THE EFFECTS OF ORGANIC FERTILIZER AND VERMICOMPOST EXTRACT ON BABY LEAF SPINACH IN A HYDROPONIC SYSTEM

A Report

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Meghan Powers

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ABSTRACT

Controlled environment agriculture is becoming increasingly important in a world where climate change has made conventional farming uncertain. Hydroponic agriculture is an attractive option due to its efficient use of nutrients and water, but is still not quite sustainable. Organic hydroponics is a possible solution to this barrier. This experiment tested the effectiveness of organic fertilizer with and without vermicompost extract (VCE) on hydroponic spinach production when compared to a conventional fertilizer control. It was hypothesized that the vermicompost would promote growth of a microbial community, including nitrifying bacteria, that would be beneficial to plant growth. Growth chamber experiments using hydroponic ponds were conducted over three consecutive harvests. Yields increased over time in organic treatments with vermicompost extract, and the Organic + 10% VCE treatment was comparable to the control by the third harvest. The Organic + 5% VCE treatment had a similar positive trend over time but overall had lower yields than the control. Organic treatments without the vermicompost additive had significantly lower yields than the control throughout the experiment. Nitrogen analysis of the nutrient solution, temporal pH data, and rhizobiome microbial assays indicated the presence of a beneficial nitrifying community in organic treatments with the vermicompost extract. These findings show that vermicompost extract may help to make organic hydroponics a viable industry through encouraging the development of a beneficial microbial community in the system.

BIOGRAPHICAL SKETCH

Meghan has loved plants since a very early age, but she did not realize her interest in agriculture until her college years. She has always been passionate about solving climate change, and had an epiphany while in a greenhouse full of flowers in her sophomore year that she could combine her love of plants with her grand plans for a sustainable world. She has been pursuing that goal ever since, and is not planning to stop any time soon. Her main research focus lies in plant-microbe interactions, the soil nitrogen cycle, and its applications for sustainable agriculture. After graduation, she plans to join industry in creating revolutionary applications for agriculture using microbes as biocontrol and fertilizer additives. In her very limited free time, Meghan tends to her houseplants and plays the viola with the Cornell Symphony Orchestra as well as in the pit band of the musical theater group Anything Goes.

ACKNOWLEDGMENTS

Many thanks to my advisors, Todd Walter and Neil Mattson, for encouraging me to explore my interests in invisible bugs and their impact on how we grow our plants. A huge debt to David de Villers, who came out of retirement to teach me how to grow spinach in buckets, provide materials for the project, and spend hours with me in a basement weighing leaves. Thanks also to Masaki Kurosaki and Jonathan Allred for continuing the greenhouse experiments while I was away over the summer. Thank you to the staff of Kenneth Post Laboratory and Cornell CEA for allowing me to use their growth chambers and various materials, and providing answers to my endless questions. The members of the Soil and Water Lab have been a wonderful network of support and resources, and were very helpful even though most had no experience in my field. Thanks to Lisa, Sarah, and the Cornell Statistical Consulting Unit for helping me with my statistics. A special thank you to Dr. Mark Bridgen, who provided me with the opportunity to fall in love with greenhouse farming. Thanks to Clearwater Organic Farms and Worm Power© for providing funding and materials for the fertilizer and amendments.

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PREFACE

This study was funded by Clearwater Organic Farms, LLC to learn the feasibility of an organic hydroponic system for green leafy vegetables. Worm Power© provided the vermicompost extract. It was inspired by Emily Wafler's M.Eng. project on compost additives to hydroponic spinach production.

INTRODUCTION

Controlled environment agriculture (CEA) includes indoor production (greenhouse or vertical farm) where water and nutrients are delivered via hydroponics (crops grown in an aqueous nutrient solution), or aeroponics (spraying water and nutrients on suspended roots). CEA has become a rapidly expanding field of interest due to its versatility and resource efficiency. Hydroponic systems have been shown to save 70-90% of water usage when compared by crop to conventional agriculture systems (Raviv and Lieth, 2008). Closed systems, such as indoor hydroponics, allow for the reuse of water and nutrients, leading to an efficient system with very little losses to the outside world. As nutrient loss through leaching and runoff, causing eutrophication, has become a global problem in conventional agriculture, the closed agricultural system has become an increasingly large part of sustainability strategies. Nutrient addition can also be precisely calculated to remove excess fertilizer addition, leading to an overall reduction in nutrient usage.

The indoor nature of hydroponic systems allows production operations to be located in areas where there is scarce arable land to reduce transportation costs and emissions, i.e. in dense urban areas, or places where conventional agriculture is becoming impossible due to global climate change. This has been a major reason why the market for hydroponically produced leafy greens has increased over recent years, as leafy greens are easily damaged through transport and have a short shelf life, making them an ideal candidate for locally grown hydroponic facilities. Spinach was chosen as the crop for these experiments due to its economic importance and interest from the project funder (Clearwater Organic Farms, LLC). USDA certified organic spinach production in New York state was 61 tons during 2008, and

sales reached $195,000 for that year (USDA-NASS). The market for organic spinach is growing, making a hydroponic spinach operation for this product an attractive opportunity.

Although water, fertilizer, and space use efficiency are benefits of adoption of hydroponics, energy use remains a difficulty. When energy needed for light is taken into account, assuming this energy comes from fossil fuels, the carbon footprint or indoor operations is an issue. Lettuce production in a plant factory system using all light needed for photosynthesis is estimated to produce 8 pounds of carbon dioxide (CO_2) per head of lettuce, and production in a CEA greenhouse in a sunny area with only supplemental light produces 0.62 pounds CO_2 per head of lettuce (Albright, 2014). This is comparable to the emissions from transport of that lettuce head across the country from a farm in California, which would release 0.70 lbs CO_2. With improvements to the efficiency of lighting, emissions from CEA will decrease, making them more sustainable in the future, but reduction of greenhouse gasses from the process is still an important focus.

One area that has already been a strategy for sustainable agriculture in conventional systems is the use of organic fertilizers, such as compost and manure. These can provide essential nutrients like nitrogen (N) and phosphorus without using the extremely resource intensive process of chemical N fixation to produce inorganic fertilizers. Compost usually contains a thriving microbial community which can help improve nutrient cycling and soil health. Organic crops can also be sold at higher prices due to their perceived higher quality, meaning that the cost of production can more easily be recovered. The U.S. National Organic Program allows hydroponic operations to be certified organic so long as their inputs and process meet program specifications (USDA, 2018).

Although these are formidable advantages to adopting organic fertilizers, this movement has hit some roadblocks with respect to implementation in hydroponic systems. Organic fertilizer contains most of its N as organic N, which is unavailable to plants without microbial digestion, and ammonium (NH_4), which is not ideal for spinach production (Shinohara, et al. 2011). Ikeda and Osawa (1981) found that N absorption in spinach systems was dominated by uptake of nitrate (NO_3) over NH_4, regardless of pH. It has become accepted that spinach favors NO_3 as a N source, and is easily damaged by excess NH_4 in the system, which causes NH_4 toxicity (Mattson, et al. 2009).

In addition, it has been shown that organic fertilizers inhibit plant growth through dissolved organic compounds which may have a phytotoxic effect (Garland, et al. 1997). This effect is compounded by the presence of organic acids from root exudates, which accumulate over time in recycled nutrient solution and inhibit growth (Lee, et al. 2006). The slow development of a functioning soil microbiome to convert these organic compounds to nutrients available for plant use makes direct use of organic fertilizer infeasible.

It is understood that a robust microbiome is needed to bridge the gap between organic nutrient addition and plant nutrient uptake through the process of mineralization and nitrification, where organic N is first converted to NH_4 and then to NO_3. This points to the presence of microbes as indicators of a productive organic hydroponic system. Microbes can enter the system through a myriad of pathways, including in the initial seeding media and through nutrient amendments. A community then establishes within the system, shifting in concert with the growing season (Alsanius, et al. 2011). This community can be partitioned into four different areas, each with different microbial loading (Strayer 1994):

1. root associate biofilms (10^7-10^{10} CFU (g fresh weight)$^{-1}$),

2. nutrient solution (10^3-10^6 CFU mL^{-1}),

3. biofilms attached to other surfaces (10^4 CFU cm^{-3}),

4. growing medium.

Due to root exudates of organic carbon and metabolic precursors, the microbial community is the most dense in the area directly surrounding the roots. Root exudates may either inhibit or promote microbial growth, actively selecting organisms to colonize the root environment (Rosberg 2014).

These microbes can be either beneficial or pathogenic. Beneficial rhizosphere microbes are usually separated into two groups based on the mechanism by which they assist plant growth. Plant growth-promoting microbes (PGPM) have a directly positive effect on plant growth, while biological control agents help to control pathogens. Inoculating systems with PGPM have been shown to increase plant growth. For example, Jiménez-Gómez, et al. (2016) inoculated spinach with a *Rhyzobium* species chosen for its colonization properties, and found that the microbe increased plant biomass.

Nitrifying bacteria are especially of interest for this study as they help to convert N from organic fertilizers into compounds available for plant uptake. Several studies have used enrichment techniques to inoculate systems with nitrifying bacteria, prior to adding organic fertilizer. Shinohara, et al. (2011) used bark compost as a nitrification inoculum for lettuce and tomato and found that microbial culture solution was absolutely necessary to keep plants alive. They also determined that an organic fertilizer with a C/N ratio < 11 would allow for NO_3 production in hydroponic systems. Saiji, et al. (2016) continued the bark compost research and found that a pH of 7.5 was optimal for nitrification. *Nitrosomonas* and *Nitrobacter* species, as well as root-associated bacteria such as *Bacillus* and *Pseudomonas*,

were observed during the nitrification process. *Bacillus* and *Pseudomonas* have been detected on the root surfaces of wheat, soybean, and lettuce crops grown in recirculating nutrient hydroponic systems, and are commonly found in association with plants (Strayer 1994). It is likely that some sort of nitrifying community is present in most hydroponic systems.

A robust indigenous microbial community has also been shown to have biocontrol effects in helping to prevent pathogens from finding a hold in the system (Lee and Lee 2015; Raviv and Lieth 2008). Hydroponic systems are uniquely vulnerable to fast-moving pathogens due to their use of recycled nutrient solution. *Pythium aphanidermatum*, a pathogen that infects root systems in aquatic environments, is particularly devastating to hydroponic crops (Brechner and de Villiers, 2007). An established microbial community competes with pathogen populations for nutrients and may protect the roots through antimicrobial production.

