The Effect of Red-rich and Blue-rich Lighting on the Microbiome of a Tomato Crop Grown Under International Space Station Conditions of High Humidity and Elevated CO_2

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Abstract

Customized lighting treatments are being investigated to optimize space crop production. The VEG-05 experiment on the International Space Station (ISS) presented here investigated the effect of red-rich and blue-rich lighting in Veggie on the microbiome of a dwarf tomato variety, *Solanum lycopersicum* cv. Red Robin. A plant and its associated microbiome drive plant growth promotion, as well as resistance to pathogens and environmental stressors. The microbiome was investigated using bacterial 16S rRNA gene and fungal ITS sequencing methods to identify bacterial and fungal communities on tomato fruit, leaves, roots, rooting substrate, and Veggie facility surfaces grown under red-rich or blue-rich lighting. The plants were also screened using culture-based methods for potential food-borne pathogens and plate counts for bacteria and fungi. Differences in microbial load were compared between lighting conditions, as well as between ISS and ground control treatments. Due to environmental stresses, fruit production was low on ISS grown plants, thus limiting the number of samples available for analyses from flight plants. This analysis determined the core microbiome and microbiological composition for tomato plants grown under a red-rich or blue-rich lighting treatment and microgravity conditions. The core microbiome for flight plants included the genera *Rhizobium*, *Azospirillum*, *Burkholderia*, *Dyadobacter*, and *Sphigomonas.* However, *Pseudomonas* was the only genus common to all ground-control plants, due low diversity on leaf samples. Culture-based pathogen screening, corroborated by 16S rRNA gene and ITS sequencing, yielded negative results. This experiment provides valuable data on a fruiting crop grown on the ISS and how the plant microbiome may change due to different lighting conditions.

1. Introduction

NASA's plans toward long-duration space missions to the moon and Mars include growing fresh, edible crops as a component of the crew food system. This capability will provide additional health-promoting nutrients, menu variety, and positive behavioral health elements while moving away from the need to supply an entire mission's food requirement (Perchonok et al., 2012). The packaged diet currently supplied to the International Space Station (ISS) crew provides adequate nutrients and quality. However, with long-term storage under ambient conditions, vitamins decrease in potency (Cooper et al., 2017; Zwart et al., 2009). Several studies support the hypothesis that dietary antioxidants can reduce cancer risks and other chronic illnesses associated with inflammation (Liu, 2003; Zhang et al., 2015). These compounds are most effective through the consumption of fresh fruits and vegetables that are high in certain phytochemicals, and the isolated pure compounds do not appear to convey the same health benefits. (Liu, 2003). NASA has implemented crop-readiness criteria to select crops that will best help fill gaps in nutritional requirements for astronauts by providing essential nutrients and beneficial phytochemicals (Massa et al., 2015; Romeyn et al., 2019; Spencer et al., 2019).

1.1. Veggie Production System: A plant growth system on the ISS

Since 2014, when the first of two plant-growth units were installed, the Veggie vegetable-production system on ISS has been used to validate candidate crops for harvest yield, microbiological food safety, and palatability. The use of Veggie as a pick-and-eat crop system to supplement the crew diet is a starting point to understand and overcome the challenges of growing crops in the spacecraft environment to move towards sustainable space crop production. Each Veggie chamber consists of an adjustable light array containing red, blue, and green LEDs,

a fan that circulates ambient air through the chamber, and containment via a flexible bellows that can be lowered during operations (Massa et al., 2016; Morrow et al., 2005; Morrow and Remiker, 2009). Plants intended as food crops are grown in bag-like containers referred to as "plant pillows", containing the growing substrate as well as controlled-release fertilizer. Seeds are planted in wicks embedded in pillows. Water is delivered indirectly from a root-mat reservoir into the pillows and directly through manual watering injections into the pillow substrate. Leafy greens such as red-romaine lettuce have been grown repeatedly over the past 10 years in Veggie, and tissue samples sent back to the Kennedy Space Center for analysis indicated a microbiologically safe product, as well as minerals and bioactive compound levels equal to, or greater than, those of ground-control plants (Bunchek et al., 2024; Hummerick et al., 2021; Khodadad et al., 2020).

1.2. Red-rich and blue-rich light spectrum in Veggie experiments VEG-04 and VEG-05

The VEG-04 experiments done in 2019 (Bunchek et al., 2024) investigated effects of increased red or blue wavelengths and harvest method on plant yield, palatability, and microbiological food safety of a mustard-type leafy green, mizuna. Results found that organoleptic sensory acceptability was not affected by light quality, and flight-grown produce scored higher than ground controls. Light quality did influence harvest yield and nutrient accumulation, with the blue-rich light treatment yielding higher biomass with repeated harvests and nutrient content of leaves. Results were inconclusive with respect to light quality on the bacterial and fungal load on plants (Bunchek et al., 2024). Testing beyond leafy greens, the VEG-05 experiment presented here investigated the effect of red-rich or blue-rich light recipes in Veggie on the growth, yield, and microbiological quality of a dwarf tomato variety, *Solanum lycopersicum* cv. Red Robin. Extensive ground evaluations were completed at Kennedy Space Center on this cultivar for

