (b)		<p>Inoculum applied with 50% N + PK and compared to full dose of NPK:</p> <ul style="list-style-type: none"> • Savings of 50% N fertilizer (40–60 kg N ha⁻¹ season⁻¹) for grain and straw yield. • Higher polysaccharides, MBC, microbial activity, and available N and P in soil. • Higher P in leaves. • Establishment of cyanobacteria in soil. 	

lichens, and mosses form cryptobiotic crusts capable of N₂-fixation as the dominant source of N for the ecosystem as well as improving moisture retention [286,287]. The role of microalgal biofilms in soil fertility, soil structure, and crop growth and protection is a relevant topic to explore in future research for agricultural and environmental sustainability [13,283].

5. Research needs and further developments

Research demonstrates that microalgae are beneficial for soil fertility, plant growth, biocontrol, and nutrient cycling in agricultural settings. Current technological challenges and knowledge gaps limit their widespread use and incorporation into agricultural practices. Some of them extend beyond the main topics of this review and include but are not limited to:

- i) Identifying potential microalgal strains and combinations of strains (microalgae-based consortia and biofilms) with synergistic effects on plant growth and soil health, and laboratory and field testing under different types of soil, crops and agroclimatic regions to evaluate agronomic efficiency and microalgal establishment in soils with different native flora [10,12,13].
- ii) Advancing technological improvements for economically feasible, large-scale production of quality inoculum, preservation and transportation [23,302]. Microalgae are versatile and grow in a broad range of nutrient sources and conditions (temperature, light, pH, salinity). To minimize inoculum production costs, identifying the most suitable culture systems (outdoor vs. indoor, open ponds vs. closed reactors) and locally available nutrient sources and options for nutrient recycling (formulated media, nutrient-rich wastewater effluents, CO₂ supply) is crucial [10,22,70]. For instance, the use of CO₂ from flue gases and wastewater streams would decrease the costs and environmental impacts of microalgal cultivation [23,288].
- iii) Evaluating different methods and timing of application/dispersal. Options to consider include soil application with either fresh or dry biomass before sowing, with or without carriers; soaking of seeds or plant cuttings with microalgal cultures or extracts; or spraying on leaves after plant emergence [22]. Options for dispersal of liquid algal suspensions on large areas include off-road tank-trucks equipped with a mechanized air-assisted sprayer; aircraft-based dispersal that would not disrupt the soil surface [70]; or center pivot sprinklers that would be compatible with ongoing farming activities [289].
- iv) Identifying low-cost effective carriers for deployment or for microalgae propagation and biofertilizer storage. Examples of low-cost carriers include wheat straw, Multani mitti (Fuller's

earth), coir pith and vermicompost, all with light weight for transportation and more than 12 months of shelf life [10]. Manure and municipal waste as biofertilizer carrier options should also be explored [22].

- v) Optimizing and monitoring the establishment of cyanobacterial strains to reduce the need for re-inoculation over time [10,60]. The lack of information on the ability of strains to persist in the soil is one of the limitations for widespread use [10]. Establishment between crop cycles might be facilitated with no-till practices, while re-inoculations every cropping season would be needed in conventionally tilled soils [160].
- vi) Elucidating the role in micronutrient enrichment of soil and plants. Intensification in crop production has depleted the soil from essential micronutrients (Zn, Fe, Mn, Cu), leading to deficient contents in the plants. Microalgal biofertilizers are a promising option to offset these deficiencies in leaves and grains of food crops and contribute to tackling malnourishment [22,30,141].
- vii) Understanding the effects of microalgal inoculations on microbial communities in soil, rhizosphere and plant tissues and how these shifts relate to plant growth parameters, crop yields and ecosystem functioning [94,148,290].
- viii) Research and development of microalgal biostimulants [76,291]. Identification of bioactive compounds that promote plant growth as evidenced in foliar applications [167,168], or that confer protection against phytopathogens and pests [9,67].
- ix) Performing detailed techno-economic analysis (TEA) and estimating GHG emissions and environmental impacts. Microalgal biofertilizers have potential to improve carbon capture and sequestration (CCS) and decrease GHG emissions from agriculture [22,23]. With the 2015 Paris Agreement implementation of carbon credits, it is important to update detailed TEA of microalgal soil amendments accounting for a host of potential CCS and GHG benefits. Cyanobacteria decreased methane production in rice soil [292], and soil inoculation with microalgal biomass have shown lower ammonia volatilization as compared to urea [285] and to commonly used organic fertilizers such as feather meal and blood meal [74]. However, adding microalgae to soil has been shown to increase heterotrophic growth with associated CO₂ production through respiration and N₂O emissions in selected experiments [285,293]. These results highlight the need of a more comprehensive scientific understanding of the role of microalgal amendments in reducing GHG emissions as compared to chemical fertilizers for different crops, soil types, and climates taking into consideration specific biomass production systems, transportation, dispersal and emissions after inoculations and during crop growth.
- x) Evaluating the effect of pesticides on natural N₂-fixing biofilms and microalgal biofertilizer applications. The use of herbicides and insecticides has increased worldwide and many of them can cause decreased growth and N₂-fixation in microalgal strains, and some pesticides interfere directly with photosynthesis [89,294]. Nevertheless, at lower doses, the use of some insecticides might stimulate cyanobacterial growth likely due to the control of algal grazers [295].
- xi) Expanding use for soil reclamation and bioremediation. Salt-affected soils (saline, saline-sodic and sodic) are an extensive and increasing problem in irrigated land. Cyanobacteria improve chemical properties of these soils (pH, electrical conductivity, exchangeable Na⁺), as well as aggregation and hydraulic properties [26,48], but conflicting results on their effectiveness as compared to chemical options (such as gypsum) and observed detrimental effects on plant growth under salt stress (possibly due to nutrient sorption by EPS), highlight the need for further research on this topic [296,297]. The use of cyanobacteria for bioremediating salt-affected soils was recently reviewed by Li

