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Evaluating Organic Fertilizers and Microbial Inoculation for Soilless and Hydroponic Crop Production

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Abstract. **Because hydroponic operations in the United States can be certified as organic, and because the price of chemical fertilizers has increased, there is an increasing interest in using organic fertilizers and beneficial microorganisms for controlled-environment agriculture. However, there is a scarcity of information regarding their effectiveness and application methodologies. We investigated the effects of inoculating *Azospirillum brasilense* and *Rhizophagus intraradices* and using organic fertilizers on growing lettuce (*Lactuca sativa* 'Cherokee') and tomato (*Solanum lycopersicum* 'Red Robin') young plants in an indoor vertical farm. Seeds were sown in rockwool substrate, with *A. brasilense* (1.05×10^8 colony-forming units·L^{-1}) or *R. intraradices* (580 propagules·L^{-1}) applied weekly via subirrigation. Seedlings received chemical fertilizer, organic fertilizer derived from corn steep liquor and fermented fish by-products, and food waste-derived organic fertilizer at 100 ppm total nitrogen every 2 or 3 days. They were grown indoors at 23 °C under light-emitting diode lighting at a photosynthetic photon flux density of 200 μmol·m^{-2}·s^{-1} with an 18-hour photoperiod. Lettuce under organic fertilizers had 75% lower shoot fresh mass and 64% less shoot dry mass compared with lettuce under chemical fertilizer. Similarly, tomato seedlings with organic fertilizers had fewer leaves, 75% less shoot fresh mass, and 67% less shoot dry mass. In both lettuce and tomato, the macronutrient and micronutrient concentrations in plant tissues were generally similar regardless of fertilizer treatments, but nitrogen use efficiency and nitrogen uptake efficiency were lower under organic fertilizers compared with those under chemical fertilizer. The inoculation of *A. brasilense* or *R. intraradices* showed limited effects on plant nutrient uptake, nutrient concentrations, and seedling growth in both lettuce and tomato. Further research is necessary to optimize application methods for organic fertilizers and beneficial microorganisms to fully harness the benefits of sustainable alternative fertilizers in soilless and hydroponic crop production.**

In controlled-environment agriculture, including indoor vertical farms and greenhouses, the use of soilless media and hydroponics has gained more popularity than traditional soil-based approaches. The use of soilless media and hydroponic techniques for crop production presents several benefits compared with traditional soil-based approaches, including the potential for increases in crop yields and quality, greater water and nutrient use efficiency, the option to recycle water and nutrients, effective environmental control, and mitigation against soilborne diseases and pests (Barbosa et al. 2015; Bergstrand et al. 2020; Lorenzo et al. 2013). Because the current food production system is facing challenges, such as decreases in freshwater resources and arable lands, climate change, rapid population growth, and food insecurity, the use of soilless and hydroponic cultivation with controlled-environment agriculture is increasing (Jan et al. 2020; Schnitzler 2013).

Although chemical fertilizer remains the primary nutrient source in soilless and hydroponic crop production, there is growing interest in using organic fertilizers (Hooks et al. 2022; Phibunwatthanawong and Riddech 2019). Organic fertilizers are made from diverse organic sources, such as animal manure, fish by-products, plant residues, food waste, and algae (Bergstrand 2022). Using organic fertilizers results in several advantages, such as nutrient recycling, diminished reliance on minerals used to produce chemical fertilizers, the supply of beneficial biostimulants, and the potential to harvest organic produce (Bergstrand et al. 2020; Hidalgo et al. 2021; Mazeh et al. 2021; Niu and Masabni 2022). However, incorporating organic fertilizers into soilless and hydroponic cultivation can pose more challenges compared with chemical fertilizers. When organic fertilizers are used, many nutrients bound within organic substances are not readily accessible for direct plant uptake, necessitating microbially mediated mineralization processes to release the nutrients (Bergstrand et al. 2020; Burnett et al. 2016). However, sterile and inert soilless substrates, such as rockwool, typically lack a robust microbial community, making it challenging to provide adequate and accessible nutrients during soilless and hydroponic crop cultivation (Grunert et al. 2016; Shinohara et al. 2011; Thomas et al. 2023).

One potential method of increasing the efficiency of organic fertilizers in soilless and hydroponic crop production is harnessing the abilities of plant growth-promoting microorganisms (PGPMs) (Bartelme et al. 2018; Paradiso et al. 2017; Sheridan et al. 2017). PGPMs are free-living or symbiotic bacteria and fungi that colonize plant roots and provide benefits to their host plants by increasing the availability of nutrients, enhancing plant nutrient uptake, regulating phytohormone production, and improving resistance to environmental stresses (Batista and Singh 2021; de Souza et al. 2015; Lopes et al. 2021). Some popular PGPMs for commercial use include the plant growth-promoting bacteria (PGPB) *Azospirillum* and arbuscular mycorrhizal fungi (AMF). Bacteria belonging to the genus *Azospirillum* have been known to promote plant growth by fixing nitrogen (N), synthesizing phytohormones (including auxins, cytokinins, gibberellins, abscisic acid, ethylene, and salicylic acid), solubilizing phosphate, and stimulating root growth (Cassán and Diaz-Zorita 2016; Fukami et al. 2018; Zeffa et al. 2019). Although most studies included cereal crops, the plant growth-promoting effects of *Azospirillum* were observed in 113 plant species of 35 botanical families (Pereg et al. 2016). The AMF colonize plant root cells by forming arbuscules for the exchange of nutrients with their host plants (Li et al. 2014). The AMF aid in plant growth by increasing the decomposition of organic materials, thus enhancing the efficiency of plants to uptake water and nutrients and synthesizing plant hormones (Begum et al. 2019; Parniske 2008). The majority of land plants establish a symbiotic relationship with AMF, which occurs in 70% to 90% of land plant species (Wang et al. 2017). During the past decades, studies of AMF inoculation involving 43 plant families, including *Asteraceae*, *Fabaceae*, *Poaceae*, and *Solanaceae*, have reported the effects of AMF on plant biomass gain, increased crop yield, and enhanced plant nutrition; however, there are variabilities of the effects of AMF on their host plants, which are associated with the host specificity (Berruti et al. 2016; Chen et al. 2018; Yang et al. 2012).

However, although the establishment, function, and efficacy of PGPM on plants can be influenced by various abiotic factors, the majority of studies of PGPM effects were conducted in traditional soil and field conditions, with relatively few investigations performed in soilless and hydroponic settings under a controlled environment (Majeed et al. 2018; Mishra and Sundari 2013; Mwashasha 2016). In general, the impact of PGPMs is more pronounced under harsh or stressful environment

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because PGPMs often play a protective role for both themselves and host plants against conditions like drought, salinity, and pathogens (Balkrishna et al. 2022). In controlled environments optimized for plant growth, PGPMs can occasionally outcompete plants for essential nutrients, such as nitrate and oxygen, negatively affecting overall plant growth (MacIntyre et al. 2011; Moncada et al. 2021). The PGPM effects tend to be more significant under soil conditions than in soilless conditions because of the presence of organic matter in the soil that serves as a nutrient source for microbes and the favorable environmental conditions in the rhizosphere that promote microbial activity (Khare et al. 2014). Therefore, it is expected that the distinct environmental characteristics under the controlled environment and soilless media will influence the interactions between plants and PGPM, subsequently affecting plant benefits derived by PGPMs (De Haas et al. 2021; Paradiso et al. 2017; Sheridan et al. 2017).

This study aimed to evaluate the impact of using organic fertilizers and PGPMs instead of chemical fertilizer for soilless and hydroponic crop production in controlled-environment agriculture. We grew lettuce (*Lactuca sativa*) and tomato (*Solanum lycopersicum*) in rockwool in an indoor vertical farm using two different types of organic fertilizers, both with and without inoculation with PGPB *Azospirillum* or AMF. We chose lettuce and tomato as our focal crops because they are among the most commonly grown using hydroponic and soilless cultivation techniques (Walters et al. 2020). Additionally, there is relatively limited information regarding the impact of PGPMs in hydroponic and soilless cultivation, especially when compared with the abundance of studies of cereal crops. By focusing on lettuce and tomato, this study sought to contribute practical insights into the potential implications of organic fertilizers and PGPMs in soilless and hydroponic crop production.