space-crop readiness, ranking it high for plant morphology (dwarf is desired), fruit yield, nutritional attributes, and sensory acceptability (Spencer et al., 2019). As a dietary supplement, tomatoes are excellent sources of nutrients and bioactive compounds like lycopene, carotenoids, Vitamins C and E, and phenolic compounds (Ali et al., 2021; Chaudhary et al., 2018; Elbadrawy and Sello, 2016; Górecka et al., 2020; Ramos-Bueno, et al,. 2017; Vats et al., 2022; Yin et al, 2024), all of which can be manipulated through custom light spectra.

1.3. Light spectrum impacts on plants and their associated microbiome

Early studies at NASA's Kennedy Space Center on the use of customized LED lighting to optimize crop production led to the application in small plant growth chambers for space agriculture (Goins et al., 2001; Kim et al., 2004a, 2004b; Massa et al., 2008). Targeted lighting recipes are used in controlled environment agriculture to promote desirable plant phenotypes, bioactive compounds, and energy efficiency. Differences in plant morphological features such as stem length, number of leaves, leaf thickness and pigmentation are impacted specifically by red and blue light wavelengths (Cammarisano et al., 2021; Carvalho and Folta, 2016; Mickens et al., 2018, 2019; Ustin and Jacquemoud, 2020). Numerous investigations have described the increased production of antioxidant compounds in plants because of exposure to red-rich or blue-rich lighting recipes (Carvalho et al., 2016; Carvalho and Folta, 2014; Lee et al., 2019). Studies demonstrate that increased blue light combined with red wavelengths increased the accumulation of chlorophyl, flavonoids, and antioxidants in lettuce (Son and Oh, 2013; Li and Kubota, 2009). Additionally, Plant-phyllosphere interactions are influenced by varying light wavelengths (Carvalho and Castillo, 2018; Gomelsky and Hoff, 2011).

Light composition can modulate the plant immune response influencing resistance to plant pathogen infection (Santamaria-Hernando et al., 2020; Xu et al., 2017). For instance, resistance to *Botrytis* infection in tomatoes was enhanced with the addition of blue light that correlated to the resultant increased antioxidant capacity of the plant (Kim et al., 2013). Khanam et al. (2005) found a similar effect using a red-light treatment on broad bean leaves infected with *Botrytis cinerea* and attributed the subsequent disease resistance to increased catalase activity in the plant. In a study by Xu et al. (2017), tomato leaves inoculated with *Botrytis cinerea* were exposed to red and purple light resulting in suppression of disease by two entirely different mechanisms, red light eliciting a plant defense mechanism and purple by photo-inhibition of the mold. Response to light can also have a direct effect on pathogenicity, motility, and growth of certain microbes, including plant and human pathogens. The effect of light on gene expression and phenotypes in the tomato pathogen *Pseudomonas syringe* pv. tomato DC3000 was investigated and revealed multiple effects depending on the wavelength exposure that maximized survival and virulence on plants (Oberpichler et al., 2008; Rio-Alverez et al., 2014; Santamaria-Hernando et al., 2018). Mussi et al. (2010) studied the response in the opportunistic human pathogen *Acinetobacter baumannii* to blue light involving a gene coding for a photoreceptor protein, which regulates motility, biofilm formation, and killing fungal filaments. The direct biocidal effect of blue light has been studied since the early 20th century as a treatment for *Mycobacterium* skin infections (Wang et al., 2017) and has been demonstrated on many potential pathogens including *Staphylococcus aureus, E. coli*, and *Pseudomonas aeruginosa* (Bache et al., 2018; Halstead et al., 2016; Haridas and Atreya, 2022; Thompson et al., 2017). It stands to reason that photosynthetically efficient light wavelengths that also reduce potential plant and food-borne pathogens in a crop-growth system would have significant benefits in a food production system.

The preparation for crop growth on ISS includes sanitization and sterilization of most materials (Massa et al., 2017), limiting the introduction of microbial contaminants whenever possible. However, agriculture in space faces factors unlike those on Earth including microgravity, exposure to spacecraft environmental sources of contamination, and plant-growth stressors. Previous studies have shown that microbial communities on crops grown in Veggie on ISS differ in composition and density from ground controls and may be influenced by environmental conditions such as elevated CO_2, localized high humidity and water stress (Bunchek et al., 2024; Hummerick et al., 2021; Khodadad et al., 2020). Understanding the consequences of light spectrum on crop microbial communities will have implications in the development of engineered microbiomes for human and plant health, and mechanisms to prevent food-borne illness.

