Evaluating desiccated cyanobacteria *Aphanizomenon flos-aquae* for use as a biofertilizer on Swiss Chard

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Abstract:

Non-toxic species of desiccated blue-green algae, or cyanobacteria, may offer potential for use as a fertilizer on irrigated crops. Here, the non-toxic cyanobacteria species *Aphanizomenon flos-aquae* (AFA), collected from Upper Klamath Lake, Oregon, was evaluated for use as a biofertilizer in a controlled experiment on Swiss chard "Bright Lights" (*Beta vulgaris* L. ssp. *cicla* L.). Two comparison groups were used in the study: group 1, fertilized with a commercially available synthetic fertilizer, and group 2, an unfertilized control. All plants were grown from seeds. With the exception of average root growth, the results found that both the synthetically fertilized and algae fertilized plants produced significantly higher growth rates than the unfertilized control group. These findings are consistent with other studies showing positive outcomes using live cyanobacteria in various cropping systems.

Introduction

The frequency, size and duration of freshwater algal blooms, both toxic and non-toxic, has been steadily rising in the U.S. and globally since 1985 (Ho et al. 2019). This increase has been generally correlated to anthropogenic drivers, such as the broad use of nitrogen-based fertilizers, alterations to terrestrial surface hydrology, rises in atmospheric carbon dioxide levels, and rising global temperatures, all of which contribute to conditions favorable for cyanobacterial algae growth (Hudnell et al. 2008; Griffith and Gobler 2019; NOAA 2019). It's expected that the frequency and intensity of freshwater cyanobacterial algae blooms will continue to increase as environmental conditions progressively favor their growth (Elliott 2012).

Non-toxin producing cyanobacterial strains may not be a direct hazard to humans and animals, but the large amount of biomass they produce can lead to significant issues for both humans and aquatic organisms. For example, private and municipal water systems sourced from algae-laden waters incur increased costs associated with monitoring and removing algae growth (EPA 2015). Other economic costs include lost revenues to tourism, recreation and commercial fishing, and reduced property values for home owners. Ecological impacts occur when algal blooms form surface mats that prevent oxygen and sunlight from penetrating into the water (Scheffer et al. 2003). As algae die off, they provide food for water-borne aerobic bacteria, which can rapidly grow in population size. Large increases in aerobic bacteria populations deplete dissolved oxygen supplies in the water, leading to hypoxic conditions (Ameida 2016; USGS [no date]). If oxygen levels fall too low, the mortality of fish and other aquatic organisms is likely to increase (Kraus et al. 2015).

While the management of toxic algal bloom biomass is particularly challenging, non-toxic biomass may provide unique opportunities that have yet to be explored. The symbiotic relationship between live diazotrophic (nitrogen-fixing) microorganisms and plants has been well established (Baldani et al. 1983; Vessey 2003; Dobbelaere et al. 2010). For example, the use of rhizobacterial "inoculants" on crop seeds is a common

practice (Vessey 2003; O'Callaghan 2016), and studies using live cyanobacterial biofertilizers on rice (*Oryza sativa* L.) and corn (*Zea mays* L.) have reported positive results (Whitton 2000; Maqubela et al. 2010; Prasanna et al. 2011; Jochum et al. 2018). However, few experimental studies have been conducted to evaluate the efficacy of dried diazotrophic cyanobacterial biomass as a topical or sub-surface biofertilizer on plants.

The purpose of this study was to evaluate the effectiveness of a desiccated non-toxic algae derived from the diazotrophic cyanobacteria *Aphanizomenon flos-aquae* (AFA) retrieved from Upper Klamath Lake, Oregon, for use as a topical biofertilizer on irrigated crop plants. Although the Earth's atmosphere is comprised of 78% elemental nitrogen (N_2) (Leslie and Bateman 2016), its chemical structure makes this form of nitrogen unusable to plants (Brady and Weil 2010). Microorganisms such as cyanobacteria have the ability to convert this "non-reactive" nitrogen to plant-usable reactive ammonium (NH_4^+) and nitrate (NO_3^-) ions (Fields 2004; Rossi et al. 2015; Singh 2016). Cyanobacteria are also photoautotrophs, producing their own carbohydrates and obtaining much of their carbon through photosynthesis. It's estimated they can assimilate ("fix") carbon dioxide (CO_2) at rates up to 50 times faster than plants, when grown under ideal conditions (Rossi et al. 2015). This suggests that cyanobacteria could play a role in lowering atmospheric CO_2 levels if produced at sufficient scales (Yuan et al. 2012). Thus, in addition to their ability to increase soil nitrogen levels, cyanobacteria can potentially help offset CO_2 emissions through carbon capture and fixation.