et al. [298]. Cyanobacteria are also known to remove organic pollutants and heavy metals and decrease bioaccumulation in plants, a field that has not been fully explored [164,299,300].

- xii) Advancing applications for drylands and for restoration of degraded soils to retain moisture, counteract erosion, and prevent desertification, all growing problems worldwide due to human impacts and climate change. More research is needed to evaluate the use of microalgae to reduce soil loss by erosive forces (i.e., wind or rainfall) [266]. Also, EPS-producing cyanobacteria used in arid soils as a matrix for the development of induced BSCs with subsequent colonization by eukaryotic microalgae, lichens and mosses, have improved soil stabilization and nutritional quality with promising field results that merit further research efforts to reverse desertification [21,70,256].

6. Conclusions

Microalgae combine a broad pool of traits of increasing relevance in a challenging agricultural scenario. A strong body of research has focused on N₂-fixing cyanobacteria-based formulations demonstrating their ability to save N from chemical fertilizers in food crops of global importance such as rice, wheat and corn, and underscore the need for technological advances for large-scale cultivation and inoculum production for widespread applications. In the soil, microalgae enhance soil structure and benefit soil fertility by providing nutrients and improving soil nutrient cycling and microbiological parameters of soil quality. Microalgae also promote plant growth by protecting against plant pathogens, producing phytohormones and other bioactive compounds, or directly associating with plant root systems. These applications open options for future developments such as identifying new growth-promoting substances (phytohormones, vitamins, aminoacids) that can be used as biostimulants, or potentially developing N-independent cereals. Research also points to the use of microalgae for reclaiming nutrients from wastewaters, by generating microalgal biomass with nutrient-rich effluents, mainly N and P, and recycling these nutrients back to the soil for crop production. This approach has also explored the incorporation of CO₂ from flue gases and presents the need for further research in the role of microalgae in mitigating agricultural GHG emissions. Additionally, microalgal biofilms and consortia show agronomic efficiency and the potential to combine microalgae with other plant-growth promoting microorganisms with synergistic effects to boost productivity.

The diversity of microalgae is still greatly unexplored and offers numerous possibilities to expand their applications as renewable resources in agriculture and as sustainable solutions for crop production, plant macro- and micronutrient enrichment, and soil protection and restoration. Growing evidence supports that microalgae are suitable platforms for the development of multiple bioproducts for agriculture such as biofertilizers, organic fertilizers, biostimulants, biocontrol agents and soil conditioners. Nevertheless, many questions remain open, such as efficiencies in different types of soils, crops and agroecological regions, best options for microalgal biomass production and management in agricultural practices, effects on plants and soil microorganisms, interactions with pesticides, use as biocontrol agents, potential for soil restoration, and role in reducing environmental impacts and mitigating climate change. The extended use of the microalgal resource would contribute to improve the ecosystem services of agriculture such as soil fertility, nutrient cycling and erosion control, which are critical needs for the agricultural sustainability of future decades. Furthermore, diversifying the range of applications of microalgae and expanding their utilization in the agricultural sector could help offset costs and strengthen the nascent algal industry in other sectors (i.e., food supplements, cosmetics, bioplastics, biofuels), and would bring environmental benefits to a larger scale.