1.4. Objectives/Hypotheses

We predict microbial community composition on tomato plants will differ through exposure to blue-rich and red-rich lighting treatments, and between flight and ground controls due to the direct environmental effects on the microorganisms' growth and physiology or as a result of concomitant changes in the plant's physiology impacting the associated microbiomes. This examination further sought to determine the potential core microbiome and microbiological food quality for tomato plants grown under ISS conditions, which included microgravity, elevated CO_2 and high localized humidity and moisture.

2. Materials and Methods

2.1. VEG-05 test overview

The VEG-05 experiment was conducted on ISS between 12/14/2022-3/24/2023 (101 days) to investigate tomato-growth responses and plant-microbiome differences due to two lighting recipes: 90%R:10%B as the "red-rich" treatment and 50%R:50%B as the "blue-rich" treatment (Fig. 1). A ground control was performed in controlled environment chambers at Kennedy Space Center providing CO_2 concentrations, relative humidity and temperature from data downlinked from the ISS, with a 48-hour delay for ground controls (Table 1). To capture humidity and temperature inside of the Veggie units, HOBO® data loggers (Onset Computer Corporation, Bourne, MA) were installed. HOBOs were installed near the base plate of the Veggie unit at the root pillow level (Fig. 2), therefore internal recorded Veggie humidity data are representative of that location only (Table 1). Before initiation of the VEG-05 experiment, verification ground testing was performed at Kennedy Space Center in controlled-environment chambers providing ISS-comparable temperatures, relative humidity, and CO_2 to help determine watering requirements for the duration of the 101-day experiments. When the tomato plants matured and fruited, three harvests were planned at day 83, 90, and 100 if tomato fruit were present. Other fruit that detached in between these periods were also saved. Water stress in plants led to flower and fruit loss. In total, from the five-surviving red-rich lighted plants in flight, only five ripe fruits were produced, and from the four-surviving blue-rich lighted flight plants, 10 fruits were produced with only six of those ripe by day 100. Additional samples were collected including leaves, roots, substrate from two plant pillows per light treatment, and surface swabs

on and around plant growth hardware, which were frozen and returned to Kennedy Space Center for processing.

2.2. Preflight preparations

2.2.1. Seed sanitization

'Red Robin' tomato seeds (*Solanum lycopersicum*, Totally Tomatoes, Randolph, WI, United States) were surface sanitized using a chlorine-gas-fuming method as described by Massa et al. (2017), except the hydrochloric acid volume was increased from 0.5 mL to 0.75 mL in 30 mL of household bleach (5.25% sodium hypochlorite). Seed-germination tests and confirmation of sanitization were performed on treated seeds. Briefly, ten seeds were placed onto tryptic soy agar (TSA) and inhibitory mold agar (IMA) and monitored daily for bacterial and fungal growth during incubation at 30° C. Germination testing was performed by placing seeds on moist, sterile filter paper in closed petri dishes sealed with Parafilm®. Germination was tracked and recorded.

2.2.2. Plant rooting pillow assembly and supporting materials

Veggie plant rooting pillows were assembled under clean laboratory conditions at the Kennedy Space Center (KSC), FL using the procedure described by Massa et al. (2017b). Each pillow contained 250 mL autoclaved, porous ceramic substrate (Turface Proleague Elite, Profile Porous Ceramics, LLC, Buffalo Grove, IL, United States) sifted to 600 µm-1mm and 1-2 mm and mixed in proportions of 1:1. The substrate was then mixed with controlled-release polymer- coated fertilizer in the following proportions per unit porous ceramic: Nutricote® 14-4-14 at 4 g/L T100 and 6 g/L T180 with 1 g/L Florikan CRF 0-0-19 + 9% Magnesium (Florikan E.S.A., Sarasota, FL, United States) and 1 g/L Maxi Cal ($CaCO_3$; Kelly's Green Team, Kirksville, MO, United

States). For each pillow, three surface-sanitized seeds were attached to germination wicks with guar gum as reported by Massa et al. (2017b). Pillows were individually sealed inside gas-impermeable bags (165 mm x 203 mm Tedlar® bags, SKC Inc., Eighty Four, PA, United States), weighed, and photographed for quality and consistency. Sanitizing wipes used to clean the Veggie facility were prepared at KSC according to the method described in Bunchek et al. (2024).

2.3. ISS operations

Before initiation of the VEG-05 experiment, light mapping was completed for each Veggie Production Unit. A LI-250A meter with a LI-190R quantum sensor (Licor, Lincoln, NE, United States) that measures photosynthetically active radiation (PAR) was used to measure the external ambient light when the Veggie unit lights were off as well as the red, blue, and green light intensity at 10 cm distance from the light banks and at five positions across each Veggie unit. The lighting set points to achieve the same PAR with each red-rich or blue-rich recipe were determined from these measurements (Table 2).

