MyLand

Live Native Microalgae Enhances Soil Health

Soil Regeneration is the Key to Restoring Ecosystems

Authored by: Kristine Nichols, PhD

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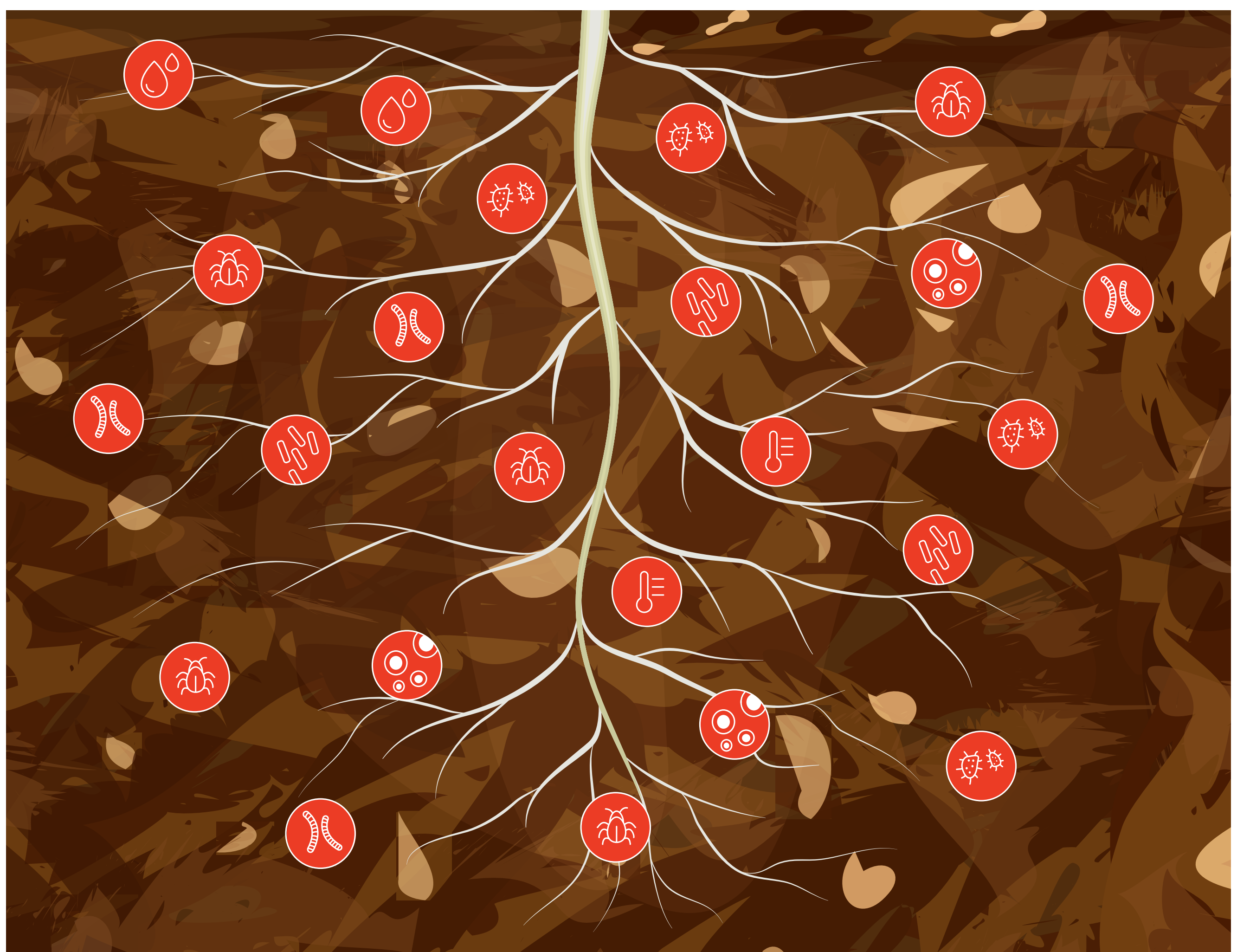
Executive Summary

Soil health is defined as "the continued capacity to function as a vital living ecosystem that sustains plants, animals, and humans" (Kibblewhite et al., 2008) while a decline in soil health is related to soil carbon loss. In the U.S., an estimated 4 ± 1 gigatons (Gt) of carbon has been lost primarily as the result of tillage during the conversion of land into cropland. Regenerative agriculture and soil management practices, such as reducing tillage and adding cover crops, decreases erosive soil loss. From 1982 to 2017, cropland soil loss to wind and water erosion in the US has decreased by 35% from 7.13 to 4.89 tons per acre per year with sustainable management (USDA, 2020).

Microalgae were essential to soil formation as land evolved and are, therefore, used as biofertilizers, biopesticides, and plant growth promoters (PGPs) in agriculture. Specifically, microalgae enhance soil health; directly and indirectly increase SOM (Soil Organic Matter); improve soil water management; increase resilience against stressors such as pests, drought, or salts; and enhance soil fertility.



> "Soil health: the continued capacity to function as a vital living ecosystem that sustains plants, animals, and humans"
>
> (Kibblewhite et al., 2008)

Therefore, they have the capacity to become an essential regenerative technique to improve soil management practices. Soil structure improves with microalgae due to the production of mucilaginous sheaths, exopolysaccharides, hydrophobic biomolecules, and other substances to form and stabilize soil aggregates. Aggregation increases porosity to enhance aeration, water infiltration, and water retention. Soil aggregates and microalgal exudates and other phytochemicals along with the bodies of microalgae or necromass contain carbon leading to higher SOM levels. Soil fertility is enhanced by microalgae because the algal biomolecules and necromass may contain nitrogen, phosphorus, sulfur, and other micronutrients which are made available by decomposition. Finally, some microalgae can fix nitrogen or solubilize phosphate to increase their availability to plants.

Microalgae enhance plant resilience against soil salinity or sodicity, pests, weather and other stressors by producing and releasing a wide variety of biomolecules (i.e., carbohydrates, proteins, enzymes, fatty acids and oils, organic acids, vitamins, hormones, nucleic acids, etc.). Microalgae boost foliar and root growth as well as crop productivity by working alone or in conjunction with other microorganisms such as nitrogen-fixing bacteria, mycorrhizal fungi, or phosphate-solubilizing bacteria. Thus, plant growth promotion results from the biochemistries of one or more microalgal biomolecules. These biomolecules may immobilize salts, buffer pH levels, and reduce salt absorption by plants. Furthermore, bacterial, fungal, viral, protozoan, and nematode pests are controlled by some of these biomolecules. Finally, mucilaginous sheaths from algae prevent pest access to plant cells by forming physical protection around foliar or root tissue. Thus, plants are more resilient against drought, UV radiation and temperature extremes as a result of algal physical protection, algal soil aggregation properties and algal exudates.