Methods & Materials

Cyanobacteria collection

AFA was collected from the east side of Shoalwater Bay in Upper Klamath Lake, Oregon from July 8th through the 10th, 2019. A Sabuy™ 18 inch fine mesh pool-cleaning net was used to collect the algae, which was then spread on screens and plastic tarps to sun dry. Minimum drying time was 24 hours with average ambient air temperatures between 13 °C (night) and 29 °C (day). Approximately 23 liters of wet algal mass was collected, yielding 2.5 kg of dry product. During collection it was noted that the algae included a variety of insectoid organisms (species unknown), making up an estimated 10% of the total wet volume. The dried algae was then stored in standard 1-gallon resealable plastic bags, with as much air removed as possible to avoid rehydration. No desiccant was used. The bags were placed in a large plastic container for transport and longer-term storage. Laboratory analysis of the algae via combustion determined it contained 10.8% nitrogen. For nitrogen determination, triplicate samples were combusted with an Exeter Analytical CE-440 Elemental Analyzer located in the Botany and Plant Pathology Department at Oregon State University. The instrument was calibrated with acetanilide standards.

Soil and container preparation and planting

On advice from U.C. Master Gardener Steven Darington (personal communication, August 27, 2019), a custom potting soil was developed, consisting of: 1-part Miracle-Gro™ Nature's Care Organic Garden Soil, 1-part Sunshine™ Sphagnum Peat Moss and 1-part #20 kiln dried crystal quartz sand. One hundred twenty mL of Gardner & Bloome™ soft rock phosphate was added to every 56.8 L of previously mixed material. A total of eighty-four 2548.3 cm^3 ("1-gallon") containers were prepared as follows: 10 cm of custom soil mix on top of 4 cm dry leaf litter (Figure 1). A 2 cm reservoir was left at the top of each container for irrigation. Organic Swiss chard "Bright Lights" (*Beta vulgaris* L. ssp. *cicla* L.), produced and sold by Seeds of Change™ were used as the experimental plant. Three seeds were equidistantly placed in each container in a radius of 5 to 6 cm and covered with ~1 cm soil.

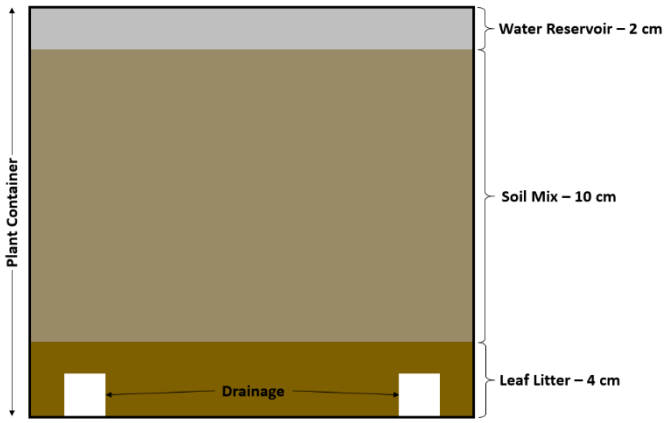

Figure 1. Plant containers each contained a bottom layer of ~4 cm dry leaf litter topped with ~10 cm custom soil mix. The presence of the leaf litter allowed for sufficient drainage with limited soil loss.

Treatment groups and fertilizer amounts

Three groups of 28 containers each were used: 1) Synthetically fertilized group (SG), 2) Algae fertilized group (AG), and 3) A control group (CG) – no fertilizer treatment. Each container in the SG received 1.68 g of Osmocote™ Flower and Vegetable time-released plant fertilizer. Product labeling indicated the fertilizer contained 14% total nitrogen, 14% phosphate and 14% soluble potash (K_2O); however, labeling also noted that only 12% *usable nitrogen* was available for plant growth due to the slow-release nature of the product. Each container in the AG received 1.86 g of dried algae (10.8% N). Both treatments were applied to the soil surface after seeding. The control group was left unfertilized. Fertilizer application amounts were calculated based on the total surface area of each container (201 cm^2) and a N concentration of 10^{-3} g N cm^{-2}:

$$\text{Synthetic: } \frac{(201\ \text{cm}^2\ X\ 10^{-3}\ \text{g N cm}^{-2})}{0.120\ \text{N}} = 1.68\ \text{g per container}$$

$$\text{Algae: } \frac{(201\ \text{cm}^2\ X\ 10^{-3}\ \text{g N cm}^{-2})}{0.108\ \text{N}} = 1.86\ \text{g per container}$$