CRedit authorship contribution statement

ALA: conceptualization, investigation, writing - original draft preparation. SLW: conceptualization, writing - review and editing. HMG: investigation, writing - original draft preparation (Section 2.1.3). BMP: writing - review and editing. RDG (deceased): conceptualization, supervision, funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The work of ALA was supported by MnDrive Global Food Ventures Program from the College of Food, Agricultural and Natural Resource Sciences (CFANS) and the Department of Bioproducts and Biosystems Engineering of the University of Minnesota. Some material in this review is based upon HMG and BMP work partially supported by the National Science Foundation under Grant No. 1632810. RDG was supported by the Department of Bioproducts and Biosystems Engineering, CFANS, University of Minnesota. SLW is a federal employee supported by the USDA Agricultural Research Service. USDA is an equal opportunity provider and employer. The authors are grateful to three anonymous reviewers for their constructive comments, which greatly improved this manuscript.

References

- [1] A. Muller, C. Schader, N. El-Hage Scialabba, J. Brüggemann, A. Isensee, K.-H. Erb, P. Smith, P. Klocke, F. Leiber, M. Stolze, U. Niggli, Strategies for feeding the world more sustainably with organic agriculture, *Nat. Commun.* 8 (2017) 1290, <https://doi.org/10.1038/s41467-017-01410-w>.
- [2] P.L. Pingali, Green revolution: impacts, limits, and the path ahead, *Proc. Natl. Acad. Sci.* 109 (2012) 12302–12308, <https://doi.org/10.1073/pnas.0912953109>.
- [3] N. Alexandratos, J. Bruinsma, *World agriculture towards 2030/2050. The 2012 revision, Agricultural Development Economics Division*, FAO, Rome, 2012.
- [4] C. Rosenzweig, J. Elliott, D. Deryng, A.C. Ruane, C. Müller, A. Arneth, K.J. Boote, C. Folberth, M. Glotter, N. Khabarov, K. Neumann, F. Piontek, T.A.M. Pugh, E. Schmid, E. Stehfest, H. Yang, J.W. Jones, Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison, *Proc. Natl. Acad. Sci. U. S. A.* 111 (2014) 3268–3273, <https://doi.org/10.1073/pnas.1222463110>.
- [5] A. Steensland, 2019 Global Agricultural Productivity Report: Productivity Growth for Sustainable Diets, and More, Virginia Tech College of Agriculture and Life Sciences, 2019.
- [6] R.A. Andersen, The microalgal cell, in: A. Richmond, Q. Hu (Eds.), *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, Wiley Blackwell, Hoboken, NJ, 2013, pp. 3–20.
- [7] L. Barsanti, P. Gualtieri, *Algae: Anatomy, Biochemistry, and Biotechnology*, 2nd, CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 2014, pp. 1–48.
- [8] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, *Renew. Sust. Energ. Rev.* 14 (2010) 217–232, <https://doi.org/10.1016/j.rser.2009.07.020>.
- [9] P. Holajjer, A. Kamra, H.S. Gaur, M. Manjunath, Potential of cyanobacteria for biorational management of plant parasitic nematodes: a review, *Crop Prot.* 53 (2013) 147–151, <https://doi.org/10.1016/j.cropro.2013.07.005>.
- [10] R. Prasanna, A. Sood, S.K. Rath, P.K. Singh, Cyanobacteria as “green” option for sustainable agriculture, in: N.K. Sharma, A.K. Rai, L.J. Stal (Eds.), *Cyanobacteria: An Economic Perspective*, John Wiley & Sons, Ltd., 2014, pp. 145–166.
- [11] R. Prasanna, S. Babu, N. Bidyarani, A. Kumar, S. Triveni, D. Monga, A. K. Mukherjee, S. Kranthi, N. Gokte-Narkhedkar, A. Adak, K. Yadav, L. Nain, A. K. Saxena, Prospecting cyanobacteria-fortified composts as plant growth promoting and biocontrol agents in cotton, *Exp. Agric.* 51 (2015) 42–65, <https://doi.org/10.1017/S0014479714000143>.
- [12] N. Karthikeyan, R. Prasanna, L. Nain, B.D. Kaushik, Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat, *Eur. J. Soil Biol.* 43 (2007) 23–30, <https://doi.org/10.1016/j.ejsobi.2006.11.001>.
- [13] R. Prasanna, F. Hossain, S. Babu, N. Bidyarani, A. Adak, S. Verma, Y.S. Shivay, L. Nain, Prospecting cyanobacterial formulations as plant-growth-promoting agents for maize hybrids. *South African J. Plant Soil* 32 (2015) 199–207, <https://doi.org/10.1080/02571862.2015.1025444>.
- [14] P.A. Roger, S.A. Kulasooriya, *Blue-Green Algae and Rice. The International Rice Research Institute, Manila, Philippines*, 1980.