Vermicompost, a two step compost process that uses, first, conventional thermophilic composting methods and then digestion by worms, is an emerging product used as an organically certified nutrient amendment to conventional agricultural systems (www.wormpower.net). Vermicompost extract (VCE) is an aqueous solution processed from finished vermicompost, which contains a similar nutrient profile and is more versatile in its use. VCE has been shown to promote plant growth in hydroponic tomato crops (Haghighi, et al. 2016). Jack (2012) also found that seed-colonizing microbes associated with VCE were able to suppress *P. aphanidermatum* infections in a cucumber model system. Worm Power© is a local company, based in Avon, NY, which produces organic vermicompost for a wide variety of industries, including CEA. They state on their website that their VCE contains “a beneficial mesophilic microbial community, including mycorrhizae” and that the VCE is beneficial to

overall plant growth and health (www.wormpower.net). Worm Power© donated VCE prepared using their traditional preparation, as well as a new preparation, for testing in this experiment. Previous research has found benefits of Worm Power© VCE along with organic fertilizer in production of vegetable transplants in an soilless potting mix (Brace 2017), however we wished to test VCE in a hydroponic setting. The possible presence of an indigenous microbial community from the VCE additive is of particular significance as it may help with plant nutrient uptake and pathogen suppression.

The focus of this project is to identify the effect of organic fertilizer and VCE additives on baby spinach growth in a hydroponic nutrient solution, as well as to identify indicators of the presence of a beneficial nitrifying microbial community in the system. The experiment reuses the same nutrient solution for three consecutive crop cycles to test the feasibility of constant crop production in an industrial setting, and to allow time for a microbial community to develop. The results will guide industry adoption of organic hydroponic nutrient solutions and inform subsequent research.

MATERIALS AND METHODS

The greenhouse experiment was conducted in a walk-in growth chamber at Cornell University, which was maintained at 72°F with 50-70% relative humidity. Spinach seeds were used from the cultivar Carmel F1, purchased from Johnny's Selected Seeds©. Eight tubs, each with a capacity of 35 L, were used as hydroponic ponds. Each tub was covered with a flat top of PVC plastic, which had two rectangular holes for flats and two smaller round holes for sampling and tubes. Tubs were filled with 35 L of nutrient solution according to the treatment plan (see Figure 1). pH control, VCE addition, and organic solutions were all tested against a

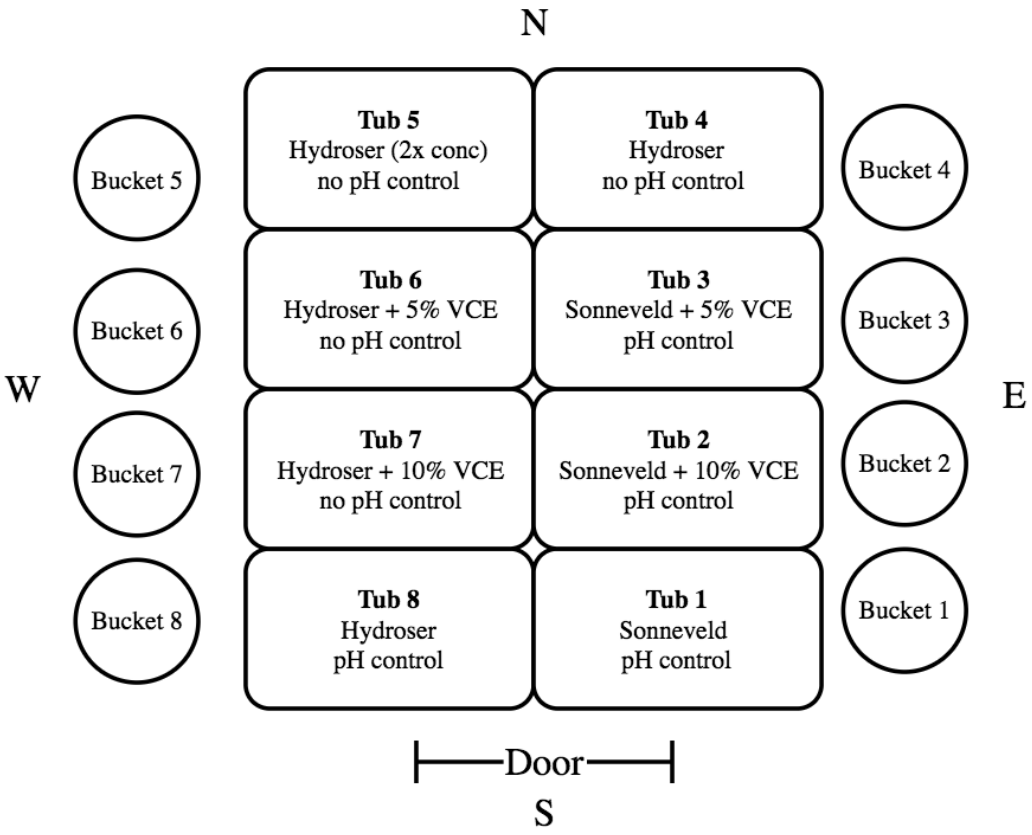

Figure 1. Schematic of growth chamber layout with 8 experimental conditions and corresponding replenishment buckets. Each tub contains two floats of 40 spinach seedlings (total of 80 per tub). Tubs were replenished daily to maintain a constant volume of 35 L. Tubs with pH control were maintained with HNO_3 and KOH to a pH of 5.8 +/- 0.4.

control of conventional Sonneveld nutrient solution (recipe adapted to spinach by Cornell University; Appendix A). Hydroser (Qingdao Seawin Biotech Group Co., LTD.), diluted to 400X according to producer specifications, was used as the organic solution (see Appendix B for product sheet). The 10% VCE treatment refers to 10% VCE used by volume as part of the nutrient solution using the original preparation methods developed by Worm Power©. The 5% VCE treatments used a new preparation of VCE which was used at 5% by volume as part of the nutrient solution. Applied at 5%, this treatment had less N overall and a greater NH_4 to NO_3 ratio than the 10% VCE treatments, which were taken from the older, less concentrated product. My experiment was testing if changing the initial concentration of VCE changed the final result on plant growth. In the pH control treatments nitric acid (HNO_3, 0.1 M) and potassium hydroxide (KOH, 0.1M) were used to keep the pH at 5.8 ± 0.4. pH and EC were monitored daily in all treatments.

Plants were germinated in sixteen Styrofoam flats and then floated in tubs with two flats per tub. Each flat was 5" wide by 8" long and contained 40 cells with 1 seed each. Each flat was filled with Lambert LM-1 germination mix to provide the necessary nutrients for seeding. Several methods (not shown in this paper) were experimented with prior to the start of planting in order to determine the best method for germination. A combination of imbibing the seeds and pre-germinating before planting in the flats was found to be the most effective. Additionally, a trial crop cycle using conventional Sonneveld's solution was completed before starting experimental cycles in order to determine germination rates and optimal crop cycle length. Figure 2 gives a timeline of the experimental design.

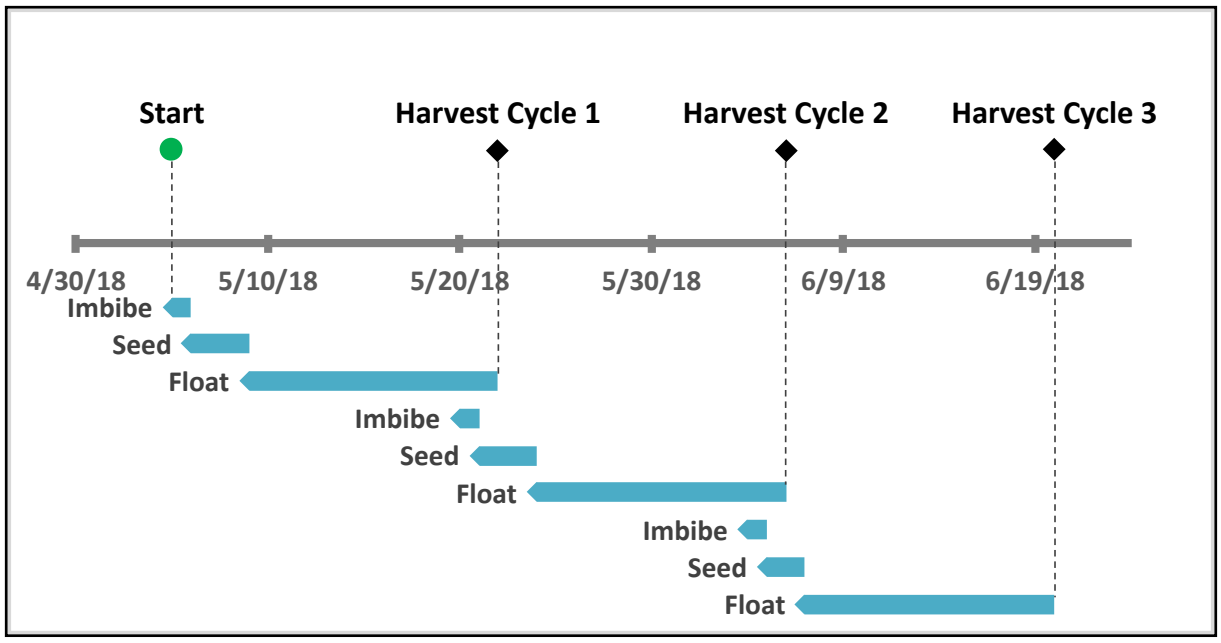

Figure 2. Timeline of experiment including all three crop cycles. "Imbibe" refers to the 12-24 hour imbibition procedure, "Seed" refers to planting the germinated seeds in flats and storing them for 24-48 hours. "Float" refers to floating the flats on the hydroponic ponds as well as the 13 day crop cycle. Note overlap of pre-germination process with previous crop cycle to model consecutive processes in the industry.

i. Seeding Methods

All working surfaces were sterilized prior to seeding. Seeds themselves were surface sterilized by soaking in 70% ethanol for 20-25 seconds and then rinsing with reverse osmosis (RO) water. Sterilized seeds were then imbibed by soaking in RO in a covered container for 12-24 hours prior to spreading. Seeds were spread in three pre-germination boxes, with 9 g of seed per box, to allow for breathing room. The boxes consisted of a plastic Tupperware® container with an added raised mesh bottom and ~500 mL of free RO water under the mesh. Seeds rested on a double layer of wetted paper towels, which had been wrung to field capacity (barely dripping) and then were positioned on the mesh (Figure 3a). Seeds were covered with an additional double layer of wetted paper towels following the same procedure. The mesh was positioned at half the height of the container to prevent splashing onto the seeds. This