Although algae have been used in agriculture for centuries, research is needed to greatly expand microalgal use to improve agricultural production and soil health. This research will demonstrate how to actualize the benefits ascribed to microalgae including how to obtain high density solutions of microalgal cells and/or biomolecules and efficiently apply these solutions to agricultural fields. The system designed by MyLand Company LLC is the only innovative system for culturing and applying high density cultures of microalgal cells to rapidly resupply degraded soils with live native algae and accelerate the recarbonization of soil for optimum soil health.

Introduction

Over the last 50 years, the total area of cultivated land worldwide has increased over 500%

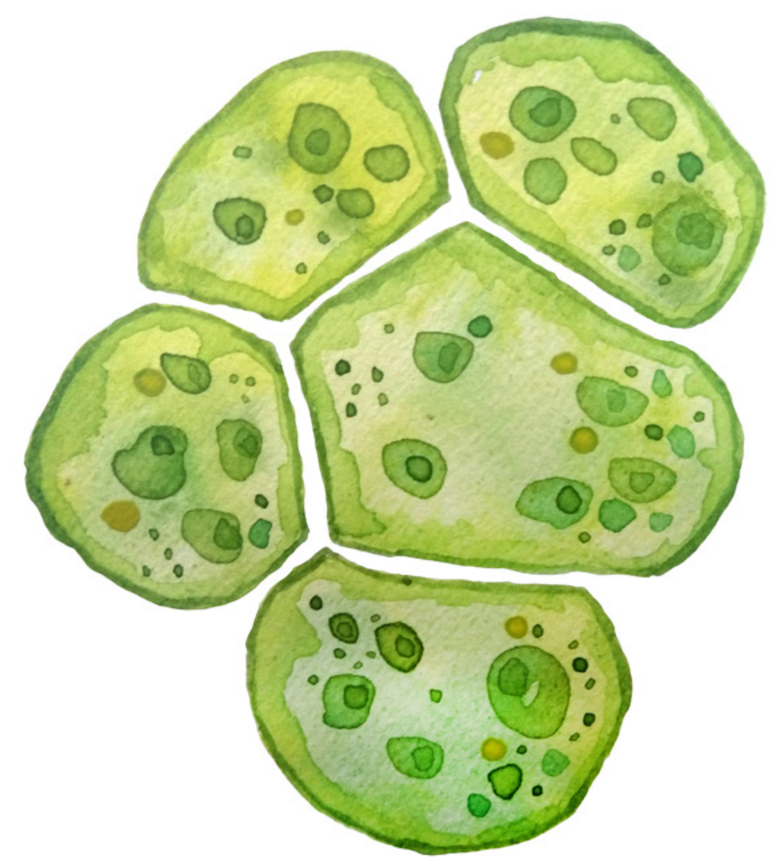
resulting a 700% increase in fertilizer use and a several-fold increase in pesticide use (Ray et al., 2020) and a loss of soil health (Kibblewhite et al., 2008; Lal et al., 2004). Soils, particularly those in arid and semi-arid regions, frequently are highly compacted; low in organic matter and fertility; saline or sodic; poorly aerated; retain less water; and easily eroded. Building soil organic matter and stimulating soil biological activity with microalgae can regenerate soils and start the regenerative agriculture process.

What are Microalgae?

Algae have been present on Earth for 2.4—3.6 billion years and are ubiquitous in both aquatic and terrestrial environments from the tropics to the poles tolerating a wide range of pH levels, temperatures, salt concentrations, and light intensities. Microalgae range in size from 1-2 micrometers to 1-2 millimeters, and ecologically are mostly autotrophs, although some are heterotrophs or mixotrophs. Furthermore, many microalgae practice mixotrophy by carrying out both photosynthesis and organic nutrient absorption. The number of algal species are estimated to be as high as a million species (Guiry, 2012) and they occur not only in water and soils but also on plants, animals, snow and ice as well as on rocks (Round, 1981). Chlorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), Euglenophyta, Pyrrophyta, Chrysophyta, Bacillarophyta (diatoms) and Cyanobacteria (blue-green algae) are microalgae phyla classified by pigmentation, life cycle and structure (Win et al., 2018., e.g. Graham et al., 2016, Lee, 2018). Topsoil population densities range from 3 and 100 million cells per gram (Chiaiese et al., 2018).

Typically, solar radiation, water, and temperature are the most important abiotic factors governing microalgal distribution, metabolism, and life strategies. Wastewaters can be used for culturing microalgae because they are an inexpensive source of necessary nutrients (Mahapatra et al., 2018). In these systems, toxic and xenobiotic compounds are broken down and nutrients are mineralized. Both living and dead algal cells are then applied to crops, seeds, foliar tissues or soil with or without other products.

Following application, the living microalgal cells will rapidly colonize the rhizosphere to enhance plant growth and development. From a fertility perspective, microalgae primarily have been exploited for the nitrogen fixing capabilities which has focused most of the research on cyanobacteria. However, contributions to phosphorus solubilization and chelation of micronutrients such as iron and calcium have gained prominence (Meeting, 1981). Some microalgal species are capable of tolerating pesticides, particularly insecticides (Win et al., 2018) while others reduce heavy metal toxicity (Hussain and Hasnain, 2011).



