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Pilot Study of Eco-Physiological Pepper Responses to Starfish-Based Organic Soil Amendments in Open-Field and Greenhouse Cultivations

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Abstract: This pilot study was conducted to compare eco-physiological responses to starfish (SF)-treated red peppers (*Capsicum annuum* L.) in organic open-field (OF) and greenhouse (GH) cultivations in commercial farmhouses, South Korea in 2019. Treatments included starfish-liquid fertilizer (LF) applied in OF (SF-OF) and GH (SF-GH) plots, and SF + seaweed in OF (SFS-OF) and GH (SFS-GH). Weekly pH levels in SFS-LF were fluctuated for a 16-week storage period at room temperature due to having higher soluble salt levels than those of SF-LF. All experimental plots were ranged on soil pH between 7.1 and 7.4. SF- and SFS-GH plots resulted in increased soil electrical conductivity, organic matter, and increased concentrations of total nitrogen, phosphorous pentoxide, potassium oxide, and magnesium oxide, as well as exhibiting a richer and more diverse bacterial community. Leaf width and length and plant height increased in plants cultivated in GH, with low canopy width and stem diameter also observed to have increased. Total fruit yields were approximately two times higher for peppers cultivated in GH compared to OF cultivated peppers.



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Keywords: liquid fertilizer; microbial biomass; nutrient; pepper; starfish

1. Introduction

Red peppers (*Capsicum annuum* L.) are an essential seasoning for all South Korean dishes and accounted for approximately 20% of the production volume and cultivation areas of vegetable commodities in 2017 [1]. However, net income rates have constantly been decreasing year on year, from 84.9% in 1980 to 66.7% in 2015, given the approximate 10 times increase in operating costs for greenhouses (GH) and global climate change affecting open-field (OF) cultivation in South Korea, having a humid, warm, East Asian monsoon climate and moderately acidic soil. Organic pepper production is an alternative way to achieve high nutritive fruit yield and fruit security without the use of synthetic inorganic fertilizers and pesticides [2]. This has provided higher premium prices and contributed to its expansion in developing countries through the use of recycled natural resources [3].

Organic peppers are cultivated in OF over a long season from spring to fall and in GH during the winter to produce year-round availability of fresh fruit, requiring additional fertilization [4]. A variety of natural sources extracted from plant and animal wastes have been extensively used on organic arable lands for additional fertilization, increasing nutrient availability and complexity of the farmland soil fauna community [5–8]. Populations of starfish (SF) have rapidly increased in recent years from rising temperatures in coastal areas of S. Korea and have been increasingly employed as biological fertilizer approved for use on organic fruit and vegetable farms in S. Korea, with little verification from the literature cited for the ecological sustainability of the practice of employing SF in OF and GH cultivations.

Soil organic matter (OM) and electrical conductivity (EC) were the key edaphic factors affecting soil bacterial ecology in GH cultivation and driving management strategies to improve bacterial diversity and fruit production [9]. Soil bacterial communities have a

direct role in mineralization and microbial biomass turnover in the plant rhizosphere, shifting the cycling of nutrient availability and OM decomposition [10,11]. SF showed high total nitrogen (T-N) concentrations, but mineralization rates and amounts of nutrients released depended on the microbial biomass and micro-environments, which varied across OF and GH cultivations [12]. The hypothesis of this pilot study was that different SF sources would have affected eco-physiological pepper responses in OF and GH systems, which was compared for mineral nutrient concentrations, soil bacterial community, and fruit productivity of red peppers.

2. Materials and Methods

2.1. Experiment Layout

The pilot study consisted of two set trials, the pre-experiment and the main experiment. The pre-experiment was conducted at a university GH facility under controlled climate conditions to investigate the effects of SF and SFS on pepper performance.