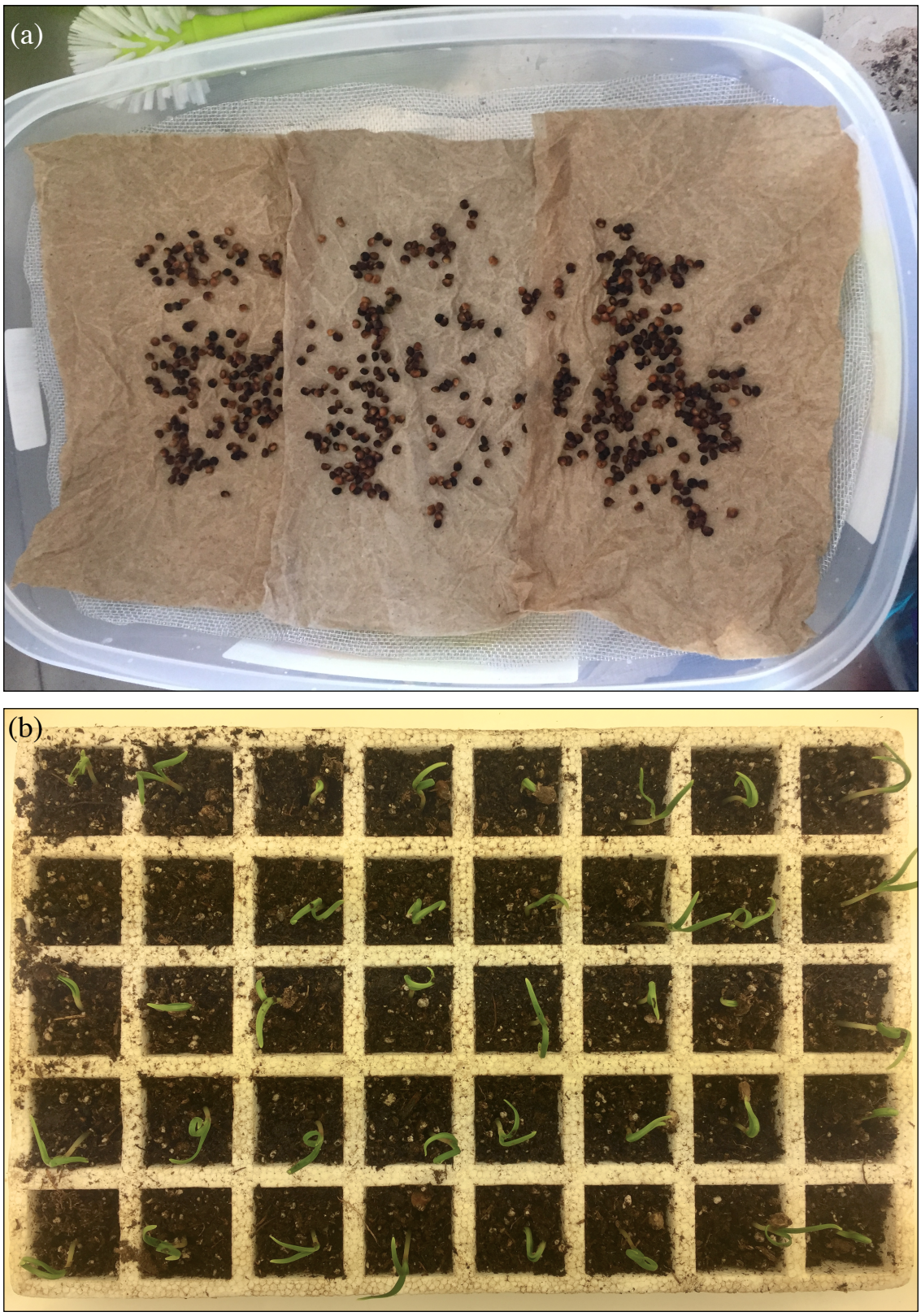

Figure 3. (a) Example of pre-germination procedure, after imbibition. Note wetted paper towels and distribution of seeds. Towels are set on a raised mesh level above free RO water. (b) Example flat filled with soil media and showing seedling emergence, 24-48 hours after planting per-germinated seeds in the flat.

setup ensured almost 100% humidity in the environment for germination without soaking the seeds. Prior testing showed that further wetting of the seeds led to decreased germination rates. The pre-germination boxes were kept in the dark growth chamber at 72°F for 24 hours.

Flats were sterilized in a 70°C oven for at least 6 hours, then filled with the soilless medium. After pre-germination was complete, seeds with visible radicles were planted by hand in the 16 flats with 40 seeds per flat, for a total of 640 plants per crop cycle. Seeded flats were then gently enclosed in a plastic bag to retain high humidity, and were stored in the dark growth chamber for 24-48 hours, until plant emergence was observed (Figure 3b). Once the seedlings emerged, the flats were floated on the hydroponic ponds.

ii. Daily Maintenance

Nutrient solution was replenished daily to maintain a constant tub volume of 35 L. Solution was added from storage containers with pre-made nutrient solution (referred to in Figure 1 as "buckets") corresponding to each treatment (Figure 1). Temperature, light levels (in μmol/s), pH, and EC were measured daily, and pH was adjusted to 5.8 ± 0.4 in the treatments that required pH control.

iii. Harvest Methods

Each crop cycle was 13 days long. On the 13th day the spinach plants were removed from the tubs and weighed to measure the fresh weight. The outside rows around each of the four flat edges were considered as edge plants and were weighed in bulk and not used as yield data. Each plant was then dried in a 70°C oven for at least 24 hours, and then weighed again to determine the dry weight. Wet weight was used in yield analysis to model industry

standards. Samples of the roots from each flat were taken during the first harvest and stored in 500 mL of 50% ethanol at -20°C for further microbial analysis. Additionally, samples of the nutrient solution from each tub, storage container, and stock reservoir were taken and sent for analysis of macro and micronutrients. The 2X Organic treatment was halted after the second harvest due to *Pythium* infection and extreme crop death.

iv. Microbial Analysis of Roots

A surface level analysis of microbial loading on the root structure of each treatment was conducted using a spectrophotometry assay (AD600). Root samples were stored in the preservation solution for several months, and then samples of both the roots and the preservation solution were analyzed for biomass. Three samples of 2 mL were collected from the preservation solution of each treatment. These were then centrifuged at 10,000 rpm for 5 minutes, and the pellet was resuspended in 1 mL of 1X phosphate buffer solution (PBS: 145 mM NaCl, 8.7 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, pH 7.4).

The total mass of the root sample for each treatment was recorded, and then a subsample of ~2.5 g was removed for microbial analysis. The roots were suspended in 10 mL of PBS, shaken vigorously for 2 min, and then left in the fridge overnight to settle. The majority of the roots were then removed, and the solution was filtered (0.45 um pore size, Pall Corporation) to concentrate the remaining biomass. The filters were then submerged in 5 mL PBS in a 15 mL tube and shaken vigorously for 30 seconds, then stored in a fridge overnight to settle. Next the filters were removed and the remaining solution was centrifuged at 10,000 rpm for 5 min. The pellet was resuspended in 1 mL of PBS, then diluted 4X to create 3 replicates of diluted sample for each treatment.

All samples were analyzed using the AD600 microbial growth assay programmed into a spectrophotometer (SmartSpec™ 3000, Bio-Rad). Pure PBS was used as a blank. As no upper bound was specified, the data were compared relatively among the samples.

RESULTS AND DISCUSSION

i. pH and EC

Daily pH and EC provided a comprehensive picture of the changes in charge and nutrient loading in the system. All treatments had a steady downward trend in EC, except for Organic + pH correction, which actually increased over time (Figure 4). This could be due to the addition of large amounts of pH correcting agents, which may have skewed the EC. The EC of the 2X Organic treatment was not compared to the rest of the treatments as it was stopped after the second cycle, and had EC levels much higher than the other treatments.

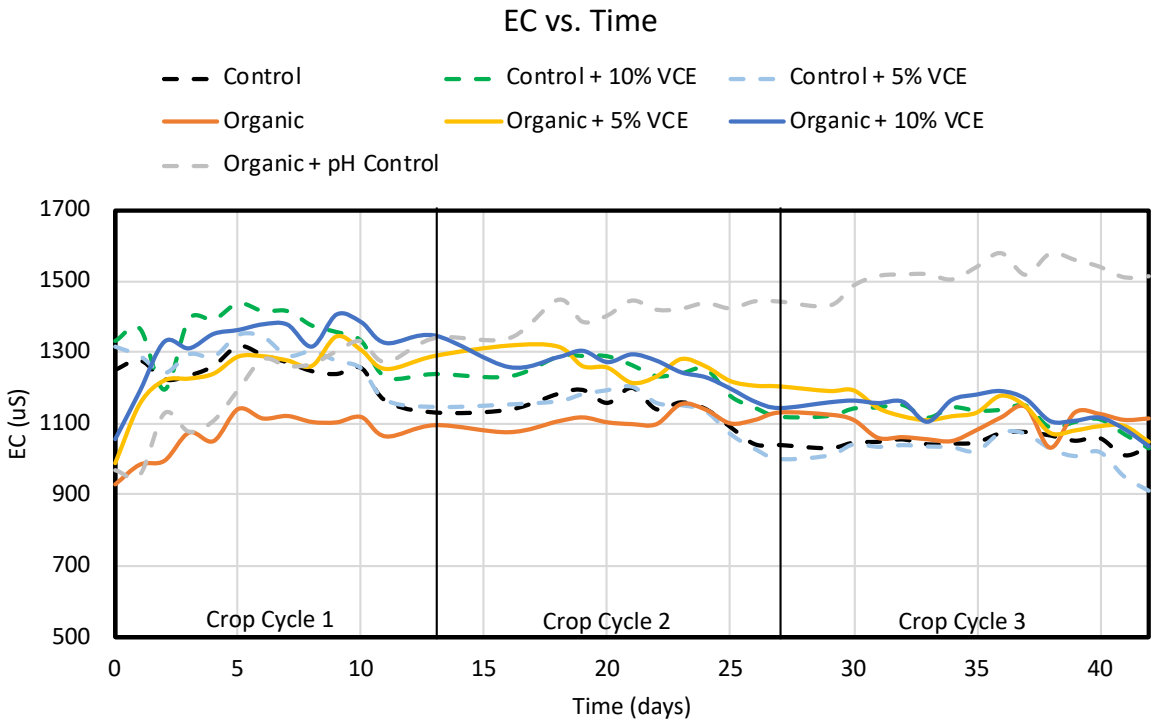

Figure 4. Daily electrical conductivity (EC; uS) measurements across three crop cycles and all treatments. Dashed line denotes daily pH correction to 5.8 +/- 0.4. Note the high EC in the organic treatment with pH correction (gray dotted line).