Growth location and container layout

The plants were grown in a residential area of Morgan Hill, California. Due to lighting constraints, the containers were placed on 2 movable platforms (A and B) that allowed for ease of movement to maintain full

sun exposure throughout the day. Lightweight screening was placed 0.6 m above each platform for shade, and both platforms were surrounded by shade cloth to reduce temperatures and to protect the plants from fauna (previous to side-netting installation container A-12C, was destroyed by wildlife). A one, two or three was assigned to each treatment type, and then a numerical randomizer was used to determine the order in which the treated containers would be placed on each platform. Once a randomized order between the three treatment groups was established it was maintained across each platform (Figure 2). Each container was physically numbered and the layout was documented for tracking purposes. All containers were planted and arranged on the platforms on September 1, 2019.

PLATFORM: A

Left Side	37S	38C	39A	40S	41C	42A
	31C	32A	33S	34C	35A	36S
	25A	26S	27C	28A	29S	30C
	19S	20C	21A	22S	23C	24A
	13C	14A	15S	16C	17A	18S
	7A	8S	9C	10A	11S	12C
	1S	2C	3A	4S	5C	6A

Right Side

PLATFORM: B

	43A						
	36S	37A	38C	39S	40A	41C	42S
Right Side	29C	30S	31A	32C	33S	34A	35C
	22A	23C	24S	25A	26C	27S	28A
	15S	16A	17C	18S	19A	20C	21S
	8C	9S	10A	11C	12S	13A	14C
	1A	2C	3S	4A	5C	6S	7A

Left Side

Figure 2. The first 3 container treatment sequences were randomized, with the established pattern following through the remainder of the layout. Treatment types are: S = Synthetic fertilizer treatment; C = Control (no fertilizer treatment); A = Algae biofertilizer treatment.

Temperature and irrigation

During the plant growth period, average high and low temperatures were 27°C (81°F) and 10°C (50°F), respectively. High temperatures reached 35°C (95°F) or higher on five occasions, and 32°C (90°F) or higher on 14 occasions (Figure 3). A *"luster leaf™" rapi-test digital moisture meter* was used to monitor daily soil moisture levels of random containers on each platform. Readings below 4 (on a scale of 0 – 9) indicated irrigation was required. The average daily irrigation amount was 188 mL, with a maximum of 500 mL on excessive heat days (>35°C) and a minimum of 150 mL on cool days (<21°C). An additional shade cloth was placed over the containers on days where the temperature exceeded 30°C. There was no measurable precipitation during the plant growth period. Most seeds germinated by day 7, and all containers had at least 1 sprout by day 10. Plants were allowed to grow for 62 days, at which point they were removed from their containers.