2.2. Treatment in Pre-Experiment

The pre-experiment was initiated to investigate the effects of organic nutrient solutions of SF and SF + seaweed (SFS) on the performance of pepper plants under controlled conditions. The central hypothesis was that nutrient release from the SF and SFS, and their effects on pepper performance would be different from each other under controlled conditions. ‘Shinhong’ red pepper seedlings, typically cultivated in South Korea, were grown in a commercial potting substrate of 10 × 30 cm density and treated with SF-LF and SFS-LF in a university GH facility for 60 days from May to July, maintaining a daily temperature between 15 and 25 °C. Root and shoot length and stem diameter on each treated plant were respectively measured with a measurer and digital Vernier calipers. Leaf chlorophyll content in the broad spread of leaves was colorimetrically estimated using a hand-held SPAD-502 m (Minolta Co., Tokyo, Japan). Root and shoot dry weights (DW) were measured with an electronic scale, calculating the shoot:root ratio. The number of fruit was counted on each plant at harvest.

2.3. Treatment in the Main Experiment

The main experiment was conducted with red peppers cultivated under OF and plastic GH systems in organically certified private farmhouses, Taean-gun, South Korea (37° N, 126° E) in 2019. The regional climate resulted in a higher average daily temperature of 19.5 °C and a lower amount of accumulated precipitation of 678.0 mm from April to October compared to those of the last 30 years from 1981 to 2010 with the annual average of 18.9 °C and 1097.7 mm [13]. Manure compost and oil cake were applied along with the annual amount of nutrients recommended for the growth of pepper plants at basal fertilization in both the OF and GH systems [1]. Black woven plastic mulch covered the peppers to prevent invasions of weed and insect populations in both systems. ‘Wonderful Guy’ pepper plants in OF (typically cultivated in OF) and ‘Super Bigarim’ in GH (typically cultivated in GH) were planted at a density of 40 cm between the plants and a density of 135 cm between plant rows on 23 February 2019. The pepper fruits were then harvested from July to October in OF and from July to December in GH.

Treatment included SF applied in OF (SF-OF) and GH (SF-GH) plots and SFS applied in the OF (SFS-OF) and GH (SFS-GH). SF-LF was manufactured as 50.4% SF, 3.4% molasses, 0.2% effective microorganism (EM), 0.8% humus, and 45.2% water for 3-year fermentation, with SFS-LF being composed of 40.3% SF, 10.1% seaweed, 3.4% molasses, 0.2% EM, 0.8% humus, and 45.2% water. One liter of LF per plant was applied weekly from 30 days after planting up to harvest with 250 times dilution, with foliar applied at 500 times dilution in turn, which was approximately 1.0 mg of T-N received for each plant. Drip irrigation was turned on in OF and GH plots when precipitation was not received on the soil surface for three consecutive days.

2.4. Nutrient Analysis in the Main Experiment

Essential mineral nutrients in the LF were analyzed according to Rural Development Administration (RDA) protocols of soil and plant analysis [14], including weekly pH and EC measurements during 14-week storage at room temperature using a pH meter (FIVEEAST FE20, Mettler Tonedo Co., Suzhou, China) and an EC meter (HI 2315 Conductivity Meter, Hanna Co., Seoul, Korea), respectively. T-N concentration in the LF was determined using a CN analyzer (Vario MAX CN, Elementar Co., Langenselbold, Germany), with an analysis of P concentration using a UV-visible spectrophotometer (UV-2450, Shimadzu, Tokyo, Japan), and exchangeable cations with an ICP-AES (Simultaneous ICP Spectrometer, SPECTRO Analytical Instruments GmbH Co., Kleve, Germany).

Soil samples were taken at a depth of 0 to 20 cm in June for soil nutrient analysis [14], and soil OM contents were estimated through oxidization via the Tyurin method [14]. Soil T-N and available P₂O₅ concentrations were analyzed with a CN analyzer (Vario MAX CN, Elementar Co., Langenselbold, Germany) and a spectrophotometer (UV-2450, Shimadzu, Tokyo, Japan) respectively, with analysis of exchangeable cations performed using an ICP-AES (Simultaneous ICP Spectrometer, SPECTRO Analytical Instruments GmbH Co., Kleve, Germany), including other essential micro-nutrients. Pepper leaves were randomly sampled in June, their leaf width and length measured, then air-dried in a dry-oven at 65 °C for 7 days and ground in a blender with four mill blades (WDL-1, Wonder blender Co., Tokyo, Japan). The ground leaves were used to analyze T-N concentration using the Kjeldahl method, with P analysis using the Vanadate method, and K, Ca, and Mg analyzed using an ICP-AES (Simultaneous ICP Spectrometer, SPECTRO Analytical Instruments GmbH Co., Kleve, Germany).