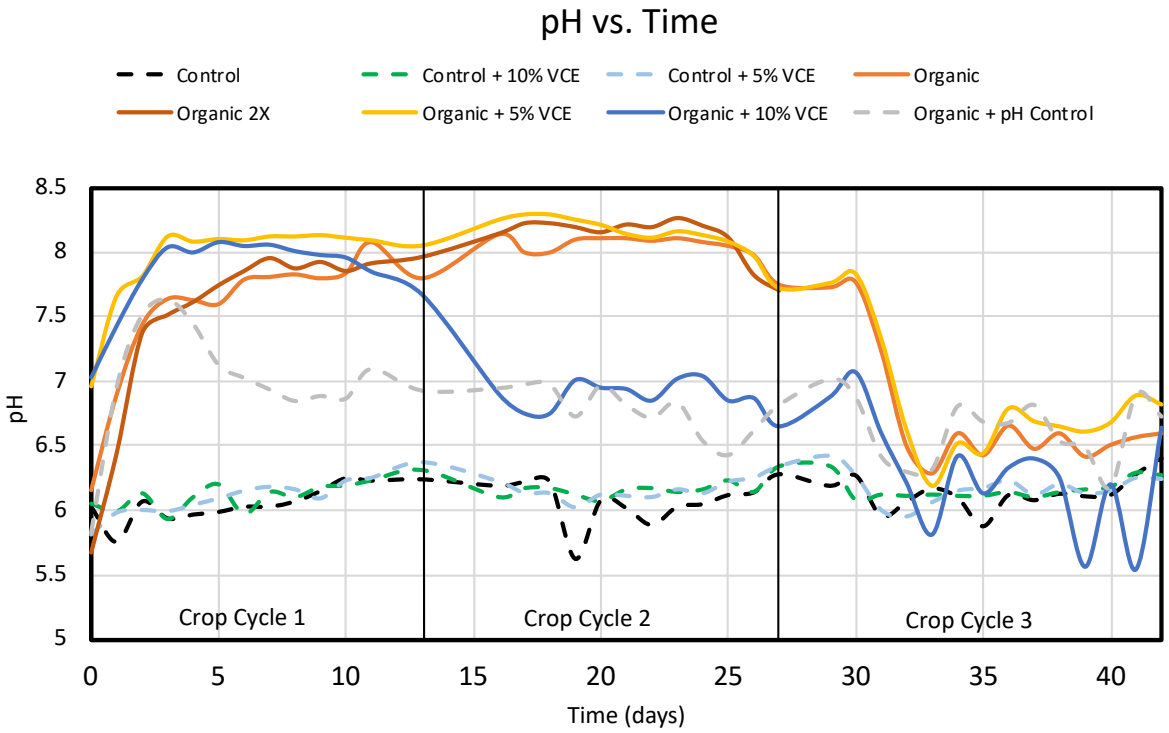

Figure 5. Daily pH measurements across three crop cycles and all treatments. Dashed line denotes daily pH correction to 5.8 +/- 0.4.

pH was adjusted in the three conventional treatments as well as one of the organic treatments. Overall, the organic treatments had wildly variable pH, and controlling it was very difficult. Large additions of HNO_3 were used to control the pH in the organic treatment, but the pH continued to climb daily. It should be noted that HNO_3 is not allowed for certified organic production, however we used HNO_3 as the organic fertilizer manufacturer noted that their fertilizer is incompatible with citric acid (the typical agent used for pH control in organic hydroponics). The pH seemed to stabilize for the organic treatments by the middle of crop cycle three, dropping down to around 6.5 for all treatments (Figure 5). The Organic + 10% VCE treatment only stayed at a high pH for the first crop cycle, and then dropped to a pH of 7 for the second crop cycle and down to close to the optimum pH by the third crop cycle.

ii. Fresh Weight Analysis

The fresh weight was measured after each harvest, for a total of three crop cycles (Figure 6). These results were then split into conventional and organic treatments in order to better compare them to the control. Statistical significance was established using mean separation comparison via Tukey's HSD test (performed using JMP software) performed on the data by treatment: fresh weight compared within each crop cycle and compared as an average across all three crop cycles. Plants that did not grow were added to the dataset as a fresh weight of 0 g to ensure that each treatment had the same number of replicates. Adding these plants also standardized for random germination rates among the samples, as the same seed was used for each planting. With these adjustments we found that each crop cycle was significantly different from the others, meaning that comparison between both treatments and crop cycles is complicated. Thus, each treatment was separated and its performance was only compared to the control for that cycle.

When all data were used on average across all crop cycles, the conventional treatments were not statistically different from the control (Figure 7b), and all of the organic treatments were significantly lower than the control (Figure 7a). However, although the Organic + 10% VCE treatment was still significantly ($p < 0.05$) lower than the control, on average it was also significantly higher than the rest of the organic treatments. This treatment also had the most extreme increase among the three crop cycles, and was not significantly different from the control by the third harvest (Figure 7a). The Organic treatment did not have an obvious positive trend across time, and the Organic + 5% VCE treatment had less of a positive trend

than the 10% VCE. This suggests that the less concentrated original product may have more of a positive impact on plant growth.

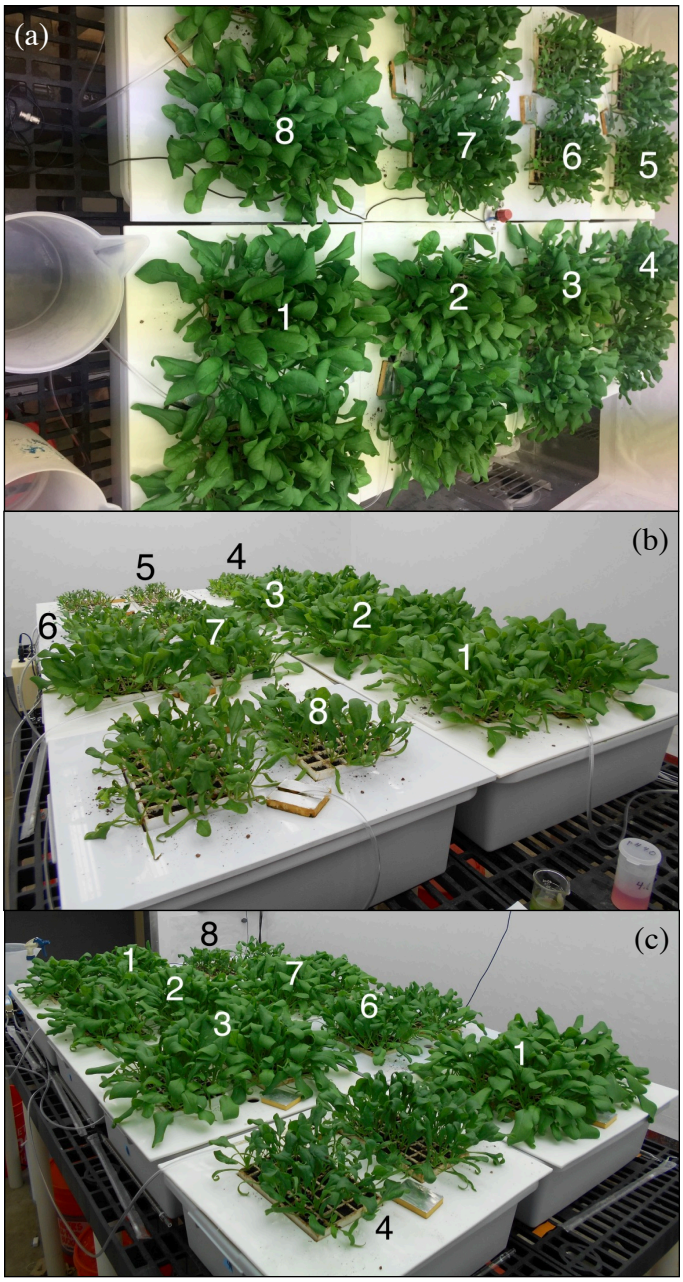

Figure 6. Photographs taken at harvest for the three crop cycles: (a) on day 13, (b) on day 27, and (c) on day 42. Treatments are labeled as such: Control (1), Control + 10% VCE (2), Control + 5% VCE (3), Organic (4), 2X Organic (5), Organic + 5% VCE (6), Organic + 10% VCE (7), and Organic + pH Control (8). Note treatment 5 (2X Organic) was replaced with treatment 1 (Control) for the third crop cycle due to disease.