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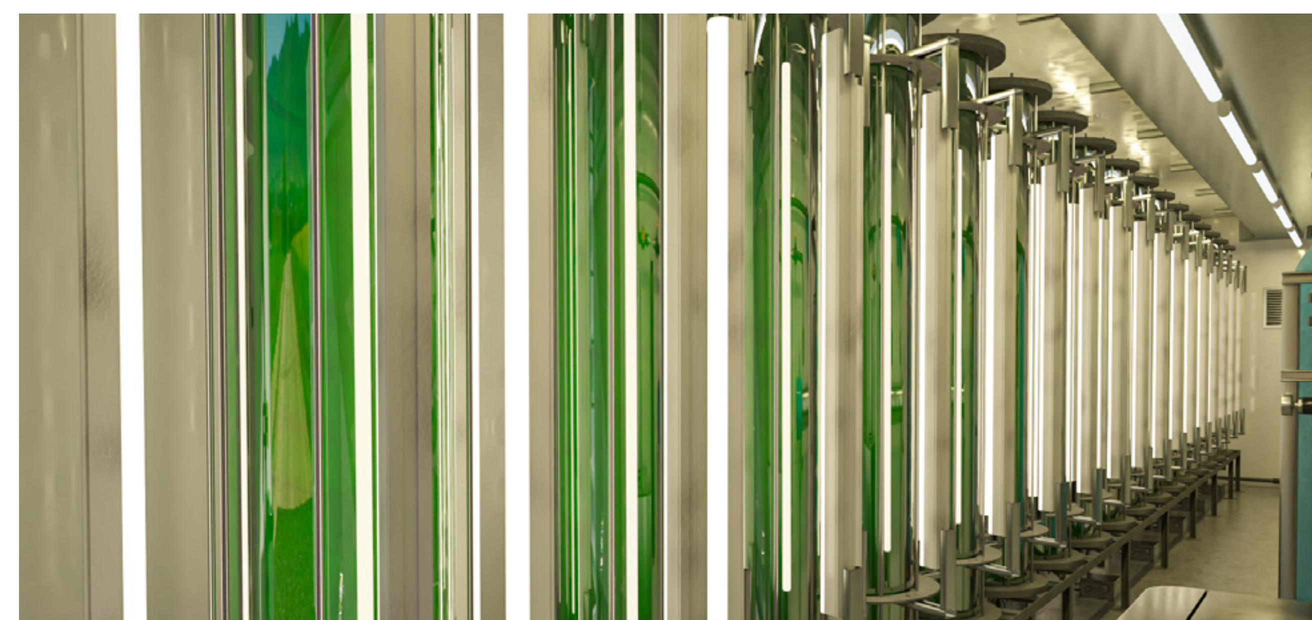
Microalgae possess chlorophyll a and b and photosynthetically produce carbohydrate, protein and oils comparable to those of plants, but importantly soil algae, when excluded from light, can grow heterotrophically on a variety of carbon sources such as glucose, glycerol, ethanol, and volatile organic acids (Chalima et al., 2017).

Exopolysaccharides (EPSs) produced by microalgae are integral to soil health by binding small soil particles together into soil aggregates. Increasing soil aggregation is elemental to expanding porosity, which in turn impacts water efficiency – water permeability or infiltration and water retention. Mucilaginous sheaths and EPSs also allow blue-green and green microalgae to survive desiccation in storage up to 70 or 59 years, respectively (Bristol, 1919; Trainor, 1970). The intracellular and extracellular biomolecules produced by microalgae act as antibacterials, antifungals, antiprotozoals, antivirals, (Alvarez et al., 2021) and nematicides (Singh et al., 2016). These microorganisms have a wide range of physiological and industrial wastewater, and production of biomolecules act as food sources for other microbes and become macro- and micronutrients or function to promote plant growth.

Due to their versatile nature – simple unicellular structure, high photosynthetic efficiency, ability for heterotrophic growth, adaptability to domestic and industrial wastewater, and production of biomolecules – microalgae are being implemented in agriculture (Chiaiese et al., 2018). In agro-ecosystems, microalgae have been widely used to improve rice crops, but they have also been used for grain crops (e.g. wheat, corn, sunflowers, peas, and chickpeas), cotton, sugar beets and sugarcane, produce crops (e.g. lettuce, cabbage, peppers, tomatoes, and radishes), ornamental flowers and trees and so forth. Studies have shown increases in plant root/shoot length, dry weight, yield, germination rate, cellular respiration, floral production, photosynthesis, chlorophyll production, and/or resilience to pests, diseases, and climate stressors (Mahapatra et al., 2018; Ronga et al., 2019). Rice yields have had a relative 14% increase in yield primarily due to cyanobacteria performing free-living and symbiotic atmospheric nitrogen gas fixation (Alvarez et al., 2021). The fixed nitrogen (ammonia, nitrate, nitrite) is necessary to produce polypeptides, free amino acids, vitamins, and auxin-like substances. These nitrogenous substances increase soil fertility either by secretion from living algal cells or through microbial degradation after algal cell death. Studies have shown that this nitrogen fixation may account for up to 100 kg N ha–1 yr–1 (Warren, 2014).

Beyond agricultural applications, microalgae produce a variety of fatty acids/oils/fats, unusual sugars, and other bioactive compounds that are used in food, aquaculture, poultry, pharmaceutical, and cosmetic industries (Alvarez et al., 2021; Priyadarshani and Rath, 2012). The research for these areas adds scientific understanding that can be applied to better improve soil health and pest management as well as reducing stressors such as UV radiation, desiccation, and temperature extremes.

In arid ecosystems, microalgae biofilms along with mosses, lichens, and fungi are components of biological crusts which typically are higher in organic matter and nutrients (Alvarez et al., 2021; Liu et al., 2013). Biocrust organisms are adapted to limited moisture, low nutrient conditions and high salt conditions. These crusts can constitute up to 70% of dryland ground cover and are a substantial portion of primary production (i.e. 6.4 to 370 kg C ha–1 yr–1) (Warren, 2014). Overall, soils under biocrusts had 11.3-fold higher organic matter, 10.4-fold higher total N, 3.25-fold higher C:N ratio, 1.46-fold higher total P, 1.57-fold higher soil moisture, and 1.13-fold lower daytime temperatures than uncrusted soil (Liu et al., 2013). Although biocrust growth is limited in agroecosystems, the addition of high-density cultures of microalgae under more direct sunlight in Arizona does support potential biocrust impacts on increased soil organic matter and reduced water evaporation with the MyLand system.



Inside the MyLand System

Why Apply Microalgae?

Microalgae such as Nostoc were used for several centuries as biofertilizers in rice fields, but only since the early 1950s, following the development of mass culture, has research been conducted on the impact of algae on crop productivity and yield (Win et al., 2018). However, this research is just beginning. For example over a recent 10-year period (ca. 2007-2017), algae as biofertilizers accounted for only 0.04% of papers in agricultural journals and only 0.09% of papers in algal journals (Mahapatra et al., 2018).

Microalgae are of interest to agribusinesses and farmers because of their biofertilization, plant growth promotion (PGP) and/or biopesticide properties, all of which increase agricultural sustainability and enhance resilience. The following roles have been described even though the specific mechanisms have not been fully identified (Abdel-Raouf, 2012b; Alvarez et al., 2021):

- Solubilizing and/or mobilizing macro- and micro-nutrients
- Acting as a source of organic matter and nutrients
- Complexing heavy metals and xenobiotics to limit their mobility and transport into plants
- Mineralizing simpler organic molecules such as amino acids for direct uptake by plants
- Protecting plants from pathogens and diseases
- Buffering pH
- Enhancing resilience against stressors such as drought or salts
- Stimulating plant growth
- Improving the physico-chemical conditions of soils, i.e. forming soil aggregates
- Providing oxygen to subsurface areas

ORGANIC MATTER Microalgae are important sources of organic matter. Some of these biomolecules as well as microalgal cells contribute significantly to sequestered soil organic matter because their cell walls contain recalcitrant algaenans (Derenne and Largeau, 2001). These biomolecules protect algal cell walls, particularly at the soil surface, and are highly aliphatic and not easily decomposable. Microalgal PGPs also are organic matter constituents, help to improve biochemical reactions and act as a carbon source (Ronga et al., 2019). Nutrient status of soil specifically nitrogen and phosphorous determines the mineralization of available carbon and thus affects the microbial community (Chatterjee et al 2017).