2.5. Bacterial Analysis in the Main Experiment

Soil at depths of 0 to 20 cm were sampled in June, which was then used to extract bacterial DNA using FastDNA[®] Spin Kit (MP Biomedicals, Seoul, Korea) at an analysis center (Chunlab, Inc., Seoul, Korea) based on the manufacturer's instructions. The V3 hypervariable region of 16S rRNA was amplified by PCR with barcoded fusion primers. The sequence data of bacterial 16S rRNA gene fragments were used to describe ACE, Chao1, operational taxonomic units (OTUs), and abundance of dominant bacterial phyla using the Mothur software package [15].

2.6. Pepper Measurement in the Main Experiment

A SPAD-502 portable chlorophyll meter (Minolta, Tokyo, Japan) and fluorometer (FluorPen P100, Photon System Inc., Drasov, Czech Republic) were used to determine the relative concentrations of chlorophyll and PS II activity, respectively, in leaves, in June. Canopy width, stem diameter, and plant height were then measured to assess plant growth among treated individuals. Total fruit yield per ha indicated the sum of accumulated fresh harvested fruit weight.

2.7. Data Analysis

Each treatment in each experiment was composed of three replications. Each dependent variable in this experiment was analyzed statistically using the general linear model procedure (PROC GLM) of SAS (Version 8.02; SAS Institute, Cary, NC, USA) to analyze the significant differences in all responses. Means were separated using a least significant differences test at $p < 0.05$. Data over time are shown as means \pm standard errors.

3. Results and Discussion

3.1. Pre-Experiment

Weekly pH increased in both LF treatments for the first 5 weeks of room temperature storage but rapidly decreased in the SFS-LF-treated plots between 5 and 8 weeks of storage (Figure 1A). High organic compounds in the SFS-LF plots would have decomposed into small molecules, generating organic acid, nitrification processes, and hydrogen ions [16] as

shown by high soluble salts contained in the SFS-LF as a marine sourced fertilizer compared to those of SF-LF (Figure 1B). Concentrations of T-N, P, K, Mg, and Cu were similar in both LF, with high concentrations of Fe, Mn, Zn, and B observed in the SF-LF (Table 1). The concentrations of Ca and Na were much higher in the SFS-LF than those of SF-LF, attributed to it containing 10% of seaweed ingredients with high Ca and Na [17]. SFS-LF increased shoot length, total length, and shoot DW of the pepper seedlings compared to those of SF-LF (Table 2), which would have been influenced by high plant growth stimulants and polysaccharides supplied from seaweed extracts in the SFS-LF [8,17–19].