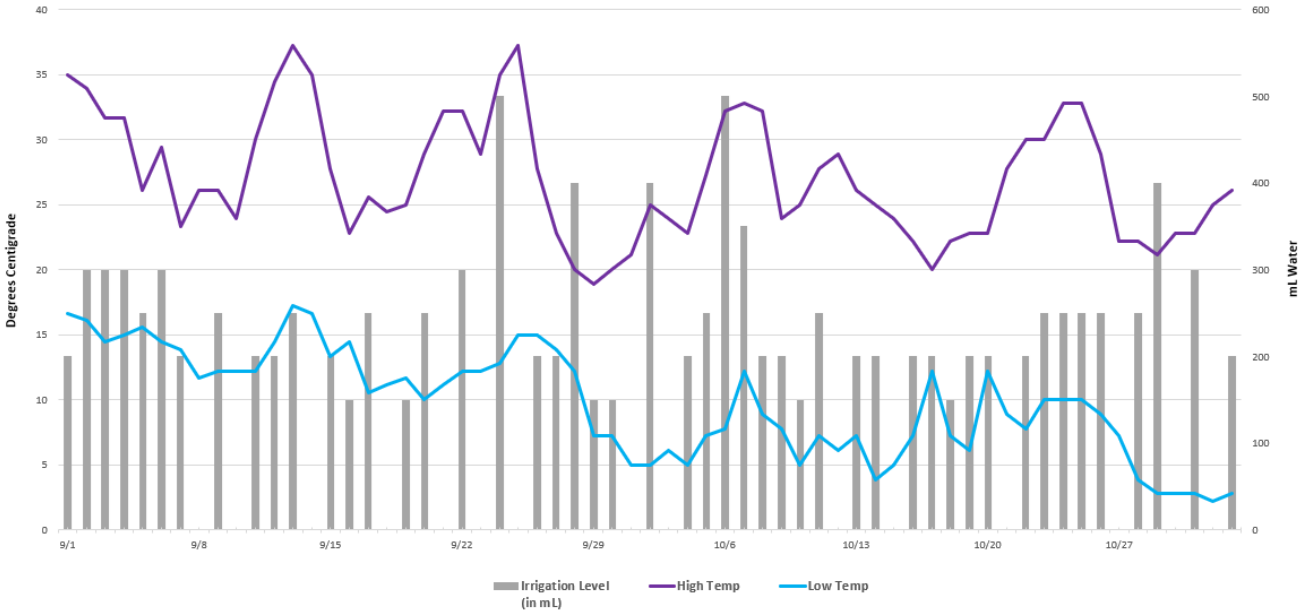

Figure 3. Daily water amounts are overlain on daily temperature fluctuations. A *"luster leaf™" rapi-test digital moisture meter* was used to monitor daily soil moisture levels of random containers on each platform. As can be seen, water needs generally increased with temperatures.

Plant growth data

On the 63rd day after planting, each plant was carefully removed from its container and thoroughly rinsed to remove as much soil and leaf litter as possible from the roots. Measurements were taken of the overall plant length, foliage length only and root length (Figure 4). The foliage was then separated from the root system at the base of the hypocotyl and each separately weighed. For dry weighing, the samples were placed in a standard kitchen oven at 77°C (170°F) for a minimum of 8 hours. Root samples maintained sufficient integrity to obtain accurate post-drying weight data; however, individual foliage samples did not remain intact, so only the total foliage dry weight by treatment type was recorded.

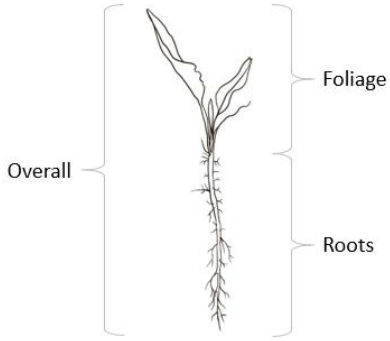

Figure 4. Plant length measurements

During harvest, it was found that many containers had grown multiple plants with the roots sufficiently entangled that attempting separation would have destroyed the sample. For example, container A-2C grew three intertwined plants, while container A-5C grew only a single plant. To generate comparative data, each weight measurement was divided by the total number of plants that grew in a given container. For example, the total wet root weight for the three plants grown in container A-2C was 43.0 g, which was then divided by 3 to yield an *average* wet root weight of 14.33 g for that container. The 14.33 g was then used for statistical analysis. Conversely, the total wet root weight for the single plant grown in container A-5C was 38.8 g, which

when divided by 1 resulted in an average wet root weight of 38.8 g for that container. This process was repeated for all containers and all weight measurements (wet foliage, wet root, dry foliage and dry root weights). Plant length measurements (foliage and roots) were simply taken of the longest plant in each container, regardless of the number of individual plants.

Statistics

R Studio (version 1.1.463 with the Mosaic package) was used for statistical analyses. A significance level of $\alpha \leq 0.05$ was used for all statistical tests and no data transformations were made. A z_α value of 1.96 was used for the 95% confidence intervals. Lower and upper boxplot error bars were calculated as the first quartile - 1.5 x the interquartile range (IQR), and the third quartile + 1.5 x IQR, respectively. If outliers were present then the error bars were adjusted up or down the closest adjacent values in the dataset. If no outliers were present the minimum and maximum values were used. With the exception of dry foliage weight (due to insufficient data), each of the plant measurements was evaluated using ANOVA (Analysis of Variance) for a difference in means. Both Tukey HSD and Holm adjusted pairwise t-tests were conducted on measurements with ANOVA results indicating a difference in means. The null and alternative hypotheses for the ANOVA tests were:

Null (H0): No difference between the means: H0: $\mu_1 = \mu_2 = \mu_3$,

Where:

μ_1 = The control group mean

μ_2 = The synthetically fertilized group mean

μ_3 = The algae fertilized group mean

Alternative (HA): At least one of the group means is different than the others.