CLIMATE RESILIENCE AND MITIGATION Overall, agriculture contributes to 10% of greenhouse gas (GHG) emissions which have magnified to have a global impact on climate (EPA, 2021 https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks). Climate change is an important topic throughout the world, and algae may play an important role. Below are some areas where algae, agriculture and climate change intersect:

- Absorbing carbon dioxide through photosynthesis
- Increasing soil carbon/organic matter storage (Alvarez et al., 2021; Tomar et al., 2020)
- Fixing atmospheric nitrogen to reduce nitrate fertilizer use and nitrous oxide emissions, particularly in rice production (Tripathi et al., 2008)
- Improving water infiltration rates and water-holding capacity to reduce water use and the carbon footprint of irrigation systems (Lewis et al., 2019; Priyadarshani and Rath, 2012)
- Enhancing soil fertility and improving pest management to reduce fossil fuel-based chemicals (Uysal et al., 2015).

Other microalgal activities have been found to stimulate the availability of P, Fe, Cu, Mo, Zn, Co, and Mn. Microalgae have been found to increase the bioavailability of phosphorus to the plants at levels higher than from synthetic fertilizers. Phosphorus solubility is increased by microalgae through the formation of biomolecules which chelate calcium and release phosphate from calcium phosphates, they exude organic acids to reduce soil pH and solubilize phosphate, and/or synthesis of phosphatase enzymes (Alvarez et al., 2021). Chelation also enhances the availability of micronutrients such as Fe, Cu, Mo, Zn,

Soil Health

In soils, microalgae make physio-chemical contributions to soil health by assisting in the formation and stabilization of soil aggregates that increase pore space and continuity. This in turn improves water infiltration and water holding capacity, aeration, nutrient cycling and seed germination (Abdel-Raouf, 2012b; Alvarez et al., 2021; Ronga et al., 2019). Microalgae physically entwine soil particles with multi-cellular filaments or by biochemically attaching organic matter, sand, silt or clay to their mucilaginous cellular sheaths or exopolysaccharides (Harper and Belnap, 2001). Also, algae release chelators that form organo-mineral complexes, and these complexes enhance aggregation by binding calcium, iron, zinc and aluminosilicate clay minerals. The accumulation of soil particles in aggregates create nutrient-rich microsites where the algal sheaths are coated with negatively-charged clay particles that then bind positively-charged nutrients (e.g., iron, copper, molybdenum, zinc, cobalt, and manganese) reducing leaching of these nutrients (Harper and Belnap, 2001). In addition to sheaths and biomolecules, Chlamydomonas sp. can effectively colonize abiotic surfaces such as fine sand particles via flagella-mediated adhesion and electrostatic interactions to further soil aggregation (Kreis et al., 2019). Soil aggregation, following microalgal application, of a sandy loam, loam, and a silty clay loam increased by 85%, 130% and 160%, respectively (Kaushik, 2014). Organic compounds exuded by microalgae (or decomposing microalgal cells) are constituents of soil organic matter that are essential for soil health (i.e. more sustainable and/or higher crop production) (Abdel-Raouf, 2012a).

Co, and Mn along with binding negatively-charged nutrients such as phosphates, nitrates, and sulfates in an exchangeable form to the chelated cations on microalgal sheaths (Chatterjee et al., 2017).

In unfavorable soil conditions, such as high or low pH, high salinity or high calcium carbonate (CaCO3) levels, both macro- and micronutrients are less likely to be available due to competitive binding and immobilization. Additional nutrient loss may occur with erosive forces, drought and high temperatures. For example, in desert ecosystems, nitrogen concentrations are known to be low because up to 77% of the nitrogen may be lost through wind erosion, ammonia volatilization, nitrification, and denitrification (Harper and Belnap, 2001). Microalgae ameliorate these unfavorable conditions through the production of organic acids, enzymes, and/or competitive chelators. Organic acids (e.g. acetic acid, citric acid, humic acid, malic acid) are natural buffers and they prevent soils from becoming too acidic or too basic by buffering the pH. Algal enzymes degrade substances and release nutrients while other biomolecules assist in the release of inorganic nutrients (e.g. Fe, Cu, Mo). Also, as described above, microalgae improve soil aggregation that, in turn, reduces erosion and nutrient loss. Microalgae, as described below, improve water management, help lower volatilization and help immobilize calcium and sodium.

SALINITY AND SODICITY Globally, nearly one billion hectares of soil are affected by salinization (Chatterjee et al., 2017). Salinity can negatively impact soil physical and chemical properties resulting in increased soil compaction and erosion. Excess salinity affects approximately 20% of irrigated arable land and is responsible for plant development damage, especially at the seedling stage. The deleterious effects of salinity on plant growth are associated with plant metabolism, nutrient deficiencies, osmotic stress, specific ion toxicities, or a combination of these factors (Chatterjee et al., 2017). Once inside a plant cell, excess salts can cause ionic stresses, largely by Na (and Cl) inhibiting major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism.

WATER MANAGEMENT Many microalgae, especially green algae, have a specific cell division process that helps form soil aggregates. Specifically, new cells are formed inside the old parent cell wall, and once formed, the new cells release enzymes that converts the parent cell wall into a sticky, mucilaginous sheath that eventually tears and releases the new cells. Soil particles adhere to remaining parts of the parental mucilaginous sheath to form soil aggregates. Soil aggregates, in turn, are less susceptible to wind and water erosion than their constituent parts. The aggregates also increase soil porosity that increase

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Salinity and sodicity: mechanisms used by microalgae

The following mechanisms are used by microalgae to tolerate salinity and sodicity (Alvarez et al., 2021; Chatterjee et al., 2017; Tomar et al., 2020):