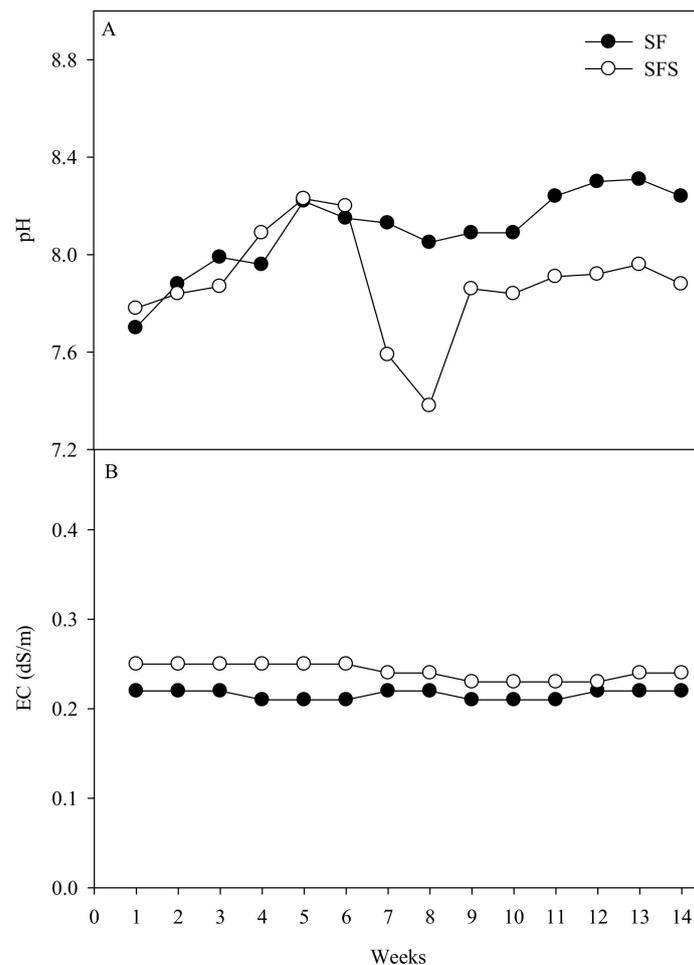


Figure 1. Weekly pH (Panel (A)) and EC (Panel (B)) in starfish (SF)- and starfish + seaweed (SFS)-liquid fertilizers at weeks of storage.

Table 1. Nutrient concentrations in liquid fertilizers with starfish (SF)- and starfish + seaweed (SFS) resources used in an experimental plot.

Treatment	T-N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B	Na
	(mg L ⁻¹)										
SF	20	70	600	250	30	3.9	0.08	0.77	0.35	8.0	0.002
SFS	20	70	610	380	30	2.6	0.06	0.49	0.35	7.4	568
Significance	ns	ns	ns	*	ns	*	*	*	ns	*	***

ns, not significantly different. *, *** Significantly different mean values (n = 3 liquid fertilizers) between treatments for each system at $p \leq 0.05$ and $p \leq 0.001$.

Table 2. Growth of red pepper seedlings as affected by starfish (SF)- and starfish + seaweed (SFS)-liquid fertilizers.

Treatment	Shoot Length	Root Length	Total Length	Stem Diameter	Shoot (S) DW	Root (R) DW	S:R Ratio	SPAD	No. Fruit
	(cm)				(g)				
SF	79.8	24.8	105	0.6	6.6	1.2	5.5	33.2	2.0
SFS	88.5	24.0	113	0.6	8.1	1.2	6.6	34.8	1.8
Significance	*	ns	*	ns	*	ns	ns	ns	ns

ns, not significantly different. * Significantly different mean values (n = 3 plants) between treatments for each system at $p \leq 0.05$.

3.2. Nutrients and Bacterial Community in the Main Experiment

Soil types in the SF-OF, SF-GH, SFS-OF, and SFS-GH plots were composed of sandy loam (sand 63.6%, silt 32.4%, and clay 4.0%), loam (sand 49.5%, silt 35.6%, and clay 14.9%), silt loam (sand 32.1%, silt 50.9%, and clay 17.0%), and silt loam (sand 21.6%, silt 67.0%, and clay 11.4%), respectively (data not presented). GH plots contained a lesser portion of sand particles than those OF plots, probably affecting salt accumulation and poor water and air circulation [11,20].

All treated plots were ranged on soil with a pH between 7.1 and 7.4 (Table 3), indicating moderately high pH levels for optimum growth of pepper plants [21]. Soil EC was increased to 3.7 dS m⁻¹ of SFS-GH plots, followed by SF-GH, SFS-OF, and SF-OF. Soil OM and concentrations of T-N, P₂O₅, K₂O, and MgO were significantly higher in the GH plots, in particular on the SFS-GH than those values observed in OF plots. The concentration of P₂O₅ was increased to 1301 mg kg⁻¹ in the SFS-GH plots and 768 mg kg⁻¹ in the SF-GH plots. Continuous application of organic fertilizer would have contributed to high OM content and phosphate salt by reducing nutrient leaching and evaporation under the rooftop covering in the GH [11,20]. Exchangeable Ca concentration in the SFS plots was attributed to high Ca supplement in the SFS [8,17–19].

Table 3. Soil mineral nutrients as affected by starfish (SF)- and starfish + seaweed (SFS)-liquid fertilizers applied in open-field (OF) and greenhouse (GH) cultivated red peppers.