Pillow installation in both Veggie units on ISS was completed on 12/14/2022; water was added to all pillows; the fan was set at low, and the lights turned on. Due to lower-than-normal humidity of incoming cabin air (i.e., ~30% versus normal 40%) from day after initiation (DAI) 0-2, pillows pre-maturely dried out, initiating additional watering tasks. In the flight units, excess water was frequently observed, which may have led to a variety of plant-stress responses including uneven plant growth, excess adventitious root formation, flower and fruit abortion, and visible microbial growth (Fig. 2). This was likely due to unconstrained capillary wicking from

183 the root mat, not countered by gravitational counterforce, resulting in visible accumulation of
184 water droplets on leaves, wicks, and other surfaces including the HOBO data loggers (Fig. 2).
185 Water accumulation was documented on the HOBO data loggers resulting in saturated humidity
186 readings at the bottom of the Veggie units. While flight plants received approximately half the
187 water that ground plants received (Table 3), the uneven growth of plants and conditions of the
188 flight environment caused possible overwatering stress to occur with flight plants. While
189 ground-control plants required water to both the root mat and plant pillows throughout their life
190 after the initial root mat fill at DAI 45, flight plants required very little water that had to be
191 manually added to pillows, relying on water addition from the root mat only.
192
193 As stated in Section 2.1, HOBO® data loggers were installed in the Veggie base plates inside
194 each Veggie unit. Each pillow was watered for the first time with 150 mL of potable water. Due
195 to excessive water in the proximity of the data loggers at pillow level (Fig. 2), data collected by
196 HOBO units were specific to that location in Veggie and did not represent humidity levels
197 throughout the Veggie chamber and in the canopy. Dry ISS air (37.6% RH) was circulated
198 through Veggie (Fig. 1) potentially lowering the RH from the saturated base to the top of the
199 plant canopy. Pillow wicks were opened by the crew at six days after initiation, and at day 10
200 pillows were thinned to one seedling.
201
202 **2.4. Sample collection**
203 Plant, pillow, water, and surface-swab samples were collected from flight and ground
204 experiments at DAI 101. Plant samples included fruit, stem with leaves, and adventitious roots,

while the wick, substrate, and roots were sampled from the pillows. Samples from the flight red-rich treatment were collected from plants in pillows 1, 3, 4, 5 and 6. Fruit was collected from plants 1 and 3. Samples from plants 7 and 10, and 11 were collected from the Blue-rich light treatment, fruit was collected from plants 7, 10, and 12 (Fig. 3). Ground control sample sets were collected from all plants. Two plant pillows from each Veggie chamber (pillows 1 and 3 from the red-rich chamber and pillows 7 and 10 from the blue-rich chamber) that contained plants yielding fruit were removed and stored at – 80°C for analysis from both flight and ground experiments. Swab samples from the flight units were taken after harvest from the surface of the plant pillows and bungees (3 each unit) while eight swabs per unit were collected from the ground control Veggie units. These included additional sample sites not collected in flight such as the bellows surfaces and fans.

Fruit, plant branches with leaves and adventitious roots were wrapped in aluminum foil and stowed in the Minus Eighty-degree Laboratory Freezer on ISS (MELFI) at -80°C. All ground control samples were immediately placed in a -80° C freezer at KSC. Frozen samples were maintained between -80 and -100°C until analysis (Fig. 3).

2.5. VEG-05 post-flight analysis

2.5.1. Microbiological analysis

All plant tissues, pillow wick, and substrate samples were placed into pre-weighed 50 mL centrifuge tubes containing 30 mL sterile phosphate-buffered saline (PBS) with sterile glass beads. Tubes were weighed to determine sample weights and mixed using the Omni BeadRuptor set to shake for three 30 s intervals at 3.1 m/s. Swab samples were placed in sterile PBS with 0.3% Tween 80 and vortexed at high speed for 30 s. Sample extracts and water samples were

diluted and plated in duplicate onto TSA and IMA. After incubation at 30 ℃, enumeration of aerobic bacteria was performed after 48 hours and fungi after 48 hours to five days. Individual colony phenotypes were selected and re-streaked for identification using Biolog Micro ID or Microseq 16S rRNA gene and Fungal D2 LSU gene sequencing. Sample extracts were also plated onto *E.coli*/Coliform and Staph express petrifilm (3M, Saint Paul, MN, United States) and buffered peptone for Salmonella enrichment following procedures adapted from the FDA bacteriological analytical manual (https://www.fda.gov/food/science-research-food/laboratory-methods-food) (Khodadad et al., 2020; Hummerick et al., 2021).

2.5.2. Microbial community sequencing

Samples were shared and acquired upon completion of the microbiological sampling. All liquid was aliquoted into microfuge tubes, centrifuged to 13K x g for 3 minutes, and pellets were collected. Downstream 16S rRNA gene processing for polymerase chain reaction and sequencing followed methods used by Khodadad et al. (2020). The 16S rRNA gene library was sequenced on an Illumina MiSeq V2-500 cycle sequencing kit and 10% Phi-X control library to increase diversity.