- Producing of extracellular polysaccharides to buffer salts
- Releasing organic acids through microbial decomposition of organic matter to react with calcium carbonate, release calcium and create the acidic version of aqueous carbon dioxide or carbonic acid
- Utilizing chelators to bind and immobilize calcium and sodium
- Synthesizing and accumulating osmoregulatory compounds such as sugars and quaternary amines to impart high osmotic tension to plant roots for absorption of water and nutrients
- Maintaining low internal sodium by either restricting uptake or efflux through algal biomolecules or in consortia activities of microalgae with other rhizosphere microorganisms
- Substituting calcium or potassium ions for sodium ions in clay complexes
- Improving permeability, aeration and water movement through soil aggregation
- Expressing of a set of salt-stress responsive proteins

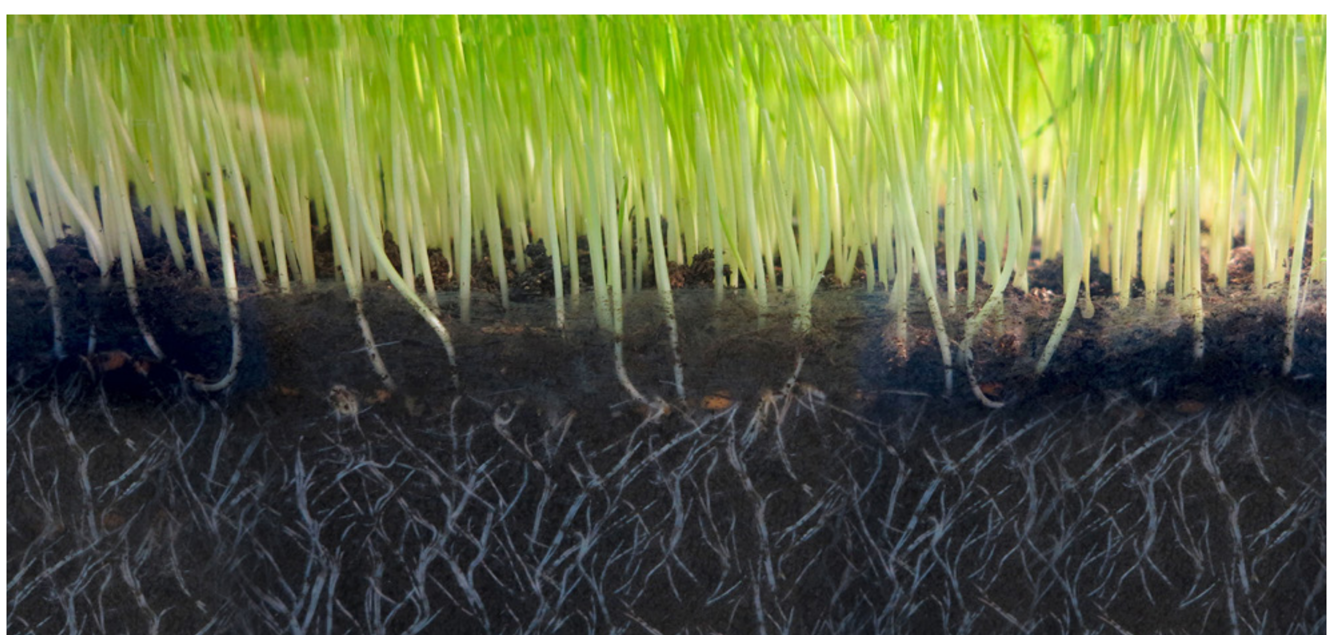
water infiltration rates and water-holding capacity. Finally, soil aggregates increase soil aeration.

Studies of soil biocrusts have found that moistened cyanobacterial biofilms made from the cell wall sheaths absorb ten times their volume in water (Colica et al., 2014; Fan et al., 2008; Johansen et al., 1984; Warren, 2014). In most cases, the sheaths and other biomolecules including exopolysaccharide increase water holding capacity due to their hygroscopic (i.e. ability to attract water molecules) nature. In a few cases, hydrophobicity (i.e. water-hating) in some of these molecules or high clay content can contribute to surface sealing (Singh et al., 2016). Too much or too little soil disturbance of biocrusts via grazing, traffic or cultivation may restrict pathways for water flow and reduce water availability by altering the concentrations of cyanobacteria, other microalgae, lichens, and mosses (Colica et al., 2014).

Humidity and water levels impact microalgal biological processes. For example, photosynthetic activity can be higher at low water levels while nitrogen fixation may be reduced (Aranibir et al., 2003). These differences make the impacts of microalgae on plants very complex. For example, in non-water-stressed tomato plants, microalgae stimulated better root length and an increased leaf number and leaf area while in water-stressed tomato plants, alleviation of water stress increased plant height (Ronga et al., 2019).

PEST MANAGEMENT Microalgae form compounds that have antibacterial, antifungal, antiprotozoal, nematicides, and antiviral properties. These compounds include exopolysaccharides, hydrophobic biomolecules, and hormones (Alvarez et al., 2021). The types of antimicrobial compounds vary greatly, and their production depends on the algal culture medium composition, incubation period, pH, temperature, and light intensity. More hydrophobic biomolecules such as polyketides, amides, alkaloids, fatty acids, indoles, and lipopeptides have effective antibacterial activity, and the production of hydrophobic substances varies with microalgal species (Ronga et al., 2019). Similar compounds also have antifungal activities particularly against soil-borne or foliar fungal pathogens (Singh et al., 2016). Crop plants improve when saprophytes—Chaetomium globosum, Cunninghamella blakesleeana, and Aspergillus oryzae —and plant pathogens, such as Rhizoctonia solani and Sclerotinia sclerotiorum, are in decline following microalgal application; some specific antifungal compounds have been isolated from the microalgae (Alvarez et al., 2021). Additional antimicrobial activities occur as a biophysical protection of plant foliar tissue and roots produced by the mucilaginous sheaths and biofilms of microalgae (Alvarez et al., 2021). Specifically, studies have found cell constituents of cyanobacteria, likely through biophysical protection, reduced the incidence of Botrytis cinerea on strawberries, Erysiphe polygoni powdery mildew on turnips and "damping off" disease in tomato seedlings (Singh et al., 2016). In grains, the alga Fischerella muscicola showed antifungal activity against Uromyces appendiculatus (brown rust) and Pyricularia oryzae (rice blast). The alga Nostoc muscorum has displayed antifungal against soil fungi, especially those producing "damping off", and Nostoc also reduced Sclerotinia sclerotiorum, or "white mold," in lettuce and vegetables forming rosette plants (Singh et al., 2016). In another study, nematode egg hatching decreased and immobility and mortality of juvenile plant parasitic nematodes increased following an application with microalgae (Win et al., 2018). Finally, in desert ecosystems, biocrusts containing microalgae reduced exotic annual grass germination that in turn reduced invasive species (Liu et al., 2013; Warren, 2014).