Treatment	pH	EC (dS m ⁻¹)	OM	T-N	P	K	Ca	Mg
			(mg kg ⁻¹)			(cmolc kg ⁻¹)		
SF-OF	7.3	0.9	14.1	8000	108	117	680	156
SF-GH	7.2	2.1	27.1	1600	768	704	1160	288
Significance	ns	ns	*	*	*	*	ns	ns
SFS-OF	7.4	1.1	19.5	1100	545	782	2540	276
SFS-GH	7.1	3.7	41.9	2400	1301	1369	2400	696
Significance	ns	*	*	*	*	*	ns	*
Fertilizer significance	ns	ns	ns	ns	*	ns	***	ns
Desired level	6.0–7.0	0.5–1.5	20.0–30.0	–	400–500	274–313	1000–1200	180–240

ns, not significantly different. *, *** Significantly different mean values (n = 3 soils) between treatments for each system at $p \leq 0.05$ and 0.01, respectively. The desired levels were adopted from RDA (2010).

SF-OF plots showed decreasing numbers of OTUs and a lower richness estimate and diversity index with respect to the bacterial community compared to those of SF-GH plots (Table 4). The bacterial community was not significantly different between the SFS-OF and SFS-GH plots. GH plots were found to show high ACE (Figure 2A), Chao1 (Figure 2B), and the number of OTUs (Figure 2C) in the bacterial community compared to OF plots, indicating greater bacterial richness and diversity. EC levels mostly affected bacterial community structure, important in nutrient cycling and mineralization, which can be a primary estimator of the bacterial activity in the soil [9,22,23]. The phyla Planctomycetes and Firmicutes are favorable to high EC in soils with warm and humid conditions [9] and were present in considerable numbers in the GH plots applied with SF and SFS (Figure 3). The phylum Firmicutes mainly contains two classes of Clostridia and Bacillus capable of degrading large organic molecules to small molecules, converting N₂ into ammonia,

and stimulating nitrification and denitrification as part of the N-cycle [20]. Acidobacteria are known for a weak tolerance of salt accumulation in the soil [9,20], which was not consistently observed in the OF and GH plots below saline soils of 4.0 dS m^{-1} [23,24].

Table 4. Soil bacterial community as affected by starfish (SF)- and starfish + seaweed (SFS)-liquid fertilizers applied in open-field (OF) and greenhouse (GH) cultivated red peppers.

LF	Number of OTUs	Good's Coverage	Richness Estimator (Chao1)	Diversity Index	
				Shannon	Inverse Simpson
SF-OF	2132	95.7	2445	6.4	0.012
SF-GH	3126	98.0	3265	6.9	0.003
Significance	*	*	*	*	*
SFS-OF	2564	97.6	2722	6.8	0.004
SFS-GH	2589	97.1	2746	6.9	0.003
Significance	ns	ns	ns	ns	ns
Fertilizer significance	ns	ns	ns	ns	ns

ns, not significantly different. * Significantly different mean values ($n = 3$ soils) between treatments for each system at $p \leq 0.05$.