Materials and Methods

Plant materials and growing environment. This study was conducted twice, in Aug 2020 and Mar 2021; in each replication, there were nine treatments (3 PGPM treatments × 3 fertilizer treatments). For each treatment, 40 rockwool cubes (A-OK Starter Plug 3.6 cm × 3.6 cm × 4.1 cm; Grodan, Milton, ON, Canada) were placed in a plastic plant tray (52.8 cm × 27.4 cm × 6.1 cm; Hawthorne, Vancouver, WA, USA), and 20 seeds of tomato 'Red Robin' (Park Seeds, Greenwood, SC, USA) and 20 seeds of lettuce 'Cherokee' (Johnny's Selected Seeds, Winslow, ME, USA) were sown in individual rockwool cubes. The seeded trays were covered with humidity domes and placed in a growing rack with three tiers inside a research indoor vertical farm on the Arizona State University Polytechnique campus (Mesa, AZ, USA). Three trays under the same fertilizer with three PGPM treatments were placed on the same tier of the growing rack. One week after sowing, when both lettuce and tomato seeds germinated, the humidity domes were removed.

The seedlings were grown at a set air temperature of 23 °C under sole-source lighting from blue + red + white LEDs (T8 Double-Row LED Indoor Grow Light; Homer Farms Inc., Mesa, AZ, USA) at a photosynthetic photon flux density (PPFD) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an 18-h photoperiod. The air temperature was continuously monitored on the center of each tier of the growing rack at the seedling height using a hygrometer thermometer (H57075; Shenzhen Intellirocks Tech Co., Shenzhen, Guangdong, China). The radiation spectrum and PPFD were measured using a spectroradiometer (PS-300; StellerNet, Inc., Tampa, FL, USA) by taking the average of nine predetermined points inside each growing rack tier at the seedling tray height. The radiation spectrum from blue + red+ white LEDs consisted of 28.0% blue (400–500 nm), 17.6% green (500–600 nm), 78.8% red (600–700 nm), and 3.6% far-red (700–800 nm) photons.

PGPM and fertilizer treatments. PGPM treatments were applied at sowing and weekly after sowing. At sowing, rockwool cubes were presoaked, and there were three trays of tap water (control), three trays of AMF solution, and three trays of PGPB *Azospirillum* solution. The AMF solution was formulated with *Rhizophagus intraradices* at a concentration of 580 propagules·L^{-1}, which was achieved by manually blending 2.36 g of mycorrhizal inoculum wettable powder (Mykos mycorrhizal wettable powder; Reforestation Technologies International, Gilroy, CA) with every 1 L of tap water, in adherence to the recommended dosage indicated on the product label. The PGPB solution was prepared using *A. brasilense* at a concentration of 1.05×10^8 CFU·L^{-1}. This was achieved by adding 1.05 mL of Azos red liquid plant growth-promoting bacteria (Reforestation Technologies International) to every 1 L of tap water according to the manufacturer's recommended dosage as stated on the product label. After sowing, the rockwool cubes were inoculated weekly through sub-irrigation with 1 L of tap water, PGPB *Azospirillum* solution, or AMF solution.

One week after sowing, when both lettuce and tomato seeds germinated, seedlings were irrigated as needed, every 2 or 3 d, through sub-irrigation with nutrient solutions made from tap water supplemented with chemical fertilizer (Chemical) [15N (3% ammoniacal N and 12% nitrate N)–$5P_2O_5$–$20K_2O$; Jack's Nutrients FeED; JR Peters, Allentown, PA, USA], organic bio-fertilizer derived from corn steep liquor and fermented fish by-products (OCF) [3N (0.16% ammoniacal N, 2.63% other water soluble N, and 0.21% water insoluble N)–$3P_2O_5$–$2K_2O$; AgroThrive Organic Bio-fertilizers; AgroThrive, Gonzales, CA, USA], or organic fertilizer derived from anaerobically digested food waste (OF) [0.06N (0.0378% ammoniacal N and 0.0222% organic N)–$0.026P_2O_5$–$0.1191K_2O$; Climate Saver, Homer Farms Inc.] at 100 ppm N (Table 1). Furthermore, 100 ppm N was selected as the typical N rate used for hydroponic and soilless lettuce and tomato transplants (Henry et al. 2018; Whipker et al. 2018).

Table 1. The calculated concentration (in ppm) of nitrogen (N), phosphorous (P), and potassium (K) concentrations of fertilizer treatment solutions made with chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF).

	N (ppm)	P (ppm)	K (ppm)
Chemical	100	15	111
OCF	100	44	55
OF	100	19	165

Before each application, the pH of PGPM treatment solutions and fertilizer treatment solutions were adjusted to a pH of 5.8 to 6.0 with diluted (1:1) 95% to 98% sulfuric acid (J.Y. Baker, Inc.) and potassium bicarbonate (Earthborn Elements; American Fork, UT, USA), and the electrical conductivity (EC) and pH of each solution were measured using a pH and EC meter (HI9814; Hannah Instruments; Woonsocket, RI, USA) (Table 2). Additionally, to determine the effects of fertilizer treatments on the dissolved oxygen level in the nutrient solution, additional fertilizer treatment solutions were made in 5-gallon buckets according to the previously described method. Then, we monitored the dissolved oxygen level of these fertilizer treatment solutions in the 5-gallon buckets every 2 d for 2 weeks after their preparation using a dissolved oxygen meter (850048; Sper Scientific, Scottsdale, AZ, USA).

Data collection and analysis. In each replication, the following seedling growth data were collected from 10 plants per treatment per species 3 weeks after sowing seeds: leaf number (leaf length ≥1.5 cm); leaf length and width for the first true leaf; relative chlorophyll concentration [Soil and Plant Analysis Development (SPAD) index] with a handheld SPAD meter (SPAD-502; Konica Minolta Sensing, Inc., Chiyoda, Tokyo, Japan); shoot fresh mass and shoot dry mass after ≥5 d in a drying oven (Hafo 1600 Oven; VWR International, LLC, Aurora, CO, USA) at 86 °C with an analytical balance (PB602-S; Mettler Toledo, Columbus, OH, USA); plant diameter (lettuce only) measured with a ruler; stem diameter (tomato only) measured with a digital caliper; and seedling height (tomato only) measured with a ruler.

To analyze plant tissue nutrients, we combined dried plant samples from each treatment within each replication and submitted them to an analytical laboratory (Plant Tissue, Waste & Compost, Solutions and Soilless Media Testing Laboratory, Agronomic Division, North Carolina Department of Agriculture and Consumer Services, Raleigh, NC, USA). In the analytical laboratory, samples were dried overnight (12–24 h) at 80 °C and then processed through a stainless steel grinder with a 20-mesh (1-mm) screen. The total N concentration was determined by oxygen combustion gas chromatography with an elemental analyzer. The total concentrations of phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese

Table 2. Means (±*SD*) of electrical conductivity (EC) and pH of plant growth-promoting microorganisms (PGPMs) treatment solutions, including tap water (control), arbuscular mycorrhizal fungi (AMF), and plant growth-promoting bacteria *Azospirillum* (PGPB), and fertilizer treatment solutions made with chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), throughout two replications (reps).

	EC ($mS \cdot cm^{-1}$)		pH	
	Rep 1	Rep 2	Rep 1	Rep 2
PGPM treatment solutions				
Tap water	0.82 ± 0.04	0.99 ± 0.08	5.84 ± 0.04	5.83 ± 0.01
AMF	1.03 ± 0.07	1.09 ± 0.04	5.94 ± 0.18	5.86 ± 0.03
PGPB	0.97 ± 0.04	0.99 ± 0.08	5.91 ± 0.15	5.84 ± 0.02
Fertilizer treatment solutions				
Chemical	1.60 ± 0.06	1.51 ± 0.15	5.85 ± 0.10	5.85 ± 0.02
OCF	1.33 ± 0.10	1.36 ± 0.14	5.84 ± 0.05	5.87 ± 0.03
OF	2.02 ± 0.08	2.13 ± 0.21	5.89 ± 0.12	5.87 ± 0.03

(Mn), zinc (Zn), copper (Cu), and boron (B) were determined with inductively coupled plasma-optical emission spectrometry (Spectro Arcos EOP and Arcos II EOP; Spectro Analytical). Because of the limited dry mass of tomato plants, total concentrations of P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B were assessed using combined samples from both replications. The N use efficiency [shoot dry mass (mg)/applied N (mg)], N uptake efficiency [shoot N content (mg)/applied N (mg)], and N utilization efficiency [shoot dry mass (mg)/shoot N content (mg)] were calculated (Anas et al. 2020; Colla et al. 2010; Goins et al. 2004; Masclaux-Daubresse et al. 2010), and each value was presented relative to that of the plants grown under chemical without PGPMs. Similar to the N uptake, the P uptake efficiency [shoot P content (mg)/applied P (mg)] and K uptake efficiency [shoot K content (mg)/applied K (mg)] were calculated, and each value was presented relative to that of the plants grown under chemical without PGPMs.