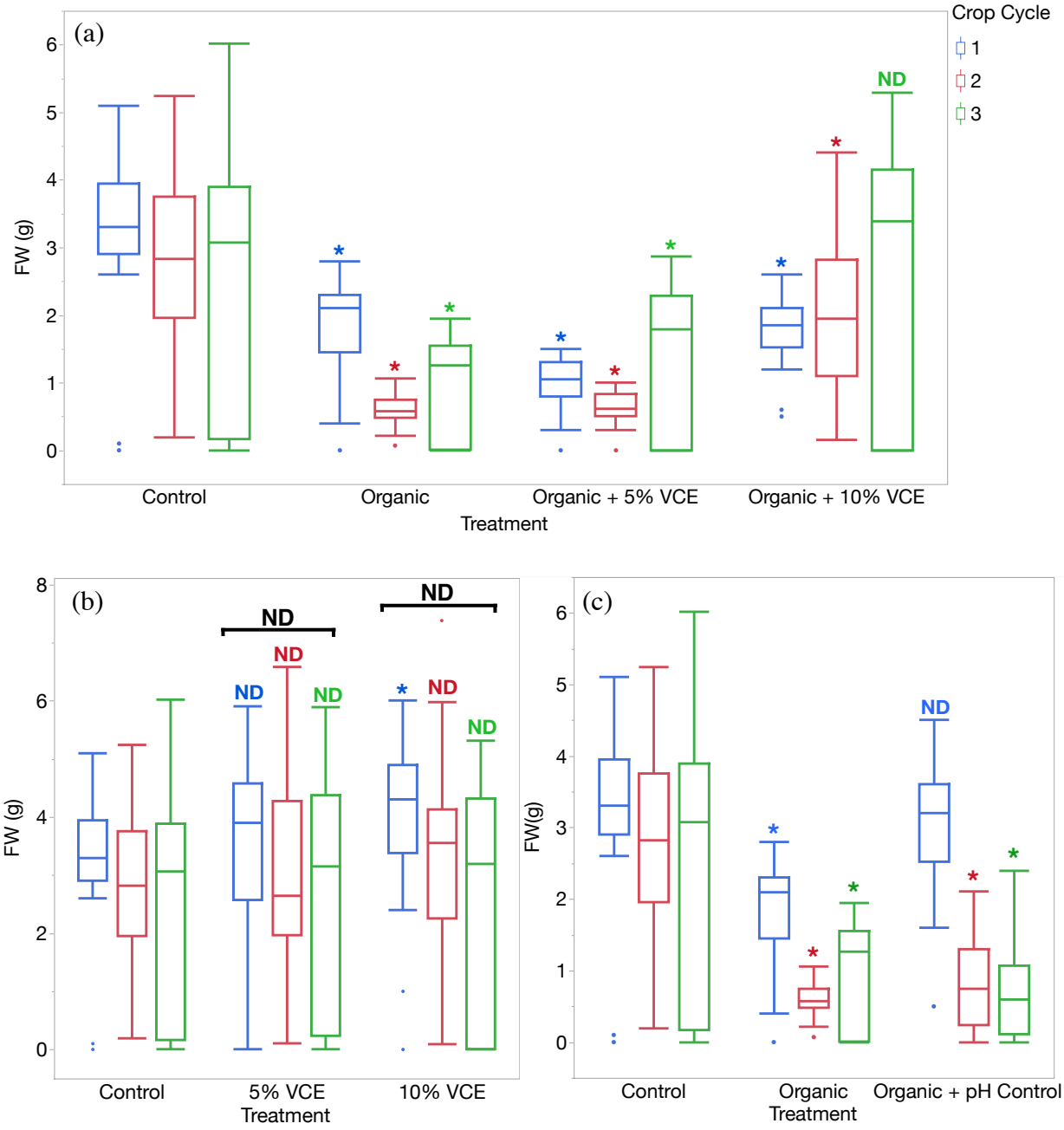

Figure 7. Organic treatment results compared with the Control (a), Control + VCE comparisons (b), and a comparison of the effect of pH control on the Organic treatment (c). A colored asterisk denotes a significant difference ($p < 0.05$) from the control for the corresponding crop cycle. ND denotes no significant difference from the corresponding control. A black ND denotes no significant difference from the control when results were averaged over all crop cycles. Note positive trend over time with addition of VCE in organic treatments. Also note change in y-axis in (b). FW = Fresh weight.

Another interesting result is the Organic + pH Control compared with the Organic and Control treatments (Figure 7c). Organic + pH control did relatively well during the first crop cycle, and the results were not statistically different from the Control, but the growth dropped drastically for the next crop cycles, becoming significantly lower than the Control and comparable to Organic without pH control. This is inconsistent with the pH and EC results, as the pH decreased and the EC increased throughout the second and third crop cycles. However, the wildly fluctuating pH, which was usually adjusted daily from 7-8 down to ~6, may have been detrimental to plant growth.

iii. Nutrient Solution Composition

Macro and micronutrients were analyzed from several sources at each harvest (days 13, 27, and 42): stock solution, treatment solution samples from the nutrient solution reservoir, and solution samples from each tub (treatment). The stock solution analysis revealed large differences in the initial N concentration and NH_4 to NO_3 partitioning based on treatment (Table 1). This shows that the production of the VCE at a higher concentration had an effect on the nutrient makeup of the solution, with a lower concentration leading to lower amounts of NH_4. These results are mirrored in the experimental trials, which showed the Organic + 5% VCE (higher concentration) treatment began with more NH_4 in the system

Table 1. Nitrogen partitioning for the four stock solutions used to make the treatment solution. Note higher amount of NO3 + NO2 for the lower concentration VCE, and the lower NH4 to NO3 ratio.

[mg/L]	Control	Organic	VCE	VCE (higher concentration)
NH_4	7.26	41.61	0.90	1.63
NO_3+NO_2	0.07	0.56	91.13	0.17
NH_4:NO_3	110.81	74.57	0.00982	9.57

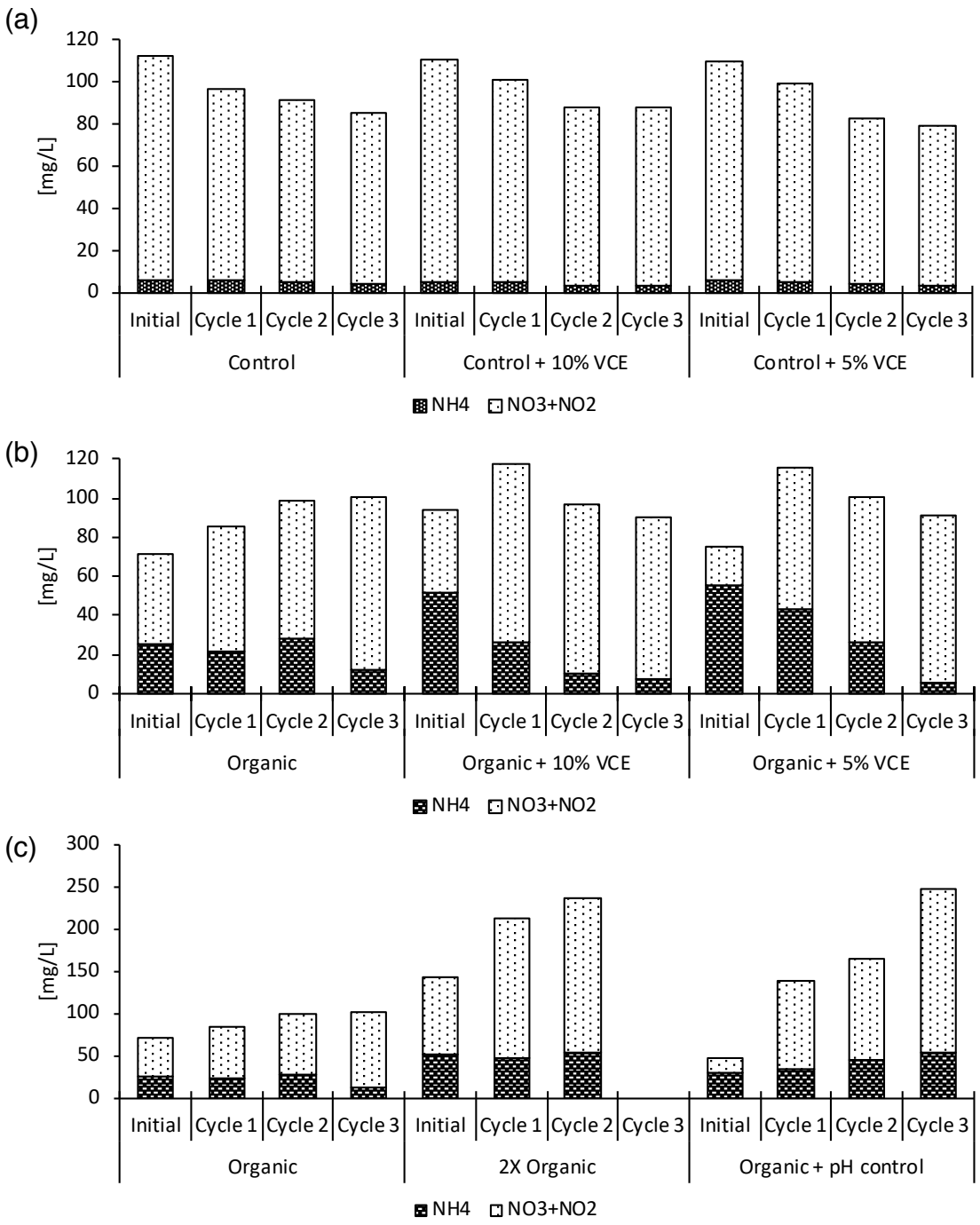

Figure 8. Nitrogen species concentrations in nutrient solutions across all three crop cycles. Ammonium (NH_4) and nitrate/nitrite ($NO_3 + NO_2$) were analyzed separately. (a) shows a comparison between the three conventional treatments. Note the decrease in $NO_3 + NO_2$ concentration over time. (b) Organic treatments vs. VCE amendments. Note decrease in NH_4 concentration in treatments with amendments. (c) compares different treatments of organic solution without amendments. The 2X Organic treatment was stopped after the second harvest due to the presence of disease in the system. Note increase in nitrogen over time and the difference in y-axis scale.

(Figure 8). All three control nutrient solutions, regardless of the addition of VCE, experienced a typical decline in N throughout the experiment. The N in these systems was dominated by $NO_3 + NO_2$ species, with a fairly constant percentage (5%) of the total N as NH_4 (Figure 8a). In contrast, the Organic treatment was about 30% NH_4 initially, an amount which varied over time (Figure 8b). Interestingly, the total N concentration in the Organic treatment increased over time, reaching levels almost consistent with the control after the third crop cycle. This may have been caused by the smaller plant mass present in the Organic treatment as the crop cycles progressed: more solution was added daily, which would increase the concentration of N if the plants were absorbing less nutrients than were added.

The combination of Organic + VCE showed linear decrease in NH_4 concentrations over time. For the Organic + 10% VCE treatment the decline had a R^2 of 0.91 and a R^2 of 0.99 for the Organic + 5% VCE treatment. The total N for these treatments also decreased over time, although these values remained slightly higher than their corresponding control treatments (Figure 8b). The obvious trends shown here could not be statistically analyzed due to the small sample size, and it is noted that all future work in this area should include more replication. These results are consistent with the pH trends over time, as plant uptake and metabolic processing of NH_4 leads to a decrease in pH, which was observed during the second and third crop cycles in organic treatments with VCE additives. This consistency, combined with the established preference of spinach for NO_3 uptake over NH_4, suggests the possible presence of nitrifying bacteria in the microbial community. We assumed that, due to the excessive aeration of the tanks, denitrification was negligible in the system.