The null and alternative hypotheses for the comparative pairwise t-test were:

Null (H0): No difference in the means between groups: H0: $\mu_{AG} = \mu_{SG} = \mu_{CG}$

Alternative Hypotheses (HA):

HA_1: A significant difference in means exists between the SG and CG: $\mu_{SG} \neq \mu_{CG}$

HA_2: A significant difference in means exists between the AG and CG: $\mu_{AG} \neq \mu_{CG}$

HA_3: A significant difference in means exists between the AG and SG: $\mu_{AG} \neq \mu_{SG}$

Results

With the exception of root length, all ANOVA tests resulted in significant findings (P-value ≤ 0.05) for a difference in means between at least two of the treatment types within each measurement group (Table 3). Holm adjusted and Tukey Honest Significant Difference (HSD) post-hoc comparative tests were run to

Table 3. F-Statistic and associated P-values for each measurement.

Measurement	$F_{(2,81)}$ Statistic	P Value
1. Foliage length	6.935	0.002
2. Root length	1.661	0.200
3. Average foliage wet weight	13.910	<0.001
4. Average root wet weight	8.074	<0.001
5. Average foliage dry weight	NA	NA
6. Average root dry weight	5.507	0.006

determine which group means were different from the others. In all cases, Holm adjusted and Tukey HSD pairwise tests resulted in the same conclusion; therefore only Tukey HSD test results are reported below. The means and standard deviations for foliage length between the control group (CG), synthetically fertilized group (SG) and the algae fertilized group (AG) were 23.1 cm ±4.0 cm, 26.4 cm ±4.9 cm and 27.1 cm ±3.8 cm, respectively. The 95% confidence intervals for the means were 23.1 cm ±1.5 cm, 26.4 cm ±1.8 cm, and 27.1 cm ±1.4 cm, respectively. Medians were 23.2 cm, 25.7 cm and 27.0 cm for the CG, SG and AG, respectively (Figure 5). Tukey HSD t-tests calculated P-values of 0.0148 for the SG vs. CG (significant), 0.0022 for the AG vs. CG (significant), and 0.7988 for the AG vs. SG (not significant). The means and standard deviations for root lengths for the CG, SG and AG were 25.9 cm ±7.0 cm, 29.0 cm ±7.0 cm and 28.7 cm ±7.3 cm, respectively. As noted previously, there was no significant difference in root lengths between treatment groups. The 95% confidence intervals for the means were 25.9 cm ±2.6 cm, 29.0 cm ±2.6 cm, and 28.7 cm ±2.7 cm, respectively. Medians were 25.4 cm, 28.3 cm and 28.6 cm for the CG, SG and AG, respectively (Figure 6). Tukey HSD t-tests calculated P-values of 0.2267 for the SG vs. CG (not significant), 0.3052 for the AG vs. CG (not significant) and 0.9825 for the AG vs. SG (not significant).

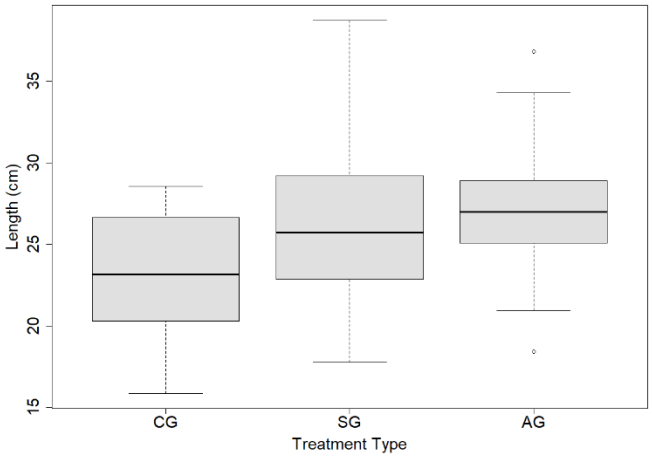

Figure 5. Foliage length medians for the CG, SG and AG: 23.2 cm, 25.7 cm and 27.0 cm, and IQR's were: 6.4 cm, 6.4 cm and 3.8 cm, respectively.