The experiment used a randomized complete block design (RCBD) with two blocks. With the RCBD design, a block is a grouping of experimental units that share similar characteristics aimed at minimizing the impact of potential sources of variability. In this context, each replication in the study was considered a block. The experimental units were plant trays, and the plant trays were considered the basis for applying PGPM and fertilizer treatments. For the plant growth analysis, within each plant tray, the observational units were 10 individual seedlings per species serving as the subsamples for analysis. This RCBD design allowed for controlled variability management, thus ensuring a comprehensive evaluation of the treatments on individual seedlings within the specified blocks. Data from two replications were pooled and analyzed with the SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA) using the PROC MIXED procedure with two fixed factors (PGPM and fertilizer treatments) and two random factors (blocks and the interaction among blocks, PGPM treatments, and fertilizer treatments). Using PROC MIXED, the pairwise comparisons between treatments were conducted by performing Tukey's honestly significant test at $P < 0.05$.

Results

Lettuce. The PGPM treatments had little to no effect on lettuce seedling growth (Fig. 1 and Table 3). Lettuce seedlings grown with OCF or OF had a smaller plant diameter (39%–55%), leaf length (37%–55%), leaf width (36%–54%), shoot fresh mass (65%–85%) and shoot dry mass (51%–78%) than those grown with Chemical. The leaf number and SPAD index were generally similar among fertilizer treatments, although the SPAD index was greater under OF with AMF than under Chemical. The OCF and OF had similar effects on lettuce seedling growth.

Tomato. The PGPM treatments did not influence tomato seedling growth (Fig. 2 and Table 4). Tomato seedlings grown with OCF or OF had 0.7 to 1.2 fewer leaves and lower shoot fresh mass and shoot dry mass (65%–81% and 54%–76%, respectively) than those grown with Chemical. Stem lengths were 33% to 51% shorter and stem diameters were 30% to 40% smaller under OCF or OF than under Chemical. The leaf lengths with OCF and OF were 33% to 53% shorter than those with Chemical, except for those with PGPB, for which the leaf lengths were similar to those with Chemical with AMF. Leaf widths with OCF and OF were similar or 25% to 65% smaller than those with Chemical. In general, OCF and OF had similar effects on the leaf number, leaf size, leaf width, shoot fresh mass, and shoot dry mass. The SPAD index values were similar regardless of microorganisms and fertilizer treatments.

Plant tissue nutrient concentrations. For lettuce seedlings, PGPM treatments did not affect the concentration of any macronutrients and micronutrients in lettuce seedlings (Table 5). Lettuce seedlings accumulated 55% to 71% less P under OF compared with Chemical and OCF, except when lettuce seedlings had similar P levels under OCF and OF with PGPB. Lettuce seedlings with OCF and OF had similar or 40% to 43% lower K concentrations than those with Chemical. Conversely, lettuce seedlings with OF had 333% to 685% higher or similar Fe levels compared to those with Chemical and OCF. Lettuce seedlings with OCF showed similar or 67% to 68% lower Zn levels compared to those with Chemical, whereas lettuce seedlings with OF had similar or 60% to 72% higher Zn than those with Chemical. The N, Ca, Mg, S, Mn, Cu, and B levels were similar among fertilizer treatments. In general, the nutrient concentrations of tomato seedlings showed a similar trend with lettuce in response to PGPM and fertilizer treatments (Table 6).

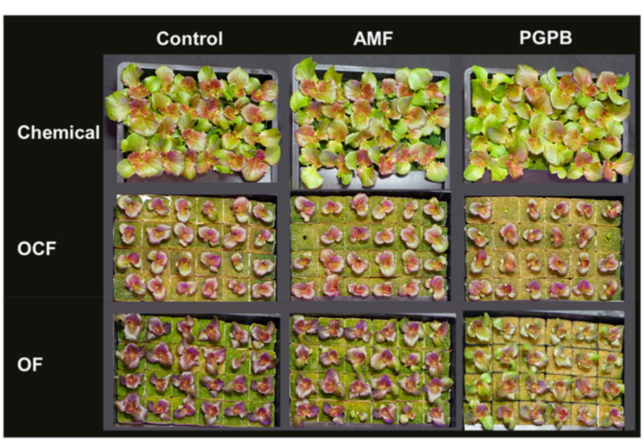

Fig. 1. Lettuce seedlings grown for 3 weeks after sowing under chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), either without (control) or with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria *Azospirillum* (PGPB).

Discussion

Effects of organic fertilizers. In this study, when lettuce and tomato seedlings were fertilized at the same 100 ppm total N concentration, the shoot fresh mass measurements of lettuce and tomato seedlings were 65% to 85% (shoot dry mass, 51%–78%) and 65% to 81% (shoot dry mass, 54%–76%) smaller under organic fertilizers, regardless of the specific type, compared to chemical fertilizer, respectively (Tables 3 and 4). Similarly, several studies reported that at the same total N level, plants have smaller biomass with organic fertilizer than with chemical fertilizer. For example, in lettuce 'Rouxai', the fresh mass was 29% to 35% smaller with fish-based organic fertilizer than with the conventional

Table 3. Effects of fertilizer treatments and plant growth-promoting microorganisms (PGPMs) treatments on plant growth characteristics of lettuce seedlings. Lettuce seedlings were grown for 3 weeks after sowing under chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), either without (control) or with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria *Azospirillum* (PGPB). Data represent the mean of two replications.

Fertilizer	PGPM	Plant diam (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	SPAD index	Shoot fresh mass (mg)	Shoot dry mass (mg)
Chemical	Control	6.5 a[i]	4.7[ii]	4.9 a	4.1 a	24.4 c	1352 a	85 a
	AMF	6.5 a	4.5	5.1 a	4.0 a	25.4 bc	1291 a	79 a
	PGPB	6.4 a	4.4	4.9 a	3.9 a	23.4 bc	1141 a	68 a
OCF	Control	3.5 b	3.1	2.7 b	2.2 b	28.2 abc	295 b	27 b
	AMF	3.9 b	3.2	3.1 b	2.5 b	26.7 abc	396 b	33 b
	PGPB	2.9 b	2.8	2.3 b	1.9 b	26.5 abc	206 b	19 b
OF	Control	3.6 b	3.2	2.7 b	2.1 b	32.1 abc	357 b	31 b
	AMF	3.5 b	3.1	2.7 b	2.0 b	34.4 a	336 b	28 b
	PGPB	3.7 b	3.5	3.0 b	2.2 b	30.5 ab	336 b	28 b
Significance								
Fertilizer		***[iii]	**	***	***	***	***	***
PGPM		NS	NS	NS	NS	NS	NS	NS
Fertilizer × PGPM		NS	NS	NS	NS	NS	NS	NS

[i] Different letters are statistically different at $P < 0.05$.

[ii] Means with no lettering were found to have no significant difference at $P < 0.05$.

[iii] NS, *, **, or *** indicate not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

nutrient solution at 150 ppm N (Lau and Mattson 2021). In a separate study, squash (*Cucurbita pepo* L.) ‘F1 Alata Yesili’ that received liquid organic fertilizer containing organic chicken manure showed an 18% reduction in shoot fresh mass compared with chemical fertilizer at 151 ppm N (Dasgan and Bozkoylu 2007). Together, these results indicate that N use efficiency, defined as the ratio of crop yield per N supplied (Anas et al. 2020; Masclaux-Daubresse et al. 2010), is generally lower under the organic fertilizers than under chemical fertilizer.

The N use efficiency in plants is determined by N uptake efficiency and N utilization efficiency (Anas et al. 2020; Masclaux-Daubresse et al. 2010). The N uptake efficiency quantifies how effectively plants absorb N from N sources, whereas N utilization efficiency describes the ability of plants to assimilate and remobilize N (Anas et al. 2020; Masclaux-Daubresse et al. 2010). Our results showed that the lower N use efficiency under organic fertilizers was mainly caused by lower N uptake efficiency, not N utilization efficiency (Table 7). The N uptake efficiency of plants depends on the types of N available (Li et al. 2013). The N is available to plants as either nitrate or ammonium. Most plants preferably uptake nitrate over ammonium, and ammonium at higher concentrations can be toxic to the plants (Li et al. 2013). Chemical fertilizers provide N mostly in the form of nitrate or ammonium ions. However, N in organic fertilizer often exists in the organic or ammonium form that is needed to be converted into nitrate for optimal plant uptake (Bi et al. 2010; Dasgan and Bozkoylu 2007; Gaskell and Smith 2007). In this study, 80% and 20% of the N in the chemical fertilizer were nitrate and ammonium, respectively. In contrast, most of the N in the food waste-based organic fertilizer existed in the ammonium (63%) and organic N (37%) forms, although the forms of N in the organic fertilizer derived from corn steep liquor and fermented fish by-products was less clear. The observed lower N uptake efficiency under organic fertilizers, compared with chemical fertilizer at the same N level, could be, at least partly, attributed to the lower nitrate concentration and higher organic and ammonium N content in organic fertilizers.