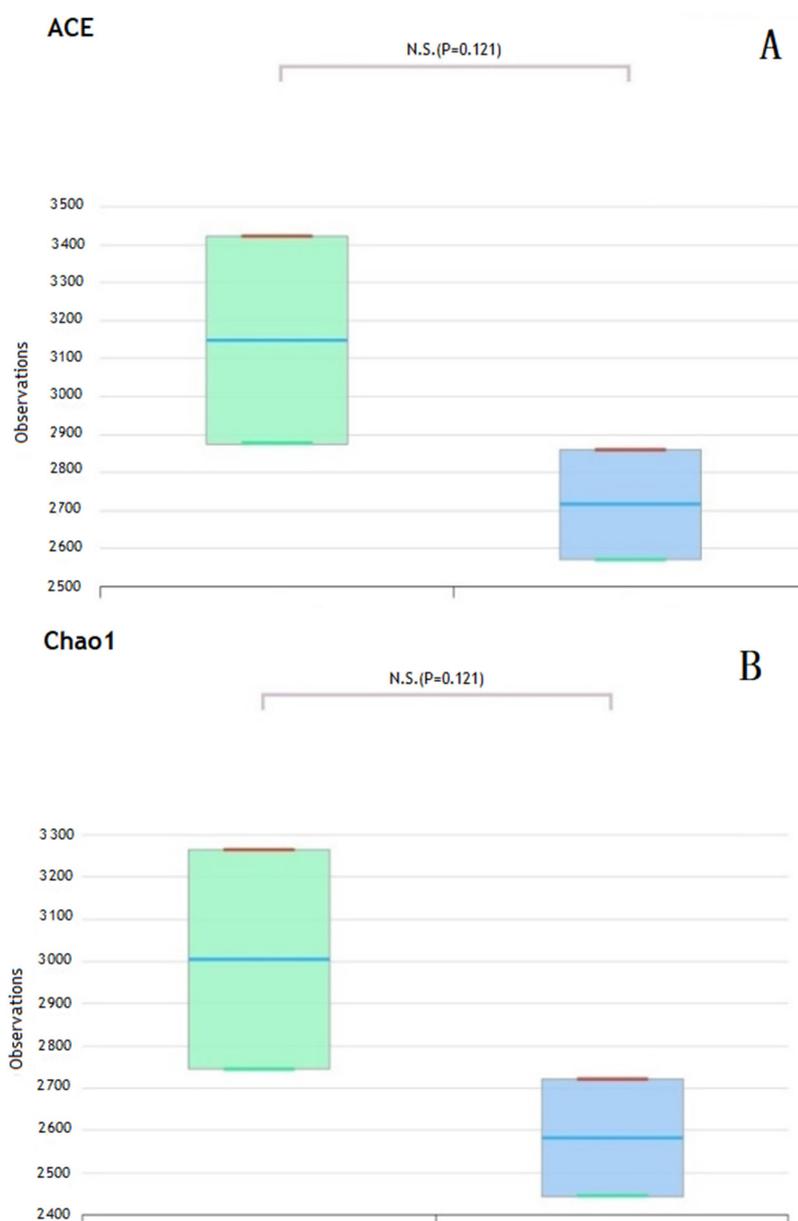


Figure 2. Cont.

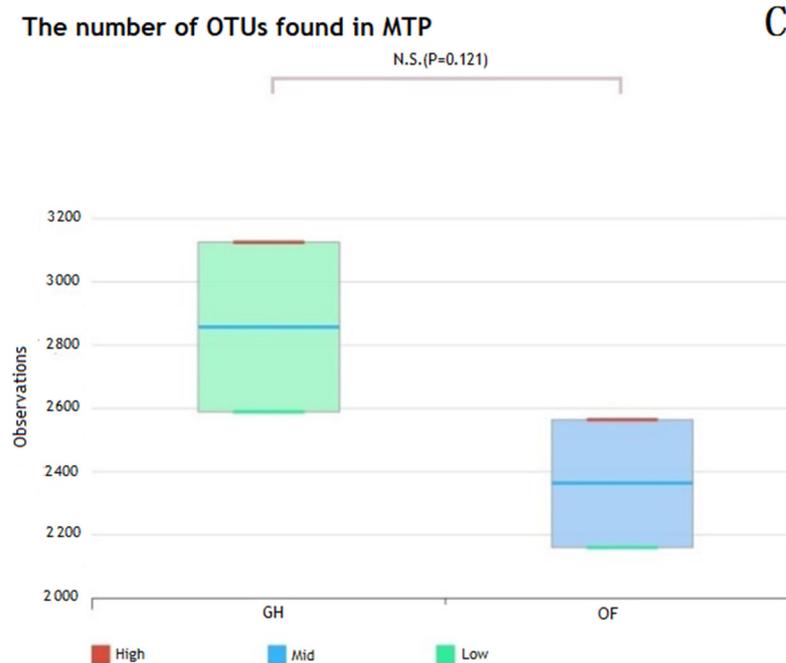


Figure 2. Bacterial diversity of ACE (Panel (A)) and Chao1 (Panel (B)) and number of operational taxonomic units (OTUs; Panel (C)) present in the soil in June as affected by open-field (OF) and greenhouse (GH) cultivated red peppers. Bars represent the standard error of the mean.