Fungal identification was completed using the ZYMO Quick-ITS Plus NGS Library Prep Kit with approximately 10 ng of DNA following manufacturer protocol (Zymo Research, Irvine, CA, USA). Briefly, 10 ng of DNA (when possible) isolated from each sample were barcoded with unique dual indices, and a PCR reaction completed as optimized for this kit. Primers covered sections of the ITS-3f (forward primer, GCATCGATGAAGAACGCAGC) and ITS-4r regions (reverse primer, TCCTCCGCTTATTGATATGC). Each sample was pooled, the

resultant library was cleaned, and quantified with the QUBIT 2.0 fluorometer and the DS high sensitivity DNA assay (Thermo Fisher, Waltham, MA, United States) and sequenced on an Illumina MiSeq with a V3-600 cycle sequencing kit and 10% Phi-X.

2.6. Statistical analysis

Microbiological counts (log transformed) between treatments and consecutive harvests in VEG-05 were compared following one-way ANOVA followed by Tukey's multiple comparisons test using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA). Analyses of the 16S rRNA gene bacterial amplicon sequences were completed using Qiime 2 V. 2024.2 through Conda V. 24.7.1 (Bolyen et al., 2019).Taxa were classified using Greengenes2 V. 2022.10 (McDonald et al., 2024). Relative abundance plots were produced in phyloseq V. 1.48.0 from QIIME2 objects. The DESeq2 package was used to identify taxa that are differentially abundant between light treatments and ground and space flight conditions (McMurdie and Holmes, 2013; Love et al., 2014). Default parameters, consisting of a Wald test with parametric fit, were kept. Plotted results from the analysis included only those having an adjusted p-value of $Padj < 0.01$. Use of the ggplot2 package V. 3.5.1 provided plot customization (Wickham et al., 2016). Heatmaps were created in R version 4.4.1 (Quast et al., 2013; R Core Team, 2024) with the following packages: Qiime2R V. 0.996, tidyverse V. 2.0.0 (Wickham et al., 2019) and phyloseq V. 1.48.0. Heatmaps were created using ampvis2 V. 2.8.9 (Andersen et al., 2018) and RColorBrewer V. 1.1-3.

ITS sequences were analyzed via Kraken 2 (Wood et al., 2019). A custom database was created with ITS specific DNA sequences retrieved from UNITE database (UNITE general FASTA

release for Fungi 2; Abarenkov et al., 2022) to map with Kraken 2. Bracken (Bayesian Re-estimation of Abundance with KrakEN) was used to calculate abundance of species from Kraken 2 output (Lu et al., 2017). Finally, reports generated from Kraken 2 mapping and Bracken's species abundance estimates were visualized using Pavian, a web application for exploring metagenomics classification results (Breitwieser and Salzberg, 2020). Heatmaps for ITS taxa were created as stated previously in R using Ampvis2 V. 2.8.9 following the same script as utilized for 16S rRNA gene visualization.

3. Results

3.1. Microbial counts and culture-based isolation

Figure 4 shows the results of total culturable microbial counts (CFU/g) on plant tissues, pillow components, and surfaces. Aerobic bacterial and fungal-plate counts were highest on the roots and wick material for both ground and flight plants. We observed no difference between red-rich and blue-rich treatments in bacterial and fungal CFU/g for flight samples. For the ground-control samples, the red-rich-treated adventitious roots had significantly lower bacterial and fungal counts than the blue-rich treatment (p=<0.0001). Fungal counts were lower on the red-rich wicks (p=0.0175), and adventitious roots (p=<0.0001). Another possible variable to consider in the comparison of the microbial growth in the two Veggie chambers aside from the light treatment is the high humidity readings taken at the bottom of the chamber and the difference between the two chambers. The HOBO humidity measured over the duration of the experiment in the ground control red-rich lighting unit was on average 12% (+/- 5.5%) lower than the blue-rich chamber ranging from 44% to 98% and 44% to 100% respectively (Supplemental Fig. 1). The only significant differences in microbial counts were lower bacterial

297 and fungal counts in adventitious roots and lower fungal counts on the wick in the red-rich
298 ground control. The difference in humidity readings between the two flight units was 9% (+/-
299 4%). Conversely, in flight, the red-rich chamber had higher HOBO humidity readings (43%-
300 100%) in comparison to the blue-rich lighting chamber (42%-99%) (supplemental Fig. 1) and no
301 difference in microbial counts was observed between samples from different flight Veggie units
302 (Fig. 4).
303
304 Fruit bacterial counts on the ground-control blue-rich treatment (n=14) ranged from 8 to 136
305 CFU/g and 12 to 1.3 x 10^3 CFU/g in the red-rich treatment (n=16). There were fewer fruit
306 harvested and analyzed from the flight plants, with only one from the red-rich treatment and four
307 from the blue-rich treatment, with bacterial counts ranging from 13 (red-rich) to 1.8 x 10^3 (blue-
308 rich) CFU/g. Fungal counts on the ground-control fruit in both treatments were low, <117
309 CFU/g, while the flight samples from the blue-rich treatment were higher than ground controls,
310 ranging from 2.5 x 10^3 to 1.3 x 10^4 CFU/g. Screening for potential pathogens yielded negative
311 results on all fruit samples, indicating an acceptable microbiological quality if the fruit were to
312 be consumed. However, the high fungal counts on the blue-rich flight samples could affect the
313 quality of the fruit if not sanitized.
314
315 The ground-control leaf, fruit, and swab samples yielded lower bacterial and fungal counts than
316 the flight samples. This trend is evident in other Veggie experiments in which edible crops were
317 grown (Bunchek et al., 2024; Hummerick et al., 2021; Khodadad et al., 2020). Access to the
318 ground control experiments is limited and Veggie chambers are housed inside a controlled