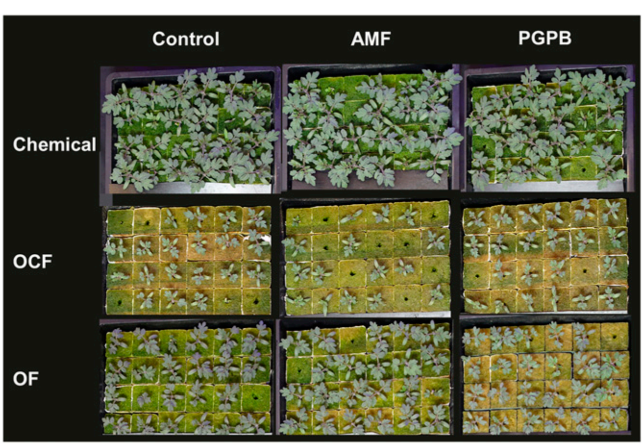

Fig. 2. Tomato seedlings grown for 3 weeks after sowing under chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), either without (control) or with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria *Azospirillum* (PGPB).

When the fertilizer effects were compared at the same N level in this study, while the macro- and micronutrient concentrations in plant tissues were generally similar among fertilizer treatments, lettuce seedlings contained less P under OF than under chemical fertilizer (Table 5). The differences in P concentrations in lettuce seedlings was associated with the concentrations of P (or N:P ratios) of the fertilizers used in this study and the uptake efficiency of P. The N:P ratios (in ppm) of Chemical, OCF, and OF were 100:15, 100:44, and 100:19, respectively (Table 1). The uptake efficiency of P was 87%–94% lower under organic fertilizers than under chemical fertilizer (Table 7). Subsequently, the lower P concentration in lettuce seedlings under OF coincided with the similar P levels between OF and the chemical fertilizer. When P level was elevated under OCF compared to the chemical fertilizer the concentrations of P in plant tissues were similar to those observed with the chemical fertilizer. Considering the essential roles of P in plant development, the lower concentrations of P in lettuce seedlings under OF could be contributing factors to the observed decreased plant growth and biomass accumulation under OF.

In soilless media and hydroponics, the dissolved oxygen level in the nutrient solution greatly affects the availability of oxygen to plant roots, thereby influencing nutrient uptake and root growth (Meselmani 2022; Moran 2018). It is recommended to maintain the dissolved oxygen concentration of nutrient solution at or above the natural saturation point (Moran 2018). However, in nutrient solutions containing organic fertilizers, microbial decomposition and organic material breakdown can decrease dissolved oxygen levels (Kano et al. 2021). In addition, organic fertilizers can promote the formation of biofilm on plant roots, further decreasing dissolved oxygen (Kano et al. 2021; Lau and Mattson 2021; Shinohara et al. 2011). The dissolved oxygen level in the nutrient solution made with

Table 4. Effects of fertilizer treatments and plant growth-promoting microorganisms (PGPMs) treatments on plant growth characteristics of tomato seedlings. Tomato seedlings were grown for 3 weeks after sowing under chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), either without (control) or with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria *Azospirillum* (PGPB). Data represent the mean of two replications.

Fertilizer	PGPM	Stem diam (cm)	Stem length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	SPAD index	Shoot fresh mass (mg)	Shoot dry mass (mg)
Chemical	Control	2.5 a[i]	3.5 a	3.0 a	4.3 a	3.5 a	53.2[ii]	680 a	67 a
	AMF	2.5 a	3.7 a	3.2 a	4.3 a	3.7 a	50.8	680 a	67 a
	PGPB	2.3 ab	3.3 ab	3.0 a	3.9 ab	3.2 ab	52.5	566 a	53 a
OCF	Control	1.5 b	2.1 c	2.1 b	2.0 c	1.4 c	41.8	135 b	18 b
	AMF	1.5 b	2.0 c	2.0 b	2.2 c	1.6 c	45.1	142 b	20 b
	PGPB	1.5 ab	1.8 c	2.0 b	1.8 c	1.3 c	43.1	126 b	16 b
OF	Control	1.6 ab	2.2 b	2.0 b	2.5 c	1.8 bc	42.1	184 b	24 b
	AMF	1.6 ab	2.2 bc	2.3 b	2.6 bc	1.9 bc	53.8	197 b	24 b
	PGPB	1.6 ab	2.4 bc	2.2 b	2.3 c	2.4 abc	52.5	169 b	21 b
Significance									
Fertilizer		***[iii]	***	***	***	***	**	***	***
PGPM		NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer × PGPM		NS	NS	NS	NS	NS	NS	NS	NS

[i] Different letters are statistically different at $P < 0.05$.

[ii] Means with no lettering were found to have no significant difference at $P < 0.05$.

[iii] NS, *, **, or *** indicate not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

organic fertilizer decreased by 1 to 6 ppm in comparison with the nutrient solution made with chemical fertilizer (Arancon et al. 2019; Kano et al. 2021). It was observed that when moderate amounts of hydrogen peroxide were added to nutrient solution containing fish emulsion, the resulting lettuce yield was comparable to that of conventional controls, possibly because of the increase in the dissolved oxygen level (Lau and Mattson 2021). In this study, following the preparation of the nutrient solution, no additional oxygen supply was provided, and the dissolved oxygen level was consistently 1.5 to 5.6 ppm lower in the nutrient solution made with organic fertilizer derived from corn steep liquor and fermented fish by-products, as well as organic fertilizer derived from food waste, compared with the nutrient solution made with chemical fertilizer (Fig. 3). Thus, there is a possibility that the reduced nutrient uptake and plant growth under organic fertilizers is at least partly associated with decreased oxygen levels in the root zone.

Effects of PGPMs. The plant growth-promoting effects of AMF and PGPB, mediated by increased nutrient uptake and plant hormone production, have been reported in a wide range of crops (Grobelak et al. 2015; Kim et al. 2017; Oljira et al. 2020; Shinohara et al. 2011). However, in this study, the application of AMF and PGPB had little to no effect on nutrient uptake and plant growth in lettuce and tomato seedlings (Tables 3–6). The beneficial effects of AMF and PGPB on plant growth can depend on root zone temperature and pH because they can affect plant–microbe interactions (Agehara and Warncke 2005; Gaskell and Smith 2007; Moncada et al. 2021; Saijai et al. 2016; Silgram and Shepherd 1999). In general, plant growth-promoting effects of AMF and PGPB increased under warm root zone temperatures (25–40 °C) and basic pH (7.5–8.5) because these conditions become more favorable for microbial activity (Curtin et al. 2012; Franzluebbers 1999; Jones and Hood 1980; Linn and Doran 1984; Lopes et al. 2021; Saijai et al. 2016). In this study, we grew lettuce and tomato seedlings at the set air temperature of 23 °C with an average nutrient solution pH of 5.8 principally for plant growth and nutrient uptake. Little effects of AMF and PGPB applications could be attributable to lower temperatures and lower pH than the ideal conditions for AMF and PGPB, which can suppress the activities of AMF and PGPB.

The little effects of AMF and PGPB on nutrient uptake and plant growth could be a result of unsuccessful inoculation of lettuce and tomato seedlings with AMF and PGPB. The plant growth-promoting effects of PGPMs require the successful inoculation of plants with microorganisms, which depend on the inoculation method, microbe inoculant density, inoculation frequency, and root colonization (Lopes et al. 2021). For example, plant growth-promoting effects from the PGPB *Phyllobacterium brassicaearum* showed dose-dependent responses with the maximum growth potential on roots of rapeseed (*Brassica napus*) from bacterial densities of approximately

Table 5. Effects of fertilizer treatments and plant growth-promoting microorganisms (PGPMs) treatments on the concentration of macronutrients [nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S)] and micronutrients [iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B)] in lettuce seedlings. Lettuce seedlings were grown for 3 weeks after sowing under chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), either without (control) or with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria *Azospirillum* (PGPB). Data represent the mean of two replications.