The organic treatments without amendments had an interesting trend of increasing N levels over time in both NH_4 and $NO_3 + NO_2$ concentrations. This can be explained in the

Organic + pH Control treatment due to the large daily additions of HNO_3 needed to lower the pH to 5.8 ± 0.4. These additions would have substantially increased the concentration of NO_3 in the system. The 2X Organic treatment has reasonably high levels of N due to its double concentration of nutrients, but the increase in $NO_3 + NO_2$ concentration without a corresponding decrease in NH_4 concentration is interesting. This could possibly be due to the extreme decrease in plant weight, meaning that the plants were using much less N for growth (Figure 8c).

Micro and macronutrient analysis was beyond the scope of this study, which focused mainly on N as a nutrient of interest. However, a cursory analysis of iron (Fe) levels was investigated, as Fe is among the micronutrients needed in greatest quantity by plants (Table 2). The overall trend for fertilizers with 10% VCE addition was a slight increase in Fe concentration over time whereas the three bolded treatments (Table 2): Organic without pH correction, 2X Organic, and Organic + 5% VCE, had a substantial drop in Fe, leading to an almost negligible concentration by the end of the second crop cycle. Interestingly, by the end of the third crop cycle, Fe concentrations had started to increase, suggesting Fe concentrations were still in flux even after 3 crop cycles. However, Fe supplied by 10% VCE appears to be

Table 2. Fe concentrations (mg/L) for treatments during all three crop cycles. Note the fluctuations in the bolded treatments.

	Initial	Crop Cycle 1	Crop Cycle 2	Crop Cycle 3
Control	1.14	1.19	1.22	1.14
Control + 10% VCE	1.07	1.20	1.22	1.15
Control + 5% VCE	1.12	1.27	1.40	1.32
Organic	**1.66**	**1.99**	**0.08**	**0.47**
2X Organic	**8.72**	**2.37**	**0.03**	**—**
Organic + 5% VCE	**1.39**	**0.71**	**0.01**	**0.53**
Organic + 10% VCE	1.72	2.30	2.87	3.80
Organic + pH control	1.60	4.02	4.17	3.99

helpful in maintaining Fe concentration. The 3 treatments with a rapid drop in Fe also had a noticeable increase in pH throughout the first and second crop cycles, averaging around a pH of 8, as well as a drop in pH during the third crop cycle. This could also explain the lack of dissolved Fe in the solution and subsequent increase after the third crop cycle, as Fe is less soluble at a higher pH. An alternate explanation could be that microbial activity was tying up Fe and removing it from solution. Overall, it appears there is some fluctuation in Fe concentration in the organic fertilizer treatments without 10% VCE which may limit available Fe, and future work should seek to develop a greater understanding of this phenomena as well as developing strategies to add an organic source of plant available Fe.

iv. Root Microbiome Analysis

Root samples collected after the first harvest were analyzed using a spectrophotometry assay (AD600) to determine microbial loading (Figure 9). These results were manipulated to find the average and standard deviation of the absorbance per gram of root sample analyzed, for three replicates of each treatment. Absorbance in this case is an indicator of microbial biomass concentration in the sample. A Tukey's test found that many of the treatments had statistically significant differences from one another (Figure 10). The assay results correspond with visual differences in the roots, especially for the Control + 5% VCE treatment (Figure 9b). A possible explanation is that the organic treatments, as well as the fertilizer, simply promoted microbial growth. This is consistent with the very high absorbance found in the Control + 5% VCE treatment, as well as significant differences from the control in the Organic + 5% VCE treatment. An interesting result is the notable decrease in absorbance between the 5% VCE addition and 10% VCE addition for both conventional and organic

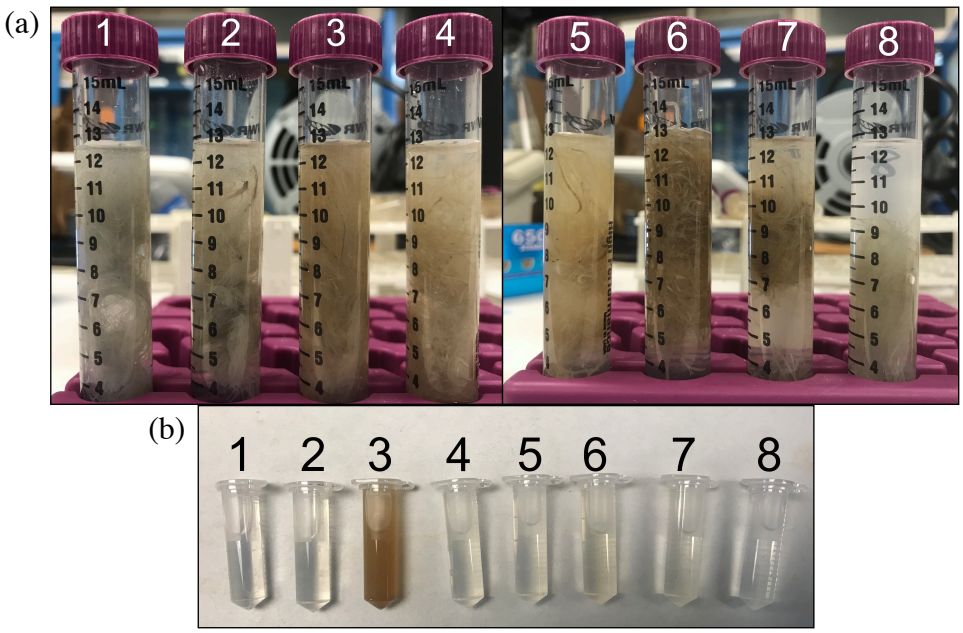

Figure 9. Root samples for microbial analysis after storage in 50% ethanol. Treatments: (1) Control, (2) Control + 10% VCE, (3) Control + 5% VCE, (4) Organic, (5) 2X Organic, (6) Organic + 5% VCE, (7) Organic + 10% VCE, (8) Organic + pH control. (a) shows subsample of roots (~2.5 g) in 1X PBS. (b) samples of ethanol solution after storage for 3 months to account for biomass that may have become detached from roots. Note visual contrast between treatments with VCE addition (2, 3, 6, and 7) and treatments without.

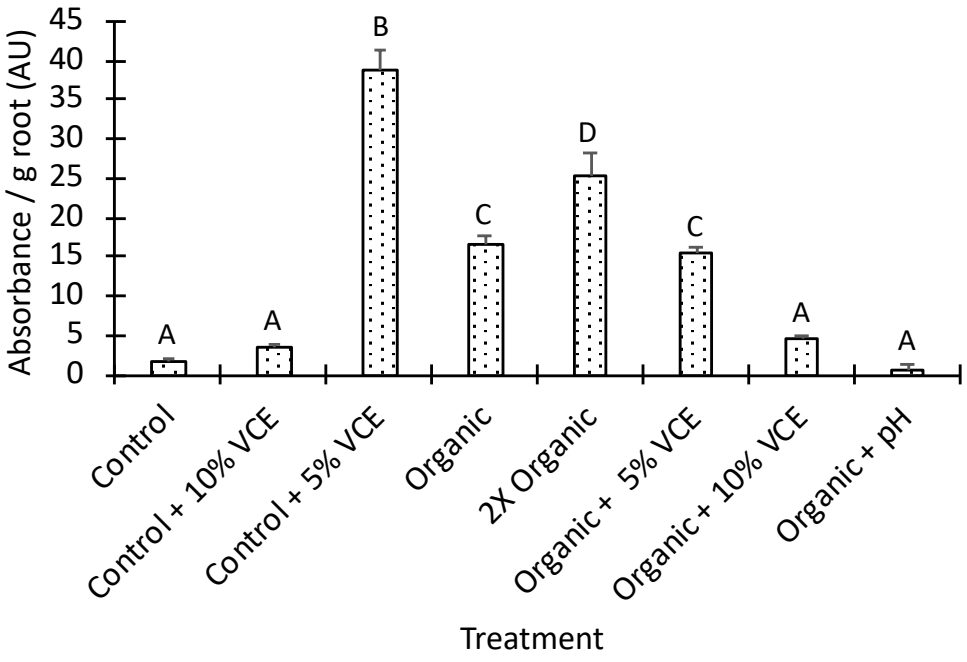

Figure 10. Combined absorbances from solution and root samples, normalized to 1 g biomass. Treatments with different letters have statistically significant differences. Error bars show standard deviation of the three replicates for each treatment.

treatments. This result disagrees with both the fresh weight and the nutrient analysis, which suggested that the 10%VCE was more effective. A plausible explanation is that the microbial community had not yet been fully established in these communities, and that the microbial loading may have increased as the plant biomass increased. Another possible explanation is that microbial loading does not necessarily correlate with plant health, supported by the fact that the 2X Organic treatment had a relatively high absorbance.

These samples are a snapshot of the root microbiome after the first crop cycle, which is before many of the changes took place that may have been caused by an established microbial community, such as a lowered pH and NH_4 concentration. The significant differences between samples at this early time point suggest a large discrepancy in microbial loading between treatments. Again, an obvious pattern was the high microbial loading in the Control + 5% VCE treatment. As the composition and makeup of the microbial community have been shown to change over time, further exploration of the temporal distribution of microbial communities in hydroponic systems should be conducted (Alsanius, et al. 2011). These results open the door for more questions with regard to the presence and impact of a microbial community in VCE additives in hydroponic systems.

CONCLUSIONS AND RECOMMENDATIONS

Hydroponically grown leafy greens are a blossoming industry due to large savings in fertilizer and water usage, lack of runoff, and proximity to local markets. High energy usage and costs offset some of these positive externalities, leading to high emissions and a struggle for profitability (Albright 2014). Organic agriculture is one solution to this problem as organic fertilizers have much lower greenhouse gas emissions during production than conventional fertilizers, and organic produce can be sold at a higher price point than conventional produce to recover revenue. With this in mind, the project sponsor was interested in the feasibility of growing spinach in a hydroponic system with organic fertilizers. Without a microbial community to convert organic nutrients to bioavailable species, especially with regards to organic N, organic fertilizer significantly lowered plant yield in hydroponic spinach. Microbial inoculation with nitrifiers has been shown to address this problem by converting organic N to NH_4 and then NO_3, which is preferred for uptake by spinach (Saijai, et al. 2016; Shinohara, et al. 2011).