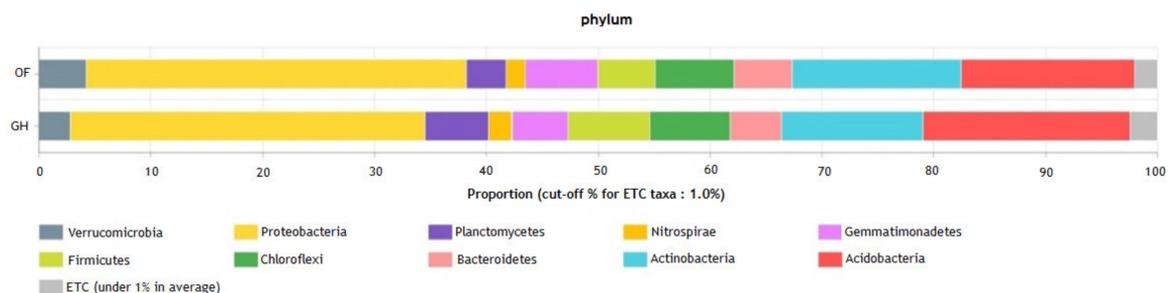


Figure 3. Relative abundance of the dominant bacterial phyla in soil on June as affected by open-field (OF) and greenhouse (GH) cultivated red peppers.

Foliar concentrations of macronutrients, except for Mg, were not significantly different for plants grown in OF and GH plots, although OF plots showed reduced soil nutrients (Table 5). Concentrations of P, Zn, Cu, and B tended to increase for plants cultivated in the OF plots. This would have been caused by the low transport of soil mineral nutrients into the plants under conditions of high relative humidity and salt accumulation in GH plots. Mineral nutrients in all the treated plants overall ranged between desired levels, except for P and Mn, which, for sufficient growth of peppers [21], were increased to ameliorate concentrations of EC, OM, and other macronutrients of the soil. Excessive Mn uptake in plants is mostly associated with high Mn solubility occurring in low soil pH or with the application of acid-forming fertilizers [24,25]. Our experiment plots showed moderately high soil pH and were fertilized with alkaline-forming SF-LF and SFS-LF to avoid excessive Mn concentration in the plants, though this could have been induced through the monthly foliar application of the LF once every two weeks during the growing season. SFS-LF treatment significantly increased soil Ca concentration as supplementing with seaweed ingredients.

Table 5. Plant mineral nutrients as affected by starfish (SF)- and starfish + seaweed (SFS)-liquid fertilizers applied in open-field (OF) and greenhouse (GH) cultivated red peppers.

Treatment	T-N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
	(mg kg ⁻¹)									
SF-OF	43,000	500	50,000	31,000	6000	201	1948	225	33	186
SF-GH	43,000	400	50,000	34,000	9000	223	2108	62	21	125
Significance	ns	ns	ns	ns	*	ns	ns	*	*	*
SFS-OF	47,000	400	60,000	34,000	6000	147	1523	56	17	274
SFS-GH	47,000	300	55,000	37,000	11,000	224	2446	47	9	252
Significance	ns	ns	ns	ns	*	*	*	ns	*	ns
Fertilizer significance	***	ns	**	*	ns	ns	ns	ns	ns	**
Desired level	21,000–54,000	300–400	44,000–56,000	11,000–27,000	3000–9000	55–174	16–53	21–74	–	17–68

ns, not significantly different. *, **, *** Significantly different mean values (n = 3 plants) between treatments for each system at $p \leq 0.05$, 0.01, and 0.001. The desired levels were adopted from RDA (2010).

3.3. Plant Growth in Main Experiment

Leaf width and length were extended in plants cultivated under GH compared to those in OF (Table 6). However, canopy width and stem diameter were smaller in the GH plants, probably due to the elongation of plant height induced from apical dominance and fruit yield from increased foliar concentrations of Mg, Zn, Cu, and B. SPAD values, non-destructive estimates of foliar T-N and chlorophyll contents, and PS II values increased in the SFS-plants compared to those of SF-plants (data not presented) with significant increases in foliar concentrations of T-N, K, Ca, and B, presumably caused by supplementing with seaweed ingredients [8,17–19].