		Macronutrients (% dry mass)						Micronutrients ($\mu g \cdot g^{-1}$ dry mass)				
Fertilizer	PGPM	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
Chemical	Control	4.44[i]	0.46 a[ii]	6.12 ab	1.26	0.29	0.30	140 b	103	57 b	10	161
	AMF	4.74	0.52 a	6.52 a	1.29	0.31	0.34	174 b	112	60 ab	10	158
	PGPB	4.63	0.52 a	6.57 a	1.27	0.30	0.33	105 b	101	53 bc	9	130
OCF	Control	3.59	0.44 ab	3.88 b	0.88	0.26	0.24	105 b	55	19 d	7	187
	AMF	3.57	0.45 ab	4.26 ab	0.88	0.26	0.24	96 b	55	20 cd	4	111
	PGPB	3.49	0.39 abc	3.76 b	0.82	0.25	0.23	108 b	46	19 d	9	138
OF	Control	4.90	0.15 c	3.77 b	0.91	0.23	0.32	416 ab	131	72 ab	8	102
	AMF	4.74	0.17 c	4.50 ab	1.20	0.33	0.34	754 a	240	91 a	14	145
	PGPB	4.69	0.20 bc	4.12 ab	0.85	0.24	0.32	316 ab	104	58 ab	8	116
Significance												
Fertilizer		*[iii]	***	***	*	NS	***	**	*	***	NS	NS
PGPM		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer × PGPM		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[i] Means with no lettering were found to have no significant difference at $P < 0.05$.

[ii] Different letters are statistically different at $P < 0.05$.

[iii] NS, *, **, or *** indicate not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

Table 6. Effects of fertilizer treatments and plant growth-promoting microorganisms (PGPMs) treatments on the concentration of macronutrients [nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S)] and micronutrients [iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B)] in tomato seedlings. Tomato seedlings were grown for 3 weeks after sowing under chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), either without (control) or with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria *Azospirillum* (PGPB). Data represent the mean of two replications.

		Macronutrients (% dry mass)						Micronutrients ($\mu g \cdot g^{-1}$ dry mass)				
Fertilizer	PGPM	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
Chemical	Control	4.78[i]	0.42	4.31	1.80	0.45	0.34	137	49	36	8	117
	AMF	5.05	0.46	4.26	1.71	0.42	0.38	144	42	36	7	134
	PGPB	5.01	0.42	4.12	1.87	0.46	0.35	159	50	38	8	135
OCF	Control	3.20	0.50	2.56	1.42	0.46	0.38	101	38	17	1	98
	AMF	2.89	0.51	2.28	1.32	0.43	0.47	84	37	22	2	195
	PGPB	2.85	0.51	2.26	1.38	0.46	0.41	96	40	18	1	103
OF	Control	4.53	0.17	3.53	1.15	0.44	0.47	321	71	79	8	113
	AMF	4.72	0.15	3.24	1.24	0.39	0.53	231	52	58	5	99
	PGPB	4.60	0.12	2.85	1.13	0.39	0.43	292	66	61	6	100
Significance												
Fertilizer		**[ii]	–[iii]	–	–	–	–	–	–	–	–	–
PGPM–		NS	–	–	–	–	–	–	–	–	–	–
Fertilizer × PGPM		NS	–	–	–	–	–	–	–	–	–	–

[i] Means with no lettering were found to have no significant difference at $P < 0.05$.

[ii] NS, *, **, or *** indicate not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

[iii] Statistical analysis was not conducted.

10^{11} colony-forming unit (CFU)$\cdot L^{-1}$ (Larcher et al. 2008). In addition, because microorganisms are usually immobile from the inoculation site to the rhizosphere, applying inoculums close to the root zone has been suggested (Lopes et al. 2021). In this study, inoculation densities were made at 580 propagules/L for AMF and 1.05×10^8 CFU$\cdot L^{-1}$ for PGPB, in line with the recommended levels for PGPM products. However, these concentration densities may not have been optimal because the most successful plant growth effects have been reported by other studies that involved strains of *A. brasilense* with densities of approximately 10^9 CFU$\cdot L^{-1}$ and successful plant growth effects from *R. intraradices* with densities at 1000 propagules$\cdot g^{-1}$ (Bashan and de-Bashan 2002; Mangmang et al. 2015; Mikiciuk et al. 2019). Furthermore, in this study, the applications of AMF and PGPB were conducted weekly solely through sub-irrigation. The relatively low inoculation density and frequency of AMF and PGPB, along with the absence of direct inoculation into the seed and seedling roots, might have contributed to the limited establishment of PGPM populations in the root zone and, consequently, hindered their subsequent growth-promoting effects.

Additionally, the limited effects observed with PGPMs might be attributed to the relatively short duration (3 weeks) of experimental periods. Although there is evidence supporting the rapid inoculation of AMF and the manifestation of plant growth-promoting effects within a short timeframe within 3 to 4 weeks after inoculation in various crops, including lettuce (Aini et al. 2019), tomato (Balliu et al. 2015; Oseni et al. 2010), cowpea (*Vigna unguiculata*) (Oyewole et al. 2017), cannabis (*Cannabis sativa*) (Kakabouki et al. 2021), leek (*Allium porrum*), (Jansa et al. 2008), and medic (*Medicago truncatula*) (Jansa et al. 2008), the effectiveness of AMF inoculation in terms of successful root colonization and plant growth promoting effects tends to increase over time after inoculation (Jansa et al. 2008; Kakabouki et al. 2021; Qin et al. 2022).

Biologically active root exudates range from discharged ions, free oxygen, water, enzymes, mucilage, and carbon compounds containing primary and secondary metabolites, such as amino acids and sugars (Hayat et al. 2017; Neumann et al. 2014). Root exudates act as key mechanisms of interactive communication signals between plant and microbes in the rhizosphere (Ortíz-Castro et al. 2009). For example, root exudates in arabidopsis (*Arabidopsis thaliana*), including malic acid, aided in the

Table 7. Effects of fertilizer treatments and plant growth-promoting microorganisms (PGPMs) treatments on the relative nitrogen (N) use efficiency, N uptake efficiency, and N utilization efficiency of lettuce and tomato seedlings and on the relative phosphorous (P) and potassium (K) uptake efficiency of lettuce seedlings. Lettuce and tomato seedlings were grown for 3 weeks after sowing under chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF), either without (control) or with arbuscular mycorrhizal fungi (AMF) or plant growth-promoting bacteria *Azospirillum* (PGPB). Data represent the mean of two replications.

		Lettuce					Tomato		
Fertilizer	PGPM	N use efficiency	N uptake efficiency	N utilization efficiency	P uptake efficiency	K uptake efficiency	N use efficiency	N uptake efficiency	N utilization efficiency
Chemical	Control	1.00 a[i]	1.00 a	1.00[ii]	1.00 a	1.00 a	1.00 a	1.00 a	1.00
	AMF	0.93 a	0.99 a	0.93	1.05 a	0.99 a	1.00 a	1.05 a	0.93
	PGPB	0.81 a	0.84 ab	0.97	0.90 a	0.87 ab	0.79 a	0.84 a	0.95
OCF	Control	0.32 b	0.25 c	1.22	0.10 b	0.40 bc	0.27 b	0.18 b	1.54
	AMF	0.39 b	0.31 bc	1.22	0.12 b	0.55 abc	0.29 b	0.18 b	1.62
	PGPB	0.22 b	0.17 c	1.25	0.06 b	0.28 c	0.24 b	0.14 b	1.67
OF	Control	0.37 b	0.39 bc	0.89	0.10 b	0.16 c	0.36 b	0.33 b	1.05
	AMF	0.33 b	0.33 bc	0.95	0.10 b	0.16 c	0.36 b	0.34 b	1.00
	PGPB	0.33 b	0.36 bc	0.96	0.12 b	0.15 c	0.31 b	0.30 b	1.02
Significance									
Fertilizer		***[iii]	***	**	***	***	***	***	**
PGPM		NS	NS	NS	NS	NS	NS	NS	NS
Fertilizer × PGPM		NS	NS	NS	NS	NS	NS	NS	NS

[i] Different letters are statistically different at $P < 0.05$.

[ii] Means with no lettering were found to have no significant difference at $P < 0.05$.