Vermicompost extract, which in this experiment was donated by Worm Power©, may promote microbial activity in the hydroponic system, which can counteract the usual phytotoxic effects of organic fertilizer by degrading phenolic compounds produced by plants and organic fertilizers (Waechter-Kristensen, et al. 1999). Nutrient analysis from the vermicompost solids, which are close in composition with the original VCE, found that the C:N ratio was 10.2 (Appendix C). This is below the C:N ratio of 11 stated to be the cutoff for microbial growth, which suggests that VCE could contain a functioning microbial community consistent with Worm Power©'s claims (Shinohara, et al. 2011). The newer VCE product (5% treatment) may have a higher C:N ratio due to its lower N count. This may have been

detrimental to the growth of microorganisms in the system and contributed to the decrease in plant productivity.

The fresh weight, pH, and N analysis suggest that in treatments containing both organic fertilizer and VCE additives the NH_4 in the system was transformed to NO_3 through nitrification during the second and third crop cycles. The pH levels dropped in correlation with the drop in NH_4 levels in these two treatments (Organic + 5% VCE and Organic + 10% VCE). The process of transforming NH_4 to NO_3 releases H^+ ions, lowering pH (Mattson 2009). Spinach has a great preference for NO_3 in hydroponic systems, and excess NH_4 levels can actually be toxic to the plant (Ikeda and Osawa 1981; Shinohara, et al. 2011; Mattson 2009). Given the tendency of spinach to reject NH_4, the downward trend in NH_4 concentrations can be attributed to the microbial activity of nitrifying bacteria. Nitrifiers prefer a pH of 7.5, which is close to the pH levels of approximately 8 observed in the organic treatments for the first crop cycle (Figure 4; Saiji, et al. 2016). A pH of 6, farther from the optimal levels, as well as a low concentration of NH_4, probably prevented nitrifiers from having a significant impact on the control treatments, even with VCE addition. Organic treatments without VCE addition were significantly lower in fresh weight than the Control due to high NH_4 levels and a lack of sufficient N mineralization and nitrification to make N available for plant uptake.

Fresh weight analysis showed that addition of VCE increased plant growth over the 3 consecutive crop cycles. At an addition of 5% VCE to the nutrient solution there was a visible trend of increasing growth over time, and this trend was larger with 10% VCE. The trend is apparent in both Control and Organic treatments, but is less visible in Control treatments, possibly due to the saturation of the solution by the control solution, which is designed to

provide all needed nutrients. The Organic +10% VCE was the most successful of all the organic treatments, ending the third crop cycle with a yield that was not significantly different from the control value. The increase in yield over time in treatments with VCE amendments also supports the hypothesis of the establishment of a beneficial community of nitrifiers, as Saiji, et al. (2016) found that nitrification was performed within a month from an inoculation of microbes without enrichment for nitrifiers. This community, derived from bark compost, is similar to that which might be present in VCE. Absorption data were also significantly higher for rhizobia samples taken after the first harvest from treatments with 5% VCE addition. This data may have been skewed but is still an interesting support point and warrants further research. N analysis, pH, fresh weight data, and some of the rhizobiome analysis all correlated with the hypothesis that a community of nitrifying bacteria was present in later crop cycles due to the addition of VCE, and was beneficial to crop health.

Although these results are promising, this experiment was small and without adequate replication. Experiments at a larger scale and with more replication are needed in order to answer the questions that these results have raised. Given spinach's aversion to NH_4 as a N source, it is important to investigate the microbial makeup of systems with high NH_4 contents to see if nitrifiers are indeed helping with plant nutrient uptake. Root analysis at different time points should be conducted to assess the relative location of such a microbial community and its relationship with plant biomass. Additionally, a longer term experiment with more consecutive crop cycles would establish if the system ever reaches a steady state, what the characteristics of such a steady state are, and if these characteristics are feasible for commercial crop production.

Given this call for further research, some tentative industry recommendations can still be made based on the current data. The organic fertilizer, Hydroser, with 10% VCE addition shows promise as a feasible and organic nutrient solution for spinach production, after an initial stabilization period. Worm Power© should continue to produce their product at using their original preparation methods as it would appear there may be lower nutrient supply or lower microbial activity with the new preparation applied at a rate of 5% by volume to the nutrient solution. The need for a time period of about a month to establish a microbial community and reach sustainable levels of crop production should be noted when making financial decisions. It may be desirable to precondition organic fertilizer with the microbial community several weeks prior to growing plants. As with all hydroponic spinach production, the crop should be monitored closely to check for signs of disease. Overall, it seems that organic hydroponic spinach production is possible with knowledge of the indigenous microbial community and its properties, and a management plan that tailors the system to microbial health.

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APPENDIX A: Sonneveld solution

Table 3. Recipe for Sonneveld solution adapted for spinach production. Notes: DTPA stands for DiethyleneTriaminePentaAcetate. Adjustments may be needed for water source, and water source will affect EC. Typical EC of the diluted solution is c. 1300 microSiemens per cm when using RO water or rain water. Inactive salts dissolved in the water, if using well or tap water, can amount to several hundred microSiemens/cm. Calcium, magnesium, and sulfate ions in hard water should be figured into the target composition of the nutrient solution. (de Villers, 2009).

Ingredient	**Chemical Name**	**Stock A (g)** (dissolved in 30 L H_2O)	**Stock B (g)** (dissolved in 30 L H_2O)
Commerical Ca $(NO_3)_{2.3}H_2O$	Calcium nitrate	2916	
Chelated - Sprint (10% Fe)	Chelated iron, Sprint FeDTPA	67	
NH_4NO_3	Ammonium nitrate	84	
Commercial K NO_3	Potassium nitrate	613	2038
*** *If using sodium molybdate as molybdate source*			
K H_2PO_4	Potassium phospate monobasic		816
Mg $SO_{4.7}H_2O$	Epsom salts		738
Mn $SO_{4.1}H_2O$	Manganese sulfate		2.56
H_3BO_3	Boric acid		5.58
$Na_2MO\ O_{4.2}H_2O$	Sodium molybdate		0.36
*** *If using ammonium molybdate as alternate molybdate source*			
$(NH_4)_6\ MO_7\ O_{24.4}H_2O$	Ammonium molybdate		0.26
$ZnSO_{4.7}H_2O$	Zinc sulfate		3.44
$CuSO_{4.5}H_2O$	Copper sulfate		0.56
K_2SO_4	Potassium sulfate		65.5

Table 4. Nutrient content of conventional Sonneveld solution, adjusted for spinach production by Cornell CEA.

Nutrient	Nutrient Concentration (mg/L)
Ca	90.0
Fe	1.12
P	31.0
Mg	12.0
Mn	0.140
B	0.160
Mo	0.020
Zn	0.130
Cu	0.020
Si	7.0
K	215.0
NH_4	9.0
NO_3	133.0
Total N	**142.0**
Total S	**18.0**

APPENDIX B: Hydroser organic fertilizer

青岛海大生物集团有限公司
QINGDAO SEAWIN BIOTECH GROUP CO., LTD.

Certificate of Analysis

Product: HYDROSER GOLD (Seaweed Fertilizer Solution A)

GURANTEED ANALYSIS		Element
Total Nitrogen (N)	3.50%	
Soluble Potash (K_2O)	1.90%	1.577%
Calcium (Ca)	6.14%	
Iron (Fe)	0.20%	
pH: 4–6 Specific Gravity: 1.30-1.35		

Non-Plant Food Ingredients		
Amino acid	0.70%	
Organic matter	8.15%	

Product: HYDROSER (Seaweed Fertilizer Solution B)

GURANTEED ANALYSIS		Element
Total Nitrogen (N)	3.50%	
Available Phosphate (P_2O_5)	1.20%	0.524%
Soluble Potash (K_2O)	2.23%	1.850%
Magnesium (Mg)	1.13%	
Sulfur (S)	1.65%	
Boron (B)	0.03%	
Cooper (Cu)	0.003%	
Manganese (Mn)	0.10%	
Molybdenum (Mo)	0.0026%	
Zinc (Zn)	0.01%	
pH: 3.5–5.5 Specific Gravity: 1.12-1.17		

Non-Plant Food Ingredients		
Alginic acid	0.20%	
Organic matter	10.32%	

APPENDIX C: Nutrient analysis of Worm Power© compost

PENNSTATE
1855

(814) 863-0841 Fax: (814) 863-4540
Agricultural Analytical Services Laboratory
The Pennsylvania State University
University Park, PA 16802
www.aasl.psu.edu

Analysis Report For:	Copy To:
Shawn Ferro Worm Power PO Box 668 Geneseo NY 14454	Tom Herlihy Worm Power PO Box 668 Geneseo NY 14454

LAB ID:	SAMPLE ID:	REPORT DATE:	SAMPLE TYPE:	FEEDSTOCKS	COMPOSTING METHOD	COUNTY
C07398	VC - 072314	10/1/2014	Finished vermicompost		Vermicomposting	

COMPOST ANALYSIS REPORT

Compost Test 1C

Analyte	Results (As is basis)	Results (Dry weight basis)
pH	7.0	—
Soluble Salts (1:5 w:w)	16.25 mmhos/cm	—
Solids	49.9 %	—
Moisture	50.1 %	—
Organic Matter	36.12 %	72.4 %
Total Nitrogen (N)	1.943 %	3.90 %
Organic Nitrogen[1]	1.943 %	3.89 %
Ammonium N (NH_4-N)	5.2 mg/kg *or* 0.0005 %	10.4 mg/kg *or* 0.0010 %
Carbon (C)	19.88 %	39.9 %
Carbon:Nitrogen (C:N) Ratio	10.20	10.20
Phosphorus (as P_2O_5)[2]	0.72 %	1.444 %
Potassium (as K_2O)[2]	1.76 %	3.53 %
Calcium (Ca)	2.27 %	4.54 %
Magnesium (Mg)	0.51 %	1.03 %
Sulfur (S)	0.32 %	0.64 %
Sodium (Na)	3514.03 mg/kg	7045 mg/kg
Aluminum (Al)	188.52 mg/kg	377.96 mg/kg
Iron (Fe)	2038.40 mg/kg	4086.73 mg/kg
Manganese (Mn)	95.45 mg/kg	191.36 mg/kg
Copper (Cu)	476.40 mg/kg	955.11 mg/kg
Zinc (Zn)	139.34 mg/kg	279.36 mg/kg
Nitrate-N	1923.35 mg/kg	3856.08 mg/kg

[1]See comments on back of report .
[2]To convert phosphorus as (P_2O_5) into elemental phosphorus (P), divide by 2.29. To convert potassium (as K_2O) into elemental potassium (K), divide by 1.20.