environment chamber which could account for lower counts on the surfaces exposed to the air only, such as leaves, and fruit.

Isolate identifications are listed in Table 4. Five genera of bacteria and three of fungi were common to both flight and ground samples, varying by species in some cases. Thirteen different bacterial isolates were identified to at least the genus level from the flight samples, while 19 were isolated and identified from the ground control samples. Six different fungal isolates were identified in the flight samples, while eight were identified in the ground samples. Screening for selected potential human pathogens, such as *E. coli*, *Salmonella* spp., and *S. aureus* yielded negative results, and this was confirmed by community 16S rRNA and ITS sequencing.

3.2. Community sequencing

Cherry tomatoes (cv. Red Robin) were grown in red-rich or blue-rich light treatments, and the impact of these light treatments on the microbiome was investigated. Table 5 shows the average number of reads for the 16S rRNA gene and ITS sequencing runs by sample type. There were some differences between the microbial communities for each light treatment, as well as differences in microbial communities between flight and ground. In general, flight samples with both light treatments had a higher abundance of genera compared to ground samples regardless of humidity differences between chambers. A Venn diagram (Fig. 5) illustrates the genera common between leaf, root, and adventitious root samples for each treatment. While ground samples shared only two genera in the red-rich light treatment and one in the blue-rich, flight red-rich and blue-rich treatment samples had a total of 13 genera, each found between leaf, root, and adventitious root samples. Among the 13 genera, a total of seven genera were found in both

342 red-rich and blue-rich flight treatments. Of these seven common bacterial genera identified by
343 the 16S rRNA gene, *Burkholderia, Rhizobium, Dyadobacter, and*
344 *Methylobacterium/Methylorubrum* were also cultured and identified in the flight samples.
345
346 To elucidate broad differences between treatments, differential abundance plots were created. On
347 average, there were more differences between comparisons of flight and ground samples within
348 each light treatment than there were differences between comparisons of red-rich flight and blue-
349 rich flight treatments, as well as red-rich ground samples vs. blue-rich ground samples (Fig. 6).
350 Figure 6. shows the differential abundance between flight red-rich and ground red-rich samples.
351 Two genera, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Burkholderia-*
352 *Caballeronia-Paraburkholderia*, had over eight-fold greater abundance in flight red-rich samples
353 than in ground red-rich samples. A total of 21 genera were identified to exhibit changes in
354 differential abundance between flight and ground experiments (Fig. 6). These 21 genera, except
355 for *Herbaspirillum*, all exhibited increased abundance in flight samples compared to ground
356 samples. *Herbaspirillum* instead consistently exhibited decreased abundance in flight samples
357 compared to ground samples. Furthermore, this trend of increased abundance in flight as
358 compared to ground was observed in most of the samples, except approximately one-third of the
359 samples in ground controls with blue-rich lighting, which exhibited decreased abundance in
360 flight samples (Fig. 6). Comparisons were also made between light treatments for each condition
361 (flight and ground) (Fig. 7). Red-rich flight compared to blue-rich flight had a greater differential
362 abundance for 13 genera (Fig. 7). In contrast, only two genera (*Helimonas* and *Brevibacillus*)
363 had a greater differential abundance in red-rich ground samples compared to blue-rich ground
364 samples (Fig. 7). High abundance in red-rich flight could be attributed to higher humidity but

365 then the same effect would be seen in blue-rich ground. To investigate whether humidity
366 impacted the microbial community, a PCoA plot was generated and indicated that conditions
367 between flight and ground samples clustered more closely than between treatments with higher
368 humidity (i.e. red-rich flight and blue-rich ground) (Supplemental Figs. 2, 3).
369
370 Further trends were observed when comparing plant parts between flight and ground samples, as
371 well as between light treatments. In general, there was greater abundance of bacteria in flight
372 samples than in ground samples (Fig. 8). Fruit from flight samples had more genera than ground
373 samples, although none were shared between flight and ground samples. As had been found in
374 previous Veggie flight experiments (Khodadad et al., 2020), leaves showed lower diversity of
375 microbes than did roots (Fig. 8). Leaves from flight treatments typically have more diversity than
376 do ground treatments (Fig. 8). While not in the flight fruit samples, *Pseudomonas* was present in
377 ground fruit samples and all leaf samples, flight and ground. However, this genus was not among
378 the cultured isolates from ground or flight samples. The most abundant genus for flight root and
379 adventitious root samples was *Burkholderia-Caballeronia-Paraburkholderia*, which was only
380 the third-most abundant genus in red-rich ground adventitious root samples. (Fig. 8).
381 *Burkholderia* species were also cultured and identified in both ground and flight samples for both
382 light treatments as well as water samples.
383
384 In comparison to the plants, the microbial populations of non-plant parts were more similar
385 between light treatments and condition (flight vs. ground). The genus *Burkholderia-*
386 *Caballeronia-Paraburkholderia* was found in much higher abundance in flight samples
387 compared to ground samples for both light treatments (Fig. 9); *Burkholderia-Caballeronia-*