[iii] NS, *, **, or *** indicate not significant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

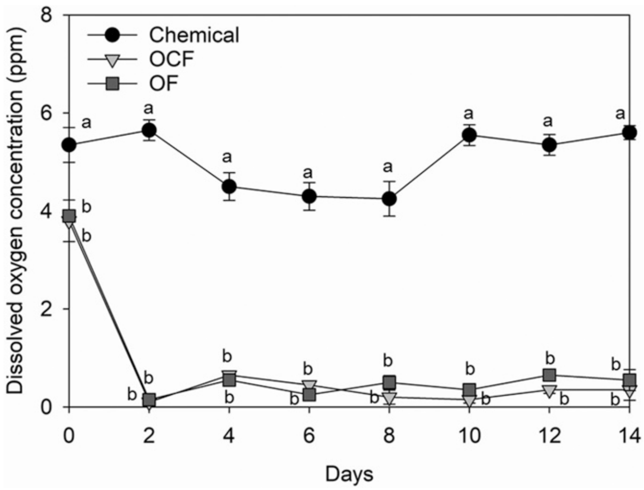

Fig. 3. Means (±*SD*) of the monitored dissolved oxygen concentration of fertilizer treatment solution made with chemical fertilizer (Chemical), organic fertilizer derived from corn steep liquor and fermented fish by-products (OCF), and organic fertilizer derived from food waste (OF) for 2 weeks after their preparation. Data represent the mean of two replications. On each day, different letters indicate a statistical difference among fertilizer treatment solutions at $P < 0.05$.

recruitment of PGPB *Bacillus subtilis* to seedling roots (Rudrappa et al. 2008). In tobacco (*Nicotiana tavacum*) and alfalfa (*Medicago sativa*), transgenic plants modified to overproduce root exudates showed increases in PGPM colonization and shoot biomass compared with control plants (López-Bucio et al. 2000; Tesfaye et al. 2003). A major factor influencing root exudation production is plant age because seedings exudate smaller amounts than mature plants (Aulakh et al. 2001; Badri and Vivanco 2009; Lucas García et al. 2001). Because the present study investigated the effects of AMF and PGPB inoculation during the young plant stage of lettuce and tomato, the small amounts of root exudates released from seedlings could less effectively facilitate the plant–microbial interaction.

Conclusions

In this study, we evaluated the potential of organic fertilizers and PGPMs as sustainable alternatives to chemical fertilizer for two popular hydroponic crops, lettuce and tomato. Our findings indicated that at the same total N concentration, the N use efficiency and N uptake efficiency were lower under organic fertilizers than they were under chemical fertilizer. Subsequently, organic fertilizer derived from corn steep liquor and fish by-product and from anaerobically digested food waste yielded comparable seedling growth in lettuce and tomato, whereas chemical fertilization increased crop yield. The inoculation of PGPB *Azospirillum* and AMF had a limited or no effect on the nutrient uptake and plant growth of lettuce and tomato seedlings, regardless of the fertilizer types used. We suggest that the limited effectiveness of PGPB *Azospirillum* and AMF in this study could be attributed to less ideal environmental conditions for PGPMs, suboptimal establishment of PGPM populations because of less effective inoculation methods and relatively shorter experimental periods, and the early developmental stage of the plants examined.

To further enhance our understanding of effective uses of organic fertilizers and PGPMs for soilless and hydroponic crop production in controlled-environment agriculture, additional research is needed to understand the specific factors that contribute to the observed low nutrient use and uptake efficiency under organic fertilizers. This investigation could involve examining the effects of application rate, nutrient release dynamics, dissolved oxygen concentrations, and microbial interactions within the root zone under organic fertilization. Considering the limited impact of PGPB *Azospirillum* and AMF on nutrient uptake and plant growth, it is necessary to explore potential optimization strategies for their application. The research focused on refining inoculation methods, exploring diverse microbial strains and their interactions with host plant species and cultivars, and considering the influence of environmental factors, may enhance the inoculation success of beneficial microorganisms and their plant growth-promoting effects. Additionally, extending the scope of soilless and hydroponic crop trials to include more species, cultivars, substrates, hydroponic systems, and environmental conditions could provide a more nuanced understanding of the applicability and generalizability of our findings.

References Cited

Agehara S, Warncke DD. 2005. Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. Soil Sci Soc Am J. 69(6):1844–1855. https://doi.org/10.2136/sssaj2004.0361.

Aini N, Yamika W, Ulum B. 2019. Effect of nutrient concentration, PGPR and AMF on plant growth, yield, and nutrient uptake of hydroponic lettuce. https://www.semanticscholar.org/paper/Effect-of-Nutrient-Concentration-%2C-PGPR-and-AMF-on-Aini-Yamika/3699aed5239703b1488f6b120191f1681a138211. [accessed 30 Jan 2024].

Anas M, Liao F, Verma KK, Sarwar MA, Mahmood A, Chen Z-L, Li Q, Zeng X-P, Liu Y, Li Y-R. 2020. Fate of nitrogen in agriculture and environment: Agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. Biol Res. 53(1):47. https://doi.org/10.1186/s40659-020-00312-4.

Arancon NQ, Owens JD, Converse C. 2019. The effects of vermicompost tea on the growth and yield of lettuce and tomato in a non-circulating hydroponics system. J Plant Nutr. 42(19):2447–2458. https://doi.org/10.1080/01904167.2019.1655049.

Aulakh MS, Wassmann R, Bueno C, Kreuzwieser J, Rennenberg H. 2001. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biol. 3(2):139–148. https://doi.org/10.1055/s-2001-12905.

Badri DV, Vivanco JM. 2009. Regulation and function of root exudates. Plant Cell Environ. 32(6):666–681. https://doi.org/10.1111/j.1365-3040.2009.01926.x.

Balkrishna A, Sharma IP, Arya V, Sharma AK. 2022. Biologicals and their plant stress tolerance ability. Symbiosis. 86(3):243–259. https://doi.org/10.1007/s13199-022-00842-3.

Balliu A, Sallaku G, Rewald B. 2015. AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. Sustainability. 7(12):12. https://doi.org/10.3390/su71215799.

Barbosa GL, Gadelha FDA, Kublik N, Proctor A, Reichelm L, Weissinger E, Wohlleb GM, Halden RU. 2015. Comparison of land, water, and energy requirements of lettuce grown using hydroponic vs. conventional agricultural methods. Int J Environ Res Public Health. 12(6):6879–6891. https://doi.org/10.3390/ijerph120606879.

Bartelme RP, Oyserman BO, Blom JE, Sepulveda-Villet OJ, Newton RJ. 2018. Stripping away the soil: Plant growth promoting microbiology opportunities in aquaponics. Front Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00008.

Bashan Y, de-Bashan LE. 2002. Protection of tomato seedlings against infection by *Pseudomonas syringae* pv. tomato by using the plant growth-promoting bacterium *Azospirillum brasilense*. Appl Environ Microbiol. 68(6):2637–2643. https://doi.org/10.1128/AEM.68.6.2637-2643.2002.

Batista BD, Singh BK. 2021. Realities and hopes in the application of microbial tools in agriculture. Microb Biotechnol. 14(4):1258–1268. https://doi.org/10.1111/1751-7915.13866.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L. 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Front Plant Sci. 10. https://doi.org/10.3389/fpls.2019.01068.

Bergstrand K-J. 2022. Organic fertilizers in greenhouse production systems – a review. Scientia Hortic. 295:110855. https://doi.org/10.1016/j.scienta.2021.110855.

Bergstrand K-J, Asp H, Hultberg M. 2020. Utilizing anaerobic digestates as nutrient solutions in hydroponic production systems. Sustainability. 12(23):10076. https://doi.org/10.3390/su122310076.

Berruti A, Lumini E, Balestrini R, Bianciotto V. 2016. Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. Front Microbiol. 6. https://doi.org/10.3389/fmicb.2015.01559.

Bi G, Evans WB, Spiers JM, Witcher AL. 2010. Effects of organic and inorganic fertilizers on marigold growth and flowering. HortScience. 45(9):1373–1377. https://doi.org/10.21273/HORTSCI.45.9.1373.

Burnett SE, Mattson NS, Williams KA. 2016. Substrates and fertilizers for organic container production of herbs, vegetables, and herbaceous ornamental plants grown in greenhouses in the United States. Scientia Hortic. 208:111–119. https://doi.org/10.1016/j.scienta.2016.01.001.

Cassán F, Diaz-Zorita M. 2016. *Azospirillum* sp. in current agriculture: From the laboratory to the field. Soil Biol Biochem. 103:117–130. https://doi.org/10.1016/j.soilbio.2016.08.020.

Chen M, Arato M, Borghi L, Nouri E, Reinhardt D. 2018. Beneficial services of arbuscular mycorrhizal fungi – From ecology to application. Front Plant Sci. 9. https://doi.org/10.3389/fpls.2018.01270.