The means and standard deviations for average wet foliage weights for the CG, SG and AG were 14.9 g ±7.7 g, 24.0 g ±11.5 g and 28.8 g ±10.4 g, respectively. The 95% confidence intervals for the means were 14.9 g ±2.9 g, 24.0 g ±4.3 g, and 28.8 g ±3.8 g, respectively. Medians were 15.2 g, 21.2 g and 27.8 g for the CG, SG and AG, respectively (Figure 7). Tukey HSD t-tests calculated P-values of 0.0029 for the SG vs. CG (significant), <0.0001 for the AG vs. CG (significant) and 0.1811 for the algae vs. synthetic groups (not significant). The means and standard deviations for average wet root weights for the CG, SG and AG were 8.2 g ±7.2 g, 13.1 g ±9.2 g and 17.0 g ±8.0 g, respectively. The 95% confidence intervals for the means were 8.2 g ±2.7 g, 13.1 g ±3.4 g, and 17.0 g ±3.0 g, respectively. Medians were 6.0 g, 10.2 g and 16.4 g for the CG, SG and AG, respectively (Figure 8). Tukey HSD tests calculated P-values of 0.0715 for the SG vs. CG (not significant), 0.0004 for the AG vs. CG (significant) and 0.1842 for the algae vs. synthetic groups (not significant).

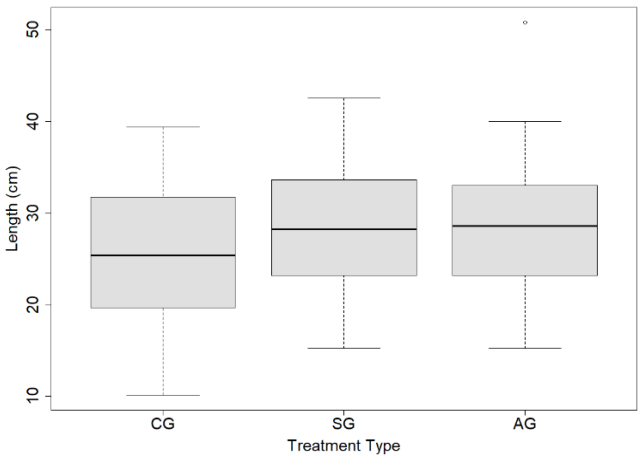

Figure 6. Root length medians for the CG, SG and AG were: 25.4 cm, 28.3 cm and 28.6 cm, and IQR's were: 12.1 cm, 10.5 cm and 9.8 cm, respectively.

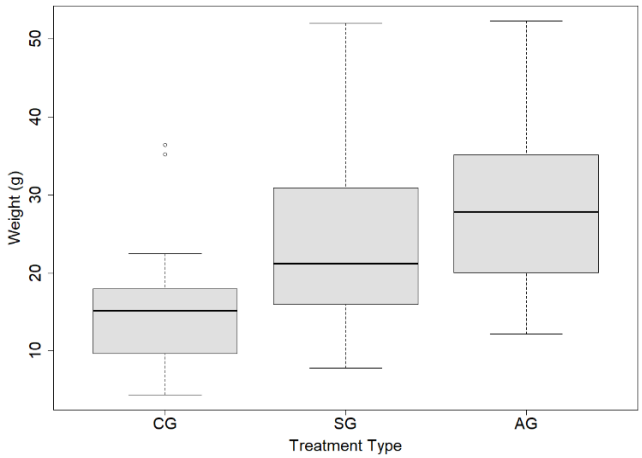

Figure 7. Average wet foliage weight medians for the CG, SG and AG were: 15.2 g, 21.2 g and 27.8 g, and IQR's were: 7.1 g, 14.4 g and 14.2 g, respectively.

Average dry foliage weight per container was not collected; therefore only summary data is reported (Table 4). Total dry foliage weight for the CG, SG and AG were 98.9 g, 129.6 g and 152.3 g, respectively. Mean dry weight per shoot (total of all dried foliage weight ÷ total number of shoots grown) was 1.5 g, 2.2 g and 3.0 g for the CG, SG and AG, respectively. The means and standard deviations for average dry root weights for the CG, SG and AG were 1.7 g ±1.9 g, 2.5 g ±2.2 g and 4.1 g ±3.7 g, respectively. The 95% confidence intervals for the means were 1.7 g ±0.7 g, 2.5 g ±0.8 g, and 4.1 g ±1.4 g, respectively. Medians were 1.3 g, 1.7 g and 2.8 g for the CG, SG and AG, respectively (Figure 9). Tukey HSD tests calculated P-values of 0.4546 for the SG vs. CG (not significant), 0.0043 for the AG vs. CG (significant) and 0.1011 for the algae vs. synthetic groups (not significant). Table 5 provides a summary of key statistical data.

Table 4. Dry foliage weight data.