PLANT GROWTH PROMOTION Microalgae produce a variety of biomolecules that act as plant growth promoters (PGPs) and that impact cellular respiration, photosynthesis, leaf chlorophyll, ion uptake, and nucleic acid and antioxidant synthesis in plants (Ronga et al., 2019). These PGPs include auxins, cytokinins, betaines, amino acids, vitamins, polysaccharides and polyamines (Ronga et al., 2019). In some cases, gibberellins, brassinosteroids, protein hydrolysates, and amino acids can be extracted from microalgal cultures and applied via sprays (Win et al., 2018). These PGPs enhance plant growth and development, mitigate injuries caused by abiotic stresses, and influence metabolic activities such as root and shoot development, leaf senescence, breaking bud dormancy, seed germination, photosynthesis, respiration, nucleic acid synthesis, and nutrient uptake. In addition, amino acids such as tryptophan and arginine impact the growth and yield of cultivated crops because these two amino acids are the metabolic precursors of aromatic secondary compounds, polyamines, and plant hormones such as auxin and salicylic acid (Chiaiese et al., 2018). These compounds impact embryogenesis, flower initiation and development, fruit setting, ripening and leaf senescence. Other PGPs include cytokinins and indole-3-acetic acid (IAA) enhance growth, yield, and nitrogen concentrations (Hussain and Hasnain, 2011), while photoprotective compounds/pigments including mycosporine-like amino acids (MAAs), scytonemin and carotenoids protect against UV radiation, oxidative stress, osmotic pressure, thermal stress and are typically used in cosmetics (Alvarez et al., 2021). Finally, plants significantly increase root exudates such as soil polysaccharides, dehydrogenase, urease, and phosphatase activities after microalgal applications.



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INTERACTIONS WITH OTHER ORGANISMS While algae, bacteria and fungi by themselves aid plant growth, the effect is enhanced when they act in concert. For example, when microalgae are combined with bacteria or fungi, then nitrogen-fixing, phosphate-solubilizing and/or other plant growth promoting substances are enhanced (Alvarez et al., 2021). In one study, mycorrhizal fungi and Rhizobium bacteria coupled with microalgae stimulated greater levels of rhizosphere activity, nutrient cycling and soil aggregation (Harper and Belnap, 2001). In a separate study, phosphate-solubilizing bacteria – Bacillus megaterium, Bacillus circulans, Bacillus subtilis, Bacillus mucilaginous and Pseudomonas striata – acted in consortia with microalgae to mobilize phosphate (Mahapatra et al., 2018).

How are Microalgae Produced and Applied?

Microalgae are cultured either in open bioreactors such as open ponds, pits, or tanks or in closed bioreactors (reference). The cultured algae are then either applied directly to the crop foliage or the cropland or applied indirectly as an extract of algal biomolecules (Lewis et al., 2019; Priyadarshani and Rath, 2012; Ronga et al., 2019). In open systems, the growth medium is directly exposed to the air where evaporation helps to regulate temperature but light limitations, aeration, evaporative water loss and contaminating algal grazers are issues. Open systems can reduce costs by using nutrient-rich wastewater as the culturing medium, but the wastewater may contain heavy metals (i.e. lead, nickel, and cadmium) that microalgae can concentrate (Ronga et al., 2019). Raceway ponds with a depth between 10 and 50 cm are the most widely used open pond system; the shallow depth allows for sufficient illumination and the paddle wheel provides mixing and circulation.

A closed photobioreactor optimizes light capture with its tubular or flat-plate design, and the closed system controls evaporation and limits contamination. Typically, photobioreactors have higher productivity and better light capture than open ponds. Table 2 below describes differences between a closed photobioreactor and an open raceway pond (Narala et al., 2016).

As described in the above sections, microalgae convey numerous benefits to agricultural crops and to soils. The microalgae are applied as living or dead cells or as algal extracts. Application is made using sprayers, liquid injectors, or irrigation systems and these applications target plant leaves, plant roots or the soil. With foliage applications, the microalgal extracts are readily absorbed by the leaf through its stomata and cuticle pores, and leaf applications are most effective in the morning when stomatal pores are completely open (Chiaiese et al., 2018; Mahapatra et al., 2018; Ronga et al., 2019). Therefore, plant responses to foliar sprays are normally more rapid than soil amendments; however, it is important to recognize that the mechanisms are quite different between aerial and soil applications. For example, foliage responds well to plant growth substances while roots respond well to phosphorous solubilization, trace metal release, and soil buffering.

TABLE 2 A comparison of factors between a closed photobioreactor and an open raceway pond.

Factor	Photobioreactor	Raceway Pond
Space Required	Moderate	High
Evaporation loss	Low	High
CO2 Sparging efficiency	High	Low
Maintenance	Difficult	Easy
Contamination risk	Low	High
Biomass quality	Reproducible	Variable
Energy input for mixing	High	Low
Operation type	Batch	Batch
Setup cost	High	Low
Maintaining continuous exponential phase	Difficult	Difficult

Table modified from Narala et al. (2016)

Microalgae application is made using sprayers, liquid injectors or irrigation systems.

Some systems involve downstream processing to either convert the liquid cultures into a solid or to create a microalgal extract containing biomolecules. The concentrated solids or extracts may be beneficial when applying the material; however, the costs associated with downstream processing are at least 20-30% of the total production cost (Silva and Silva, 2013). Concentrating techniques include centrifugation, drum drying, and lyophilization (freeze-drying) (Ronga et al., 2019). For efficacy of microalgal extracts, cells are lysed or ruptured, usually during centrifugation, flocculation with clays, crop residue, or fly-ash) or gravity sedimentation followed by liquid nitrogen cryo-freezing, mill grinding, pressure or enzymatic digestion (e.g. protease, cellulase, β-glucosidase, xylanase, β-glucanase, and pectinase) (Ronga et al., 2019; Win et al., 2018). Flocculating agents, used to concentrate microalgal cells, are capable of increasing the shelf-life of microalgal biofertilizers (Win et al., 2018). Although these solid products have a longer shelf life than liquid cultures, the solid products must be suspended in water for application.

Summary and Conclusions

Benefits of Microalgae as a Biological

Microalgae are ubiquitous in nature, from aquatic habitats to arid environments. The occurrence of algae in soils has been known for a long time (e.g. Bristol, 1919, Bristol-Roach, 1927b, Petersen, 1935, Metting 1981, Starks et al., 1981, Ettl and Gärtner, 1995). In more recent years, algae have increasingly become known as organisms that improve agriculture. The biofertilizer, PGP and biopesticide properties of microalgae enhance the accrual of soil organic matter and improve water use efficiencies, rooting, crop yields, crop quality, pest tolerance, as well as drought and salt resistance (Chatterjee et al., 2017; Derenne and Largeau, 2001; Mahapatra et al., 2018; Priyadarshani and Rath, 2012; Ronga et al., 2019).