Colla G, Suárez CMC, Cardarelli M, Rouphael Y. 2010. Improving nitrogen use efficiency in melon by grafting. HortScience. 45(4):559–565. https://doi.org/10.21273/HORTSCI.45.4.559.

Curtin D, Beare MH, Hernandez-Ramirez G. 2012. Temperature and moisture effects on microbial biomass and soil organic matter mineralization. Soil Sci Soc Am J. 76(6):2055–2067. https://doi.org/10.2136/sssaj2012.0011.

Dasgan HY, Bozkoylu A. 2007. Comparison of organic and synthetic-inorganic nutrition of soilless grown summer squash. Acta Hortic. 747:523–528. https://doi.org/10.17660/ActaHortic.2007.747.68.

De Haas BR, Van Gerrewey T, Perneel M, Van Labeke MC, Geelen D. 2021. Beneficial microbes for improving circularity and yield in hydroponic crop cultivation. Acta Hortic. 1317:149–156. https://doi.org/10.17660/ActaHortic.2021.1317.18.

de Souza R, Ambrosini A, Passaglia LMP. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol. 38(4):401–419. https://doi.org/10.1590/S1415-475738420150053.

Franzluebbers AJ. 1999. Microbial activity in response to water-filled pore space of variably eroded southern Piedmont soils. Appl Soil Ecol. 11(1):91–101. https://doi.org/10.1016/S0929-1393(98)00128-0.

Fukami J, Cerezini P, Hungria M. 2018. *Azospirillum*: Benefits that go far beyond biological nitrogen fixation. AMB Express. 8:73. https://doi.org/10.1186/s13568-018-0608-1.

Gaskell M, Smith R. 2007. Nitrogen sources for organic vegetable crops. HortTechnology. 17(4):431–441. https://doi.org/10.21273/HORTTECH.17.4.431.

Goins GD, Yorio NC, Wheeler RM. 2004. Influence of nitrogen nutrition management on biomass partitioning and nitrogen use efficiency indices in hydroponically grown potato. J Am Soc Hortic Sci. 129(1):134–140. https://doi.org/10.21273/JASHS.129.1.0134.

Grobelak A, Napora A, Kacprzak M. 2015. Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. Ecol Eng. 84:22–28. https://doi.org/10.1016/j.ecoleng.2015.07.019.

Grunert O, Hernandez-Sanabria E, Vilchez-Vargas R, Jauregui R, Pieper DH, Perneel M, Van Labeke M-C, Reheul D, Boon N. 2016. Mineral and organic growing media have distinct community structure, stability and functionality in soilless culture systems. Sci Rep. 6:18837. https://doi.org/10.1038/srep18837.

Hayat S, Faraz A, Faizan M. 2017. Root exudates: Composition and impact on plant–microbe interaction, p 179–193. In: Ahmad I, Husain FM (eds). Biofilms in plant and soil health. John Wiley & Sons, Ltd., Hoboken, NJ, USA. https://doi.org/10.1002/9781119246329.ch10.

Henry J, Whipker BE, Owen WG, Currey C. 2018. Nutritional monitoring series: Lettuce (*Lactuca sativa*). https://hortamericas.com/wp-content/uploads/2018/04/e-gro-Nutritional-Factsheet-Lettuce.pdf. [accessed 19 Jan 2024].

Hidalgo D, Corona F, Martín-Marroquín JM. 2021. Nutrient recycling: From waste to crop. Biomass Convers Biorefin. 11(2):207–217. https://doi.org/10.1007/s13399-019-00590-3.

Hooks T, Masabni J, Sun L, Niu G, Hooks T, Masabni J, Sun L, Niu G. 2022. Effects of organic fertilizer with or without a microbial inoculant on the growth and quality of lettuce in an NFT hydroponic system. Technol Hort. 2(1):1–8. https://doi.org/10.48130/TIH-2022-0001.

Jan S, Rashid Z, Ahngar T, Iqbal S, Naikoo A, Majeed S, Bhat T, Gul R, Nazir I. 2020. Hydroponics – A review. Int J Curr Microbiol Appl Sci. 9:1779–1787. https://doi.org/10.20546/ijcmas.2020.908.206.

Jansa J, Smith FA, Smith SE. 2008. Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol. 177(3):779–789. https://doi.org/10.1111/j.1469-8137.2007.02294.x.

Jones RD, Hood MA. 1980. Effects of temperature, pH, salinity, and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. Microb Ecol. 6(4):339–347. https://doi.org/10.1007/BF02010496.

Kakabouki I, Mavroeidis A, Tataridas A, Kousta A, Efthimiadou A, Karydogianni S, Katsenios N, Roussis I, Papastylianou P. 2021. Effect of *Rhizophagus irregularis* on growth and quality of *Cannabis sativa* seedlings. Plants. 10(7):7. https://doi.org/10.3390/plants10071333.

Kano K, Kitazawa H, Suzuki K, Widiastuti A, Odani H, Zhou S, Chinta YD, Eguchi Y, Shinohara M, Sato T. 2021. Effects of organic fertilizer on bok choy growth and quality in hydroponic cultures. Agronomy. 11(3):491. https://doi.org/10.3390/agronomy11030491.

Khare E, Chopra J, Arora NK. 2014. Screening for MCL-PHA-producing fluorescent pseudomonads and comparison of MCL-PHA production under iso-osmotic conditions induced by PEG and NaCl. Curr Microbiol. 68(4):457–462. https://doi.org/10.1007/s00284-013-0497-0.

Kim SJ, Eo J-K, Lee E-H, Park H, Eom A-H. 2017. Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. Mycobiology. 45(1):20–24. https://doi.org/10.5941/MYCO.2017.45.1.20.

Larcher M, Rapior S, Cleyet-Marel JC. 2008. Bacteria from the rhizosphere and roots of *Brassica napus* influence its root growth promotion by *Phyllobacterium brassicacearum*. Acta Botanica Gallica. 155(3):355–366. https://doi.org./10.1080/12538078.2008.10516116.

Lau V, Mattson N. 2021. Effects of hydrogen peroxide on organically fertilized hydroponic lettuce (*Lactuca sativa* L.). Horticulturae. 7(5):106. https://doi.org/10.3390/horticulturae7050106.

Li S-X, Wang Z-H, Stewart BA. 2013. Responses of crop plants to ammonium and nitrate N, p 205–397. In: Sparks DL (ed). Advances in agronomy. Volume 118. Academic Press, Newark, DE, USA. https://doi.org/10.1016/B978-0-12-405942-9.00005-0.

Li T, Lin G, Zhang X, Chen Y, Zhang S, Chen B. 2014. Relative importance of an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) and root hairs in plant drought tolerance. Mycorrhiza. 24(8):595–602. https://doi.org/10.1007/s00572-014-0578-3.

Linn DM, Doran JW. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci Soc Am J. 48(6):1267–1272. https://doi.org/10.2136/sssaj1984.03615995004800060013x.

Lopes MJS, Dias-Filho MB, Gurgel ESC. 2021. Successful plant growth-promoting microbes: Inoculation methods and abiotic factors. Front Sustain Food Syst. 5. https://www.frontiersin.org/articles/10.3389/fsufs.2021.606454.

López-Bucio J, de La Vega OM, Guevara-García A, Herrera-Estrella L. 2000. Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. Nat Biotechnol. 18(4):450–453. https://doi.org/10.1038/74531.

Lorenzo RD, Pisciotta A, Santamaria P, Scariot V. 2013. From soil to soil-less in horticulture: Quality and typicity. Ital J Agron. 8(4):e30. https://doi.org/10.4081/ija.2013.e30.

Lucas García JA, Barbas C, Probanza A, Barrientos ML, Gutierrez Mañero FJ. 2001. Low molecular weight organic acids and fatty acids in root exudates of two *Lupinus* cultivars at flowering and fruiting stages. Phytochem Anal. 12(5):305–311. https://doi.org/10.1002/pca.596.

MacIntyre OJ, Trevors JT, Stasiak M, Dixon MA, Cottenie K. 2011. Application of plant growth promoting rhizobacteria in a hydroponics system for advanced life support in space. Acta Hortic. 893:1285–1292. https://doi.org/10.17660/ActaHortic.2011.893.151.

Majeed A, Kaleem Abbasi M, Hameed S, Yasmin S, Hanif MK, Naqqash T, Imran A. 2018. Pseudomonas sp. AF-54 containing multiple plant beneficial traits acts as growth enhancer of *Helianthus annuus* L. under reduced fertilizer input. Microbiol Res. 216:56–69. https://doi.org/10.1016/j.micres.2018.08.006.