Measurement	CG	SG	AG
Total Dry Weight	98.9 g	129.6 g	152.3 g
Total number of shoots (all containers)	66	58	51
Mean dry weight per shoot	1.5 g	2.2 g	3.0 g

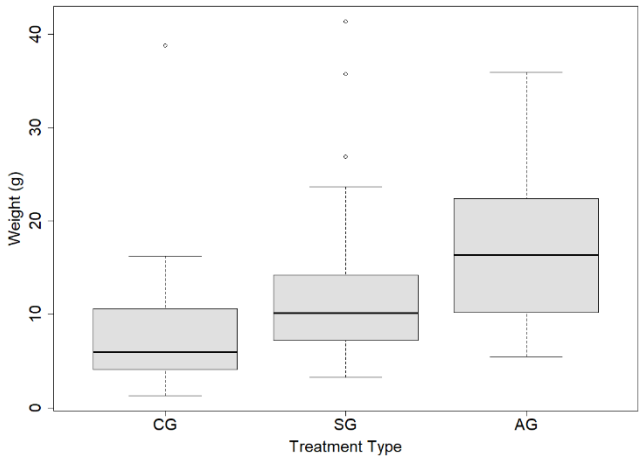

Figure 8. Average wet root weight medians for the CG, SG and AG were: 6.0 g, 10.2 g and 16.4 g, and IQR's were: 6.4 g, 6.9 g and 11.8 g, respectively.

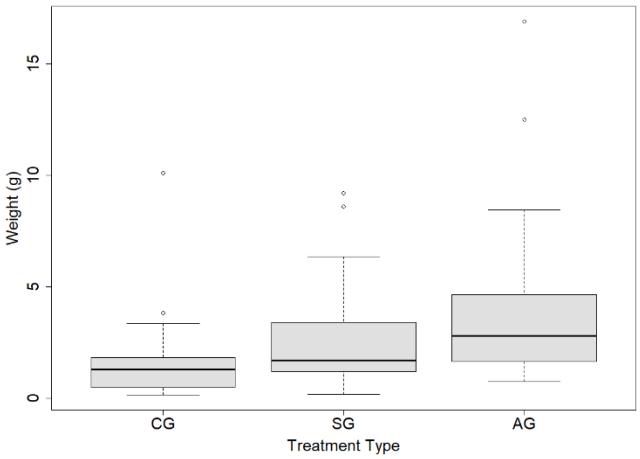

Figure 9. Average dry root weight medians for the CG, SG and AG were: 1.3 g, 1.7 g and 2.8 g, respectively. CG, SG and AG IQR's were: 1.2 g, 2.2 g and 2.9 g, respectively.

Table 5. Summary statistics. CG = Control Group, SG = Synthetic Fertilized Group, AG = Algae Biofertilizer Group. * Indicates statistical significance at $\alpha = 0.05$

Measurement	ANOVA $F_{(2,81)}$ Stat	ANOVA P-value	Treatment	Median	Mean	SD	Tukey P-values CG	Tukey P-values SG	95% CI 2.5%	95% CI 97.5%
1. Foliage length *(cm)*	6.935	0.002*	CG	23.2	23.1	4.0	--	--	21.6	24.6
			SG	25.7	26.4	4.9	0.0148*	--	24.6	28.2
			AG	27.0	27.1	3.8	0.0022*	0.7988	25.7	28.5
2. Root length *(cm)*	1.661	0.200	CG	25.4	25.9	7.0	--	--	23.3	28.5
			SG	28.3	29.0	7.0	0.2267	--	26.4	31.6
			AG	28.6	28.7	7.3	0.3052	0.9825	26.0	31.4
3. Average foliage wet weight *(g)*	13.910	<0.001*	CG	15.2	14.9	7.7	--	--	12.0	17.8
			SG	21.2	24.0	11.5	0.0029*	--	19.7	28.3
			AG	27.8	28.8	10.4	<0.0001*	0.1811	25.0	32.6
4. Average root wet weight *(g)*	8.074	<0.001*	CG	6.0	8.2	7.2	--	--	5.6	10.9
			SG	10.2	13.1	9.2	0.0715	--	9.7	16.6
			AG	16.4	17.0	8.0	0.0004*	0.1842	14.1	20.0
5. Average foliage dry weight *(g)*	NA	NA	CG	*n/a*	1.5	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
			SG	*n/a*	2.2	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
			AG	*n/a*	3.0	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*
6. Average root dry weight *(g)*	5.507	0.006*	CG	1.3	1.7	1.9	--	--	1.0	2.4
			SG	1.7	2.5	2.2	0.4546	--	1.7	3.3
			AG	2.8	4.1	3.7	0.0043*	0.1011	2.7	5.5