Although scientific research in this specific field of expertise is still in its early stages, microalgae have been applied as amendments in cropping systems for over 2000 years, particularly in rice production. The economic benefits of microalgal application come not only from production increases but from reduced fertility expenses and soil improvement. Also, microalgae can induce tolerance to biotic and abiotic stressors, including pests and diseases, drought, or salts. Existing studies show boosts in plant shoot and root growth, grain or fruit production, and grain or fruit quality; however, the results have not always been consistent and additional study is needed.

The MyLand Difference

Unlike other companies marketing microalgae bottled products, MyLand Company LLC in Phoenix, AZ is not marketing microalgae or a microalgal product but rather MyLand market unique photobioreactor systems that continuously produce live native microalgae for agricultural crops located on a customer's farm. These Systems are designed to produce high-density microalgal cultures that are applied to crop fields at the grower's desired rate and frequency. For example, the grower might apply their algae via irrigation system (e.g. flood, drip, center pivot or sprinkler). In addition to not selling a product, the MyLand difference utilizes native microalgae cultured from soil and/or water samples collected from each farm; these native algae are applied as live algae to the grower's soil. Our design is supported by scientific study. Win et al. (2018) found native species of Anabaena spp. were successful in promoting soil fertility, and they also reduced compaction and were more resilient to herbicides and drought. Native algae are adapted to the soil texture and climatic conditions and they interact naturally with other soil organisms (Alvarez et al., 2021).

> The economic benefits of microalgal application come not only from production increases but from reduced fertility expenses and soil improvement.

Conclusions

Microalgae have been a component of agricultural systems for centuries and since the 1950's these organisms have been specifically applied to cropping systems. Agricultural research studies based on microalgal amendments are increasing yearly and the data already confirm that microalgae enhance crop production, crop quality and soil health. Higher yields have been recorded following microalgal applications, and the studies show increases in protein, vitamin, and antioxidant concentrations in fruits and grains. Studies also show increased soil health parameters (e.g. organic matter levels, macro- and micronutrient content, and soil aggregation) and decreased heavy metal toxicity, salinity and sodicity. Cyanobacteria have the capacity to fix atmospheric nitrogen which provides additional fertility. Finally, microalgae have high concentrations of proteins, carbohydrates, lipids, vitamins, minerals, and other beneficial biomolecules. Some of these biomolecules may be exuded during algal growth and act as plant hormones or other PGPs. Exopolysaccharides act as aggregators or glues. As academic and private sector research shows, microalgae are not a gimmick because they play a key role in soil health with regenerative properties for depleted soils. All these characteristics make microalgae a strong candidate to be marketed to agribusinesses and MyLand is leading the way by using live native microalgae which promotes healthy soil, healthy food, healthy people, healthy planet. To learn more on how MyLand is changing soil, visit MyLand.ag.

Appendix A

GLOSSARY

Algaenans are resistant biopolymers in the cell walls of green algae.

Aliphatic compounds are comprised of hydrocarbon chains and are not easily dissolved in water.

Autotrophic organisms produce their own food using light, water, carbon, and/or other chemicals. **Photoautotrophs** use light as the energy source for producing food while chemoautotrophs use chemicals such as methane as their energy source.

Biofertilizers are products containing living microorganisms or natural substances able to improve chemical and biological soil properties, stimulate plant growth, and restore soil fertility.

Biostimulants are organic products such as hormones that stimulate the growth and development of crops under both optimal and stressful conditions.

Chelators are molecules that binds metal ions. The bonds created by chelators vary in strength and weak chelation may make the metal ions exchangeable or available for sharing bonds to bind anions weakly to the metal cations.

Exopolysaccharides are high-molecular-weight biopolymers produced during the growth or propagation of microorganisms including microalgae. These compounds are excreted from the cells into the surrounding environment or be loosely attached to the cell wall.

Exudates are molecules released by organisms into the surrounding environment.

Flocculants are substances that clump molecules together, so they settle out of solution by gravity more easily.

Heterotrophic organisms cannot produce their own food; they obtain their energy and nutrients by eating other organisms or by absorbing organic molecules.

Hydrophobic molecules repel water. Examples include fats and oils typically found in membranes and on surfaces.

Hygroscopic molecules attract and hold water molecules from the surrounding environment.

Mucilage is a thick, gluey coating made from carbohydrates, proteins and/or lipids. many algal cells produce mucilage around their cells, and the mucilage may bind molecules and particles, may be hydrophobic, etc.

Photobioreactor is a manufactured device or system that uses light to cultivate photoautotrophic organisms.

Plant-Growth Promoter (PGP) is a natural biomolecule, typically an organic acid, hormone, or vitamins that stimulate plant growth.

Plant Growth Regulator (PGR) is a natural or synthetic plant hormone that alter the growth or physiological processes of the plant.

Recalcitrant compounds resist decomposition or degradation.

Salinity containing high concentrations (i.e. the electrical conductivity or salt content is 4 mmho cm-1 or greater) of salt ions such as sodium, calcium, potassium, magnesium or chloride. In saline soils, clay particles are dispersed, clogging soil pores, reducing hydraulic conductivity and reducing aeration.

Sodicity occurs when sodium ions dominate over other salt ions in saline soil; sodicity can raise soil pH to 10 and greatly impact soil structure.

Soil Aggregate is clod or conglomeration where sand, silt and clay particles as well as organic matter adhere to each other more strongly than the surrounding environment. The adhesion comes from ionic binding mostly between cations and organic matter, microbial including microalgal polysaccharides, and/or hydrophobic molecules.

Soil Health is the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans.

Xenobiotics are foreign bodies in an ecosystem; they also may be unexpected or unnatural compounds.