APPENDIX D: Full nitrogen analysis data

Table 5. NH_4 concentrations (mg/L) for all treatments across all three crop cycles.

	Crop Cycle			
Treatment	Initial	1	2	3
Control	6.49	5.84	5.14	4.74
Control + 10% VCE	5.40	5.00	3.87	3.93
Control + 5% VCE	6.46	5.00	4.00	3.65
Organic	25.75	21.99	28.12	11.91
Organic + 10% VCE	51.61	26.61	10.28	7.13
Organic + 5% VCE	55.49	43.58	26.10	5.69
2X Organic	51.50	47.01	52.99	—
Organic + pH Control	19.18	104.56	120.49	193.00

Table 6. NO_3 + NO_2 concentrations (mg/L) for all treatments across all three crop cycles.

	Crop Cycle			
Treatment	Initial	1	2	3
Control	106.24	91.29	86.34	80.92
Control + 10% VCE	105.34	96.03	83.95	84.59
Control + 5% VCE	103.08	94.26	79.03	75.70
Organic	45.62	63.03	70.39	88.66
Organic + 10% VCE	42.53	90.51	86.17	83.25
Organic + 5% VCE	19.29	72.25	74.16	85.50
2X Organic	91.23	165.87	182.87	—
Organic + pH Control	19.18	104.56	120.49	193.00

APPENDIX E: Full micro and macronutrient analysis

Table 7. Dissolved nutrient analysis after crop cycle 1.

Sample	Al 308.215	As 189.042	B 249.773	Ba 455.404	Be 313.042	Ca 211.276	Cd 214.438	Co 228.616	Cr 267.716	Cu 324.754	Fe 259.941	K 766.491	Li 670.780
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Control	0.02	0.00	0.20	0.01	0.00	99.25	0.00	0.00	0.00	0.03	1.19	168.58	0.02
Control + 10% VCE	0.03	0.00	0.21	0.01	0.00	99.51	0.00	0.00	0.00	0.06	1.20	187.42	0.02
Control + 5% VCE	0.03	0.00	0.20	0.01	0.00	99.43	0.00	0.00	0.00	0.10	1.27	160.26	0.02
Organic	0.02	0.00	0.55	0.00	0.00	121.91	0.00	0.00	0.00	0.05	1.99	38.31	0.02
2X Organic	0.02	0.00	1.20	0.00	0.00	236.34	0.00	0.00	0.00	0.09	2.37	155.38	0.02
Organic + 5% VCE	0.02	0.00	0.52	0.00	0.00	111.87	0.00	0.00	0.00	0.09	0.71	75.34	0.02
Organic + 10% VCE	0.02	0.00	0.51	0.00	0.00	116.65	0.00	0.00	0.00	0.05	2.30	88.00	0.02
Organic + pH control	0.03	0.00	0.56	0.00	0.00	125.78	0.00	0.00	0.00	0.05	4.02	51.39	0.02

Sample	Mg 279.079	Mn 257.611	Mo 202.095	Na 330.298	Ni 231.604	P 213.618	Pb 220.353	S 182.034	Se 196.090	Si 251.612	Sr 421.552	Ti 334.941	V 292.464	Zn 213.856
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Control	10.04	0.02	0.02	8.57	0.00	28.37	0.00	18.86	0.00	0.06	0.65	0.00	0.00	0.07
Control + 10% VCE	12.80	0.01	0.02	35.55	0.00	25.63	0.00	21.49	0.00	1.03	0.60	0.00	0.00	0.08
Control + 5% VCE	11.55	0.02	0.02	22.37	0.00	25.57	0.00	18.96	0.00	0.70	0.60	0.00	0.00	0.09
Organic	18.31	0.77	0.06	20.12	0.00	4.80	0.00	31.94	0.00	0.21	0.03	0.00	0.00	0.26
2X Organic	40.48	2.04	0.13	43.75	0.00	2.53	0.00	70.70	0.00	0.30	0.04	0.00	0.00	0.51
Organic + 5% VCE	18.97	0.53	0.06	31.87	0.00	3.62	0.00	31.08	0.00	0.89	0.03	0.00	0.00	0.29
Organic + 10% VCE	20.37	0.04	0.05	42.32	0.00	5.08	0.00	31.80	0.00	0.90	0.04	0.00	0.00	0.25
Organic + pH control	17.83	0.43	0.05	20.60	0.00	6.93	0.00	31.62	0.00	0.17	0.03	0.00	0.00	0.27

Table 8. Dissolved nutrient analysis after crop cycle 2.

Sample	Al 308.215	As 189.042	B 249.773	Ba 455.404	Be 313.107	Ca 211.276	Cd 214.438	Co 228.616	Cr 267.716	Cu 324.754	Fe 259.941	K 766.491	Li 670.780
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Control	0.03	0.00	0.21	0.02	0.00	109.92	0.00	0.00	0.00	0.03	1.22	108.77	0.02
Control + 10% VCE	0.04	0.00	0.21	0.02	0.00	109.20	0.00	0.00	0.00	0.06	1.22	116.25	0.02
Control + 5% VCE	0.04	0.00	0.20	0.02	0.00	110.59	0.00	0.00	0.00	0.09	1.40	87.12	0.02
Organic	0.03	0.00	0.56	0.00	0.00	119.82	0.00	0.00	0.00	0.02	0.08	31.37	0.02
2X Organic	0.03	0.00	1.15	0.00	0.00	229.68	0.00	0.00	0.00	0.06	0.03	141.68	0.02
Organic + 5% VCE	0.03	0.00	0.53	0.00	0.00	112.56	0.00	0.00	0.00	0.04	0.01	63.94	0.02
Organic + 10% VCE	0.03	0.00	0.54	0.00	0.00	128.56	0.00	0.00	0.00	0.07	2.87	41.85	0.02
Organic + pH control	0.03	0.00	0.59	0.00	0.00	132.74	0.00	0.00	0.00	0.05	4.17	38.19	0.02

Sample	Mg 279.079	Mn 257.611	Mo 202.095	Na 330.298	Ni 231.604	P 213.618	Pb 220.353	S 182.034	Si 212.412	Sr 421.552	Ti 334.941	V 292.464	Zn 213.856
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Control	8.12	0.00	0.02	9.56	0.00	25.66	0.00	19.22	0.07	0.74	0.00	0.00	0.05
Control + 10% VCE	10.48	0.00	0.02	39.00	0.00	21.59	0.00	21.80	1.14	0.67	0.00	0.00	0.07
Control + 5% VCE	9.40	0.00	0.02	23.99	0.00	22.08	0.00	19.27	0.83	0.67	0.00	0.00	0.06
Organic	17.26	0.20	0.06	21.13	0.00	2.87	0.00	32.52	0.11	0.03	0.00	0.00	0.14
2X Organic	39.63	0.86	0.12	43.63	0.00	2.48	0.00	69.22	0.27	0.05	0.00	0.00	0.31
Organic + 5% VCE	18.41	0.04	0.06	33.26	0.00	3.10	0.00	31.21	1.00	0.04	0.00	0.00	0.17
Organic + 10% VCE	20.91	0.18	0.05	46.55	0.00	4.82	0.00	33.52	0.97	0.06	0.00	0.00	0.26
Organic + pH control	18.28	0.39	0.06	22.74	0.00	6.59	0.00	33.03	0.16	0.04	0.00	0.00	0.28

Table 9. Dissolved nutrient analysis after crop cycle 3.

Sample	Al 308.215	As 189.042	B 249.773	Ba 455.404	Be 313.107	Ca 211.276	Cd 214.438	Cr 267.716	Cu 324.754	Fe 275.573	K 766.491	Li 670.780
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Control	0.03	0.00	0.18	0.02	0.00	104.21	0.00	0.00	0.03	1.14	87.53	0.02
Control + 10% VCE	0.03	0.00	0.18	0.02	0.00	105.34	0.00	0.00	0.06	1.15	89.37	0.02
Control + 5% VCE	0.03	0.00	0.18	0.02	0.00	106.07	0.00	0.00	0.09	1.32	58.43	0.02
Organic	0.02	0.00	0.56	0.00	0.00	128.67	0.00	0.00	0.03	0.47	20.24	0.02
Organic + 5% VCE	0.02	0.00	0.50	0.00	0.00	122.23	0.00	0.00	0.05	0.53	33.44	0.02
Organic + 10% VCE	0.03	0.00	0.53	0.01	0.00	133.62	0.00	0.00	0.07	3.80	7.01	0.02
Organic + pH Control	0.03	0.00	0.55	0.00	0.00	130.76	0.00	0.00	0.05	3.99	37.96	0.02

Sample	Mg 279.079	Mn 257.611	Mo 202.095	Na 330.298	Ni 231.604	P 213.618	Pb 220.353	S 182.034	Si 212.412	Sr 421.552	Ti 334.941	V 292.464	Zn 213.856
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Control	7.32	0.00	0.02	11.30	0.00	24.12	0.00	19.92	0.06	0.58	0.00	0.00	0.08
Control + 10% VCE	9.99	0.01	0.01	43.39	0.00	20.16	0.00	23.67	1.14	0.52	0.00	0.00	0.08
Control + 5% VCE	8.02	0.01	0.02	26.55	0.00	20.32	0.00	19.75	0.92	0.54	0.00	0.00	0.07
Organic	18.77	0.31	0.06	25.38	0.00	4.88	0.00	35.98	0.10	0.05	0.00	0.00	0.24
Organic + 5% VCE	19.07	0.65	0.05	36.62	0.00	5.91	0.00	33.06	1.03	0.06	0.00	0.00	0.24
Organic + 10% VCE	19.27	0.64	0.05	51.66	0.00	3.33	0.00	36.83	0.63	0.07	0.00	0.00	0.28
Organic + pH Control	18.53	0.47	0.05	25.73	0.00	6.67	0.00	34.73	0.21	0.05	0.00	0.00	0.30