Table 6. Vegetative growth as affected by starfish (SF)- and starfish + seaweed (SFS)-liquid fertilizers applied in open-field (OF) and greenhouse (GH) cultivated red peppers.

Treatment	Leaf (cm)		Canopy Width (cm)	Stem Diameter	Plant Height
	Width	Length			
SF-OF	4.8	9.4	80.8	2.2	120
SF-GH	5.5	11.8	70.0	1.7	160
Significance	*	*	*	*	*
SFS-OF	4.5	9.5	85.8	2.3	140
SFS-GH	5.3	11.3	71.3	1.7	158
Significance	*	*	*	*	*
Fertilizer significance	ns	ns	ns	ns	ns

* Significantly different mean values (n = 3 plants) between treatments for each system at $p \leq 0.05$.

Total fruit yield increased approximately two times for peppers cultivated in GH compared with that of OF peppers (Figure 4), probably due to extended harvest duration, higher soil fertility, reduced water loss, and controlled external environmental factors in GH plots [12,26–28]. Pepper yields of various varieties increased from 50% to 250% in GH plots with protected cultivation compared to those of OF, as shown in other pepper studies [12,26]. OF plots experienced abrupt, climate change-induced, warmer and drier weather events during the growing season more frequently than those of the last 30-years [13,28], further lowering fruit productivity, regardless of LF nutrition sources.

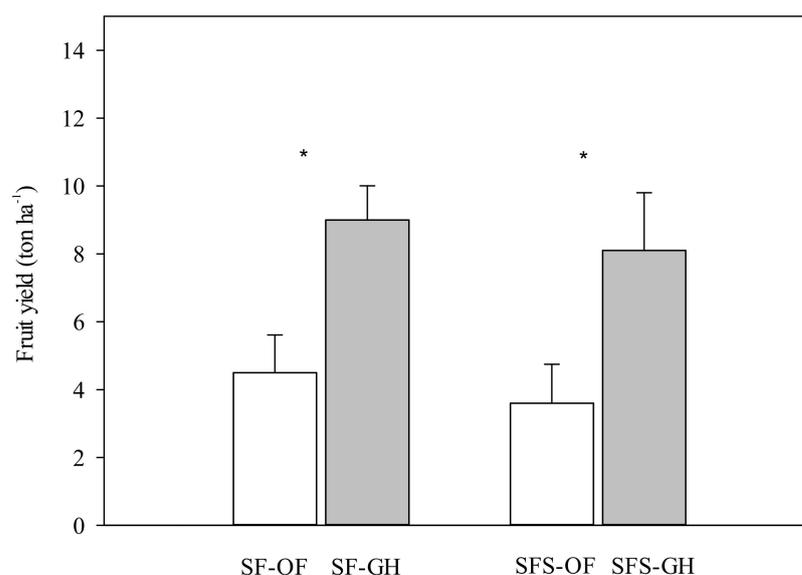


Figure 4. Fruit yield as affected by starfish (SF)- and starfish + seaweed (SFS)-liquid fertilizers applied in open-field (OF) and greenhouse (GH) cultivated red peppers. Bars represent the error of the mean. * significantly different mean values (n = 3 plants) between treatments for each system at $p \leq 0.05$.

4. Conclusions

The eco-physiological pepper responses to SF and SFS were not overall significantly different in OF and GH plots, although SFS fertilizers increased some mineral nutrition in soil and plants. Moderately high soil EC and OM levels in the GH plots, regardless of SF and SFS, would have improved the composition of their soil bacterial community and improved nutrient cycling and ecological stability, largely enhancing fruit productivity. Although the use of starfish as raw material in the production of liquid fertilizers will be subject to government regulations in some countries, the recycling of marine by-products for SF or SFS for use as fertilizer would be recommended to organic farmers in developing countries. However, the pilot study might not be sounded due to the low sample size and absence of control plots, although the pre-experiment was initiated to investigate the effects of organic nutrient solutions under controlled conditions. The effects of SF sourcing and cultivation method on ecological stability and sustainable fruit production should be further studied in the long term to further understand the interaction effects between the external factors, including light, temperature, and water, and the internal factors of nutrient cycling and soil fauna.

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Conflicts of Interest: The author declares no conflict of interest.

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