Appendix B

RESEARCH STUDY RESULTS

1. *Dunaliella* spp. and *Phaeodactylum* spp. extracts mitigated salt stress during bell pepper seed germination by forming organic biomolecules that reduced superoxide radical production and peroxidation (Ronga et al., 2019).
2. *Spirulina* spp. and *Chlorella* spp. were applied to wheat to improve tolerance to salinity through the stimulation of higher antioxidant and protein content (Ronga et al., 2019).
3. *Chlorella vulgaris* increased germination height and growth rate in wheat and corn plants particularly when grown phototrophically (Uysal et al., 2015).
4. *Chlorella pyrenoidosa* cultured in dairy waste-water effluent increased root and shoot length in rice by 30% (Win et al., 2018).
5. *Chlorella* sp. incubated on agricultural soil for 42 days increased photosynthetic activity by 3.5 times but also increase CO2 levels by 1.1 g CO2C m−2 which indicates increases in heterotrophic activity. Also, phospholipid fatty acid (PLFA) analyses showed that the suspension accelerated the development of a stable community of eukaryotic and prokaryotic microorganisms in the soil surface, whereas bacterial PLFA biomarkers were significantly associated with eukaryote biomarkers on the study level.
6. *Anabaena laxa* increased chickpea yield by 50% (Win et al., 2018).
7. Sugar and carotenoid concentrations increased in tomato fruits treated with dry biomass of *Nannochloropsis* spp., *Ulothrix* spp. and *Klebsormidium* spp. (Ronga et al., 2019).
8. Seedling growth was improved with a significant enhancement of soluble carbohydrate, soluble protein and total free amino acids from *Chlorella vulgaris* applied to lettuce seeds. When application was made to soil, there was an increase in fresh and dry weight as well as pigment content (Ronga et al., 2019).
9. When *Acutodesmus dimorphus* was applied to pots 22 days prior to transplanting tomato seedlings, there were more branches and flowers than with a no algae control. Similar studies showed faster germination rates when used as a seed primer and increases in plant height and a greater number of flowers and branches after foliar application (Ronga et al., 2019).
10. In rice, *Nostoc* spp., *Hapalosiphon* spp., and *Aulosira* spp. application improved seed germination, root and shoot growth, weight of rice grains and protein content. Research showed that these benefits came from the presence of root-promoting hormones such as auxins, cytokinins and gibberellic acid (Ronga et al., 2019).
11. *Arthrospira* spp. was applied to red beet (*Beta vulgaris*) leaves every two weeks in an organic cropping system and resulted in higher dry and fresh weights compared to the control. *Arthrospira* spp. was foliarly applied every two weeks to red beet (*Beta vulgaris* L.) in an organic cropping system and resulted in higher dry and fresh weights compared to the control (Ronga et al., 2019).
12. Following application of *Arthrospira* spp. and *Scenedesmus almeriensis* extracts at 10 g L−1 at 0, 14, 28, 35, and 42 days after transplanting to ornamental leaves, there were higher rates of root, leaf and shoot growth, earlier flowering, more flowers per plant, and higher plant water content (Ronga et al., 2019).
13. *Arthrospira* spp. was applied as a foliar spray on tomato and pepper plants at different growth stages; the application improved plant size by 20 and 30%, respectively. The effects on root weight were more marked for tomato plants (+230%) than for pepper plants (+67%). Also, the size and number of nodes per plant improved by 57% and 100% for tomatoes and by 33% and 50% for peppers (Ronga et al., 2019).
14. The MyLand system uses high density cultures of microalgae and concerns exist as to potential toxicity concerns. In the Ronga et al. (2019) study, the microalgal extracts did not have phytotoxic impacts on germination.
15. *Calothrix elenkenii* controls damping off by the soil-borne plant pathogen *Rhizoctonia solani* (Singh et al., 2016).
16. *Fischerella muscicola* reduces brown rust, powdery mildew and rice blast (Singh et al., 2016).
17. *Nostoc muscorum* negatively impacted cottony rot and damping off by the soil-borne plant pathogen *Rhizoctonia solani* in vegetables and flowers (Singh et al., 2016).
18. Under optimal conditions, *Nostoc* could produce 8.66 ug/ml IAA, and sprouting in a taro field was effectively promoted (Win et al., 2018).Under optimal conditions, Nostoc could produce 8.66 lg/ml IAA, and sprouting in a taro field was effectively promoted (Win et al., 2018).
19. *Scytonema hofmanni* produced a gibberellin-like plant growth regulator that enabled hormone homeostasis of rice seedlings under salt stress (Win et al., 2018).A gibberellin-like plant growth hormone was produced by *Scytonema hofmanni* have produced gibberellin-like plant growth regulators enabled the hormone homeostasis of rice seedlings under salt stress (Win et al., 2018).
20. Soaking seeds of some wheat, soybean, clover, and rice crop cultivars in *Nostoc* spp. and/or *Anabaena flos-aquae* filtrates containing phytohormones (e.g. abscisic acid, gibberellic acid, and indole acetic acid) and other metabolites increased germination (Abdel-Raouf et al. 2012).
21. *Plectonema, Nostoc, Calothrix, Scytonema, Hapalosiphon, Microchaete*, and *Westiellopsis* are microalgal species that occur in salt affected soils. When these algae were cultured and added to saline soils, they helped ameliorate the saline soils (Chatterjee et al., 2017; Lewis et al., 2019).
22. *Arthrospira* spp. grows at high pH and/or high salt concentrations (i.e. salt levels ranging from 7-15 g L-1) (Uysal et al., 2015). [So what? So does Dunaliella.] *Arthrospira* spp. may grow under pH and/or salt extremes (i.e. salt levels ranging from 7-15 g L-1) (Ronga et al., 2019).

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Robert A. Andersen PhD — Robert has a primary interest in the systematic biology (nomenclature, phylogeny, taxonomy) of golden algae (heterokont and haptophyte algae) and was the co-author and editor of Algae Culturing Techniques (2005) along with over 250 additional publications. Dr. Andersen was the Director of the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (now NCMA) for 20 years and has taught students at DePaul University, Arizona State University, and Michigan Technological University. Robert has a B.S. degree in Botany from the North Dakota State University, a M.A. in Aquatic Biology from the St. Cloud State College, and a PhD in Botany/Phycology from the University of Arkansas. He is currently a Senior Research Scientist at the University of Washington where he continues his research systematics biology.

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Andrew Ayers — Andrew has a 30-year career studying and exploiting the commercial potential of microalgae. As the Director of Algae Impact, Andrew focuses on optimizing the efficiency and effectiveness of algae strains cultured in the System. He previously worked at the University of Hawaii, the University of Texas, and Arizona Fish and Game developing algae for the sustainability of fish hatcheries. Private sector applications include using natural selection methodologies to develop algae cells for use in nutraceuticals, pharmaceuticals, and natural fertilizers.

Matthew Olson — Matthew began his professional career at the Arizona Department of Agriculture after he received his B.S. in Plant Biology from ASU and joined Heliae Development, LLC in 2009. He has utilized his laboratory, production, operations, and management skills to work on international transfer of technologies to companies in Mexico and Japan, where he was responsible for process design, development, implementation, and training. Matthew joined the MyLand team in late 2019 with key focuses on the biological aspects of the production process and commercial agriculture R&D.

MyLand

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