Mangmang JS, Deaker R, Rogers G. 2015. Optimal plant growth-promoting concentration of Azospirillum brasilense inoculated to cucumber, lettuce and tomato seeds varies between bacterial strains. Isr J Plant Sci. 62(3):145–152. https://doi.org/10.1080/07929978.2015.1039290.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. Ann Bot. 105(7):1141–1157. https://doi.org/10.1093/aob/mcq028.

Mazeh M, Almadi L, Paoletti A, Cinosi N, Daher E, Tucci M, Lodolini EM, Rosati A, Famiani F. 2021. Use of an organic fertilizer also having a biostimulant action to promote the growth of young olive trees. Agriculture. 11(7):593. https://doi.org/10.3390/agriculture11070593.

Meselmani MAA. 2022. Nutrient solution for hydroponics. IntechOpen. https://doi.org/10.5772/intechopen.101604.

Mikiciuk G, Sas-Paszt L, Mikiciuk M, Derkowska E, Trzciński P, Głuszek S, Lisek A, Wera-Bryl S, Rudnicka J. 2019. Mycorrhizal frequency, physiological parameters, and yield of strawberry plants inoculated with endomycorrhizal fungi and rhizosphere bacteria. Mycorrhiza. 29(5):489–501. https://doi.org/10.1007/s00572-019-00905-2.

Mishra N, Sundari SK. 2013. Native PGPMs as bioinoculants to promote plant growth: Response to PGPM inoculation in principal grain and pulse crops. Int J Agri Food Sci Technol. 4(10):1055–1064.

Moncada A, Miceli A, Vetrano F. 2021. Use of plant growth-promoting rhizobacteria (PGPR) and organic fertilization for soilless cultivation of basil. Scientia Hortic. 275:109733. https://doi.org/10.1016/j.scienta.2020.109733.

Moran N. 2018. Why add dissolved oxygen? https://www.producegrower.com/article/hydroponic-production-why-add-dissolved-oxygen/. [accessed 19 Jan 2024].

Mwashasha R. 2016. The effect of inoculating plant growth promoting microorganisms on rice production. Int J Agron Agric Res. 9(3):34–44. https://www.yumpu.com/en/document/read/60883902/the-effect-of-inoculating-plant-

growth-promoting-microorganisms-on-rice-production. [accessed 19 Jan 2024].
Neumann G, Bott S, Ohler M, Mock H-P, Lippmann R, Grosch R, Smalla K. 2014. Root exudation and root development of lettuce (*Lactuca sativa* L. cv. Tizian) as affected by different soils. Front Microbiol. 5. https://doi.org/10.3389/fmicb.2014.00002.
Niu G, Masabni J. 2022. Hydroponics, p 153–166. In: Kozai T, Niu G, Masabni J (eds). Plant factory basics, applications and advances. Academic Press, London, UK. https://doi.org/10.1016/B978-0-323-85152-7.00023-9.
Oljira AM, Hussain T, Waghmode TR, Zhao H, Sun H, Liu X, Wang X, Liu B. 2020. Trichoderma enhances net photosynthesis, water use efficiency, and growth of wheat (*Triticum aestivum* L.) under salt stress. Microorganisms. 8(10):1565. https://doi.org/10.3390/microorganisms8101565.
Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J. 2009. The role of microbial signals in plant growth and development. Plant Signal Behav. 4(8):701–712. https://doi.org/10.4161/psb.4.8.9047.
Oseni T, Shongwe N, Masarirambi M. 2010. Effect of arbuscular mycorrhiza (AM) inoculation on the performance of tomato nursery seedlings in vermiculite. Int J Agric Biol. 12:1560-853012.
Oyewole BO, Olawuyi OJ, Odebode AC, Abiala MA. 2017. Influence of arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. Biotechnol Rep. 14:8–15. https://doi.org/10.1016/j.btre.2017.02.004.
Paradiso R, Arena C, De Micco V, Giordano M, Aronne G, De Pascale S. 2017. Changes in leaf anatomical traits enhanced photosynthetic activity of soybean grown in hydroponics with plant growth-promoting microorganisms. Front Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00674.
Parniske M. 2008. Arbuscular mycorrhiza: The mother of plant root endosymbioses. Nat Rev Microbiol. 6(10):763–775. https://doi.org/10.1038/nrmicro1987.
Pereg L, de-Bashan LE, Bashan Y. 2016. Assessment of affinity and specificity of *Azospirillum* for plants. Plant Soil. 399(1):389–414. https://doi.org/10.1007/s11104-015-2778-9.
Phibunwatthanawong T, Riddech N. 2019. Liquid organic fertilizer production for growing vegetables under hydroponic condition. Int J Recycl Org Waste Agric. 8(4):369–380. https://doi.org/10.1007/s40093-019-0257-7.
Qin M, Li L, Miranda JP, Tang Y, Song B, Oosthuizen MK, Wei W. 2022. Experimental duration determines the effect of arbuscular mycorrhizal fungi on plant biomass in pot experiments: A meta-analysis. Front Plant Sci. 13:1024874. https://doi.org/10.3389/fpls.2022.1024874.
Rudrappa T, Czymmek KJ, Paré PW, Bais HP. 2008. Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol. 148(3):1547–1556. https://doi.org/10.1104/pp.108.127613.
Saijai S, Ando A, Inukai R, Shinohara M, Ogawa J. 2016. Analysis of microbial community and nitrogen transition with enriched nitrifying soil microbes for organic hydroponics. Biosci Biotechnol Biochem. 80(11):2247–2254. https://doi.org/10.1080/09168451.2016.1200459.
Schnitzler WH. 2013. Urban hydroponics for green and clean cities and for food security. Acta Hortic. 1004:13–26. https://doi.org/10.17660/ActaHortic.2013.1004.1.
Sheridan C, Depuydt P, De Ro M, Petit C, Van Gysegem E, Delaere P, Dixon M, Stasiak M, Aciksöz SB, Frossard E, Paradiso R, De Pascale S, Ventorino V, De Meyer T, Sas B, Geelen D. 2017. Microbial community dynamics and response to plant growth-promoting microorganisms in the rhizosphere of four common food crops cultivated in hydroponics. Microb Ecol. 73(2):378–393. https://doi.org/10.1007/s00248-016-0855-0.
Shinohara M, Aoyama C, Fujiwara K, Watanabe A, Ohmori H, Uehara Y, Takano M. 2011. Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. Soil Sci Plant Nutr. 57(2):190–203. https://doi.org/10.1080/00380768.2011.554223.
Silgram M, Shepherd MA. 1999. The effects of cultivation on soil nitrogen mineralization. Adv Agron. 65:267–311. https://doi.org/10.1016/S0065-2113(08)60915-3.
Tesfaye M, Dufault NS, Dornbusch MR, Allan DL, Vance CP, Samac DA. 2003. Influence of enhanced malate dehydrogenase expression by alfalfa on diversity of rhizobacteria and soil nutrient availability. Soil Biol Biochem. 35(8):1103–1113. https://doi.org/10.1016/S0038-0717(03)00162-7.
Thomas P, Knox OGG, Powell JR, Sindel B, Winter G. 2023. The hydroponic rockwool root microbiome: Under control or underutilised? Microorganisms. 11(4):835. https://doi.org/10.3390/microorganisms11040835.
Walters KJ, Behe BK, Currey CJ, Lopez RG. 2020. Historical, current, and future perspectives for controlled environment hydroponic food crop production in the United States. HortScience. 55(6):758–767. https://doi.org/10.21273/HORTSCI14901-20.
Wang W, Shi J, Xie Q, Jiang Y, Yu N, Wang E. 2017. Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. Mol Plant. 10(9):1147–1158. https://doi.org/10.1016/j.molp.2017.07.012.
Whipker BE, Cockson P, Henry J, Owen WG. 2018. Nutritional monitoring series: Tomato transplants (*Solanum lycopersicum*). https://urbanagnews.com/wp-content/uploads/2018/05/Nutritional-Factsheet-Tomato-Transplants.pdf. [accessed 19 Jan 2024].
Yang H, Zang Y, Yuan Y, Tang J, Chen X. 2012. Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: Evidence from ITS rDNA sequence metadata. BMC Evol Biol. 12(1):50. https://doi.org/10.1186/1471-2148-12-50.
Zeffa DM, Perini LJ, Silva MB, de Sousa NV, Scapim CA, de Oliveira ALM, do Amaral Júnior AT, Azeredo Gonçalves LS. 2019. *Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes. PLoS One. 14(4):e0215332. https://doi.org/10.1371/journal.pone.0215332.