Paraburkholderia was also found in higher abundance in swab and water samples of red-rich flight samples compared to blue-rich flight samples. For the water samples, *Ralstonia was* found in high abundance in the flight samples but was not very abundant in the ground samples for either light treatment. While *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* was common in the flight plant parts, it was not found in the top 20 genera for flight non-plant parts. In ground samples, "*Candidatus Obscuribacter*" was the dominant genus in both red-rich and blue-rich light treatments.

Analysis of fungal microbial samples involved sequencing the ITS region. Average fungal reads per sample type were lower than bacterial 16S rRNA gene reads (Table 5). Across most plant sample types, *Penicillium* was the most dominant genus identified (Supplemental Fig. 4). Following *Penicillium*, *Fusarium* was found in flight blue-rich samples but not in flight red-rich samples. No fungal sequences were recovered from ground red-rich fruit samples; *Blumeria* was the only fungal genus found in ground blue-rich fruit samples. As was the case for 16S rRNA gene reads, non-plant parts had a higher abundance of reads than plant parts (Supplemental Fig.. 5). Flight blue-rich substrate, wick, and swab samples had greater fungal diversity with the identification of *Trichoderma*, *Hypoxylon*, *Cladorrhinum*, and *Ustilaginoidea*. For ground samples, red-rich substrate had greater abundance of fungal genera compared to blue-rich substrate samples; conversely, blue-rich wick had more genera than red-rich wick samples.

4. Discussion

The work reported here addresses the microbiome present on tomato plants and supporting materials under two lighting regimes in the Veggie units on the International Space Station and ground controls. Characterizing the microbes present on crops and surrounding surfaces serves two objectives, assurance of the microbiological food safety and quality of crops, in this case

411 tomatoes, that are intended to be consumed by the astronauts, and understanding environmental
412 impacts on the microbiome and microbial growth. The environmental conditions on the ISS, in
413 many cases, can provide harsher growing conditions for plants than terrestrial analogs. These
414 challenges extend to microgravity, elevated CO_2 and water stress. Plant growth and the bacterial
415 and fungal communities were affected by water delivery and water behavior in microgravity,
416 confounding the examination of single-treatment differences between the red-rich and blue-rich
417 lighting recipes. In the ISS Veggie units, water accumulated on pillow surfaces, leaves, and
418 adventitious roots, and environmental data loggers. Interior humidity measurements were not a
419 reliable representation of the RH throughout the Veggie chambers as the data loggers were
420 installed at the base of each Veggie unit. These measurements indicated excessively high RH in
421 the case of the red-rich flight unit and the blue-rich ground unit reaching 100%, but with the air
422 flow through the unit, presumably the humidity would be less than those values. The roots
423 remained wet as indicated by the excessive formation of adventitious roots on flight plants, a
424 known response to flooding conditions in tomato as an adaptive escape mechanism for
425 oxygenation (Mhimdi and Pérez-Pérez, 2020). Excessive water accumulation on leaves and
426 stems because of reduced airflow on plants grown in Veggie occurred for a crop of Zinnia grown
427 as a technological demonstration (VEG-01C) in 2015. The plants exhibited disease symptoms
428 such as tissue necrosis, chlorosis, leaf curling, and visible fungal growth, which was identified as
429 *Fusarium oxysporum* from samples sent back to KSC (Schuerger et al., 2021; Urbaniak et al.,
430 2018). In ground-based studies it was determined that the water-stressed plants were susceptible
431 to an opportunistic *Fusarium* infection since unstressed plants failed to develop the infection
432 (Schuerger et al., 2021). Comparative genomics of this isolate determined a close relationship
433 with *Fusarium oxysporum* IMV-00293, an isolate cultured from after the Chernobyl nuclear

plant disaster (Urbaniak et al., 2018). The VEG-05 tomato plants were similarly exposed to excessive moisture build up resulting in leaf guttation and water coated tissues, however, obvious fungal disease symptoms or profuse growth on plant tissues did not develop. In the VEG-05 experiments, the fans functioned optimally to move the drier ISS air through the chamber from bottom to the top possibly mitigating the proliferation of fungal growth. *Fusarium solani*, a potential pathogen of the Solanaceae (Coleman, 2016) was cultured from VEG-05 flight samples and the genus identified with ITS sequencing. Furthermore, *Fusarium oxysporum* was cultured and identified from ground controls. Since the VEG-01C technical demonstration with Zinnia, *Fusarium* has routinely been isolated from Veggie samples from a variety of leafy green technical demonstrations and experiments on board the ISS (Bunchek et al., 2024; Hummerick et al., 2021; Khodadad et al., 2020).