Discussion

The purpose of this study was to evaluate the effectiveness of using desiccated non-toxic algae for use as a topical biofertilizer on irrigated crop plants. Algal biomass collected from Upper Klamath Lake in Southern Oregon was dried on-site. A controlled experiment was then conducted to evaluate the efficacy of the algal biomass against a common synthetic fertilizer product using Swiss chard "Bright Lights" (*Beta vulgaris* L. ssp. *cicla* L.) as the experimental plant species. Various plant measurements were taken at the end of the growth cycle and statistical analysis was performed to identify any significant differences in growth patterns between the three groups: Synthetically fertilized group (SG), Algae fertilized group (AG) and the Non-fertilized control group (CG).

The algae fertilized group (AG) produced higher levels of plant growth compared to the control group (CG) in all measurements except root length. In the case of average wet root weight and average dry root weight, the synthetically fertilized group (SG) did not materially outgrow the CG, while the AG did. However, statistical analysis found no significant difference between the AG and SG for these two measurements (at $\alpha = 0.05$). ANOVA tests could not be performed on the dry foliage weight data, but the means suggest that the AG produced a higher level of foliage mass compared to the CG (3.0 g vs. 1.5 g, respectively). This is also supported by the statistically significant finding of a difference in average foliage wet weights (P=<0.0001).

Confidence intervals for leaf length indicate that the variation (or spread) of data for the synthetically fertilized group (SG) was slightly higher, 1.8 cm, compared to the control group (CG) and the algae fertilized

group (AG) at 1.5 and 1.4, respectively. This trend is similar for the average foliage wet weight (SG = 4.3, CG = 2.9 and AG = 3.8) and the average root wet weight (SG = 3.4, CG = 2.7 and AG = 3.0). However, the AG had a significantly larger margin of error for the average dry root weight: AG = 1.4, SG = 0.8 and CG = 0.7 compared to the SG and CG. Plant moisture content and variation in growth levels between the groups may account for these differences in spread. The high variability seen in the algae fertilized group of the dry root weight measurement also suggests that variation in moisture content may have played a role in the average wet weight distributions.

ANOVA and pairwise statistical analyses between the algae-fertilized group and the control group indicate that the null hypotheses (H0) can be rejected in favor of the alternative hypothesis (HA_2) of a difference in means for the measurements of foliage length (P=0.0022), average foliage wet weight (P=<0.0001), average root wet weight (P=0.0004) and average root dry weight (p=0.0043). The null hypothesis cannot be rejected in the case of root length (P=0.3052), and there is insufficient data to make a determination on dry foliage weight. ANOVA and pairwise statistical analyses of the synthetically-fertilized group and the control group indicate that the null hypotheses can be rejected in favor of the alternative hypothesis (HA_1) for the measurements of foliage length (P=0.0148), and average foliage wet weight (P=0.0029). The null hypothesis cannot be rejected in the case of root length (P=0.2267), and there is insufficient data to make a determination on dry foliage weight. There were no significant differences found between the SG and AG for all measurements. Overall, these findings suggest that using desiccated cyanobacteria ("algae") as a biofertilizer on irrigated crop plants may provide an alternative to growers interested in reducing their footprint, while maintaining crop yields.

Although the nature of this study differs from live algae-based experiments (both aquatic and dry-land), the results do correlate with other findings. For example, Bidyarani et al. (2016) found that chickpeas inoculated with *Anabaena laxa* and *Anabaena Rhizobium* increased plant biomass by up to 50% over plants grown without inoculation. In another study, Nain et al. (2010) found that various (non-toxic) live cyanobacteria significantly increased wheat seed germination and plant mass when grown in clay pots. Studies on rice production also show a significant relationship between the presence of live cyanobacteria and higher yields (Prasanna et al., 2011; Nilsson et al. 2002; Jochum et al., 2018).

Recommendations for subsequent research on the use of desiccated algal biomass on crop production are: 1) Scaled-up field-level controlled experiments comparing desiccated algal biofertilizer with other forms of synthetic fertilizers; 2) Field-level controlled experiments comparing desiccated algal biofertilizers on different crop types and under different irrigation regimes; 3) Small-scale studies using other desiccated cyanobacteria species common in freshwater algae blooms; 4) Small-scale studies using desiccated toxic algae strains for effects on plant growth and plant toxicity.

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