Surfaces, fruit, and leaves on flight plants supported higher numbers of bacteria and fungi than did the ground controls, which can be explained in part by the availability and accumulation of water on these surfaces as well as differences in temperature; flight Veggie units were 2 to 3 degrees (°C) higher on average during the 16-h light cycle, providing more favorable conditions for bacterial and fungal proliferation. The ground-control procedures limit human exposure and experiment manipulation in controlled environment chambers housing the Veggie units. Efforts are made to minimize contamination using pre-entry adhesive mats, protective disposable coverings, and gloves. The Veggie chambers on ISS are not isolated and are exposed to the ISS environment, including air exchange into the plant-growth area, potentially increasing the likelihood of microbial introduction post-experiment initiation. Engineers and scientists perpetually live on-board while conducting research in a multitude of fields, including life and

457 physical sciences (Mayorova et al., 2014). With frequent launches to the ISS, payload and crew
458 changeover introduces additional human-associated microbes that become members of the ISS
459 microbiome (Avila-Herrera et al., 2020; Hospodsky et al., 2012), potentially different than those
460 in the ground control chambers on Earth. Among the cultured genera*, Bacillus, Paenibacillus,*
461 *Microbacterium, Rhizobium, Burkholderia, Curtobacterium, Fusarium, Paecilomyces*, and
462 *Penicillium* were found in both flight and ground samples, perhaps indicating the ubiquitous
463 nature of these microorganisms in the environment including the Veggie plant growth chambers
464 (Hummerick et al., 2021; Khodadad et al., 2020).
465
466 In this study, we did not distinguish between epiphytes and endophytes; it is possible for
467 microbes to colonize vertically (i.e., through the seed) or horizontally (i.e., through air or water
468 transmission) (Frank et al., 2017; Maignien et al., 2014; Mercier and Lindow, 2000). More
469 detailed temporal sampling would be required to determine the source for many of the taxa
470 reported here. *Pseudomonas* was found in all leaf samples regardless of condition (flight or
471 ground) or light treatment, which is consistent with other studies on the tomato microbiome
472 (Chaudry et al., 2021; Dong et al., 2019) and has been shown to act as a plant-growth-promoting
473 microbe (Chandra et al., 2020; Ghadamagahi et al., 2022). Mehlferber et al. (2023) created a
474 commercial synthetic microbial community as a foliar spray isolated from tomatoes grown in a
475 field at the University of California, Davis (Davis, California, United States). Seven of the
476 constituents in this synthetic microbial community were found in the VEG-05-grown plants
477 including *Massilia*, *Pseudomonas*, *Curtobacterium*, *Rhizobiaceae*, *Comamonadaceae*, and
478 *Methylobacterium*.

The use of differential abundance analysis provided information to distinguish between microbiome impacts from flight and ground conditions from those of red-rich light to blue-rich light treatments. Thus, similarities in differential abundance for a given microorganism suggests an effect mediated by flight and ground environments rather than a result of lighting treatments. This analysis then affirms the observation that flight conditions support higher plant microbial abundance than do ground conditions. Members belonging to the phyla Pseudomonadota and Actinomycetota are dominant amongst the 21 genera in higher abundance in flight samples and are documented to be major constituents of the tomato rhizosphere and roots (Naumova et al., 2022; Zhang et al., 2022). This suggests that the higher microbial abundance observed in flight samples is a result of increased root colonization by typical soil-borne microbes.

The development of a typical tomato microbial community on the ISS flight plants implies the source of these microorganisms is limited to seed endophytes and the ISS environment i.e., air, water, surfaces, and crew since the components of the plant growth pillows are sterilized on Earth before flight to mitigate the introduction of human pathogens and phytopathogens. Many of the microorganisms identified in our analyses have also been identified on the ISS. Pseudomonadota, including the genera *Ralstonia*, *Methylobacterium*, *Sphingomonas*, *Burkholderia*, and *Pseudomonas* are dominant in ISS potable and stored water, as well as terrestrial water systems (Castro et al., 2004; Ichijo et al., 2022; Yamaguchi et al., 2014) and were identified in our study as a component of the tomato microbiomes, both ground and flight. Several studies have been aimed at defining the ISS microbiome (Avila-Herrera et al., 2020; Checinska et al., 2015; Ichijo et al., 2022; Venkateswaran et al., 2017; Yamaguchi et al., 2014) identifying many of the microorganisms as human-associated, leading to the conclusion that the crew are the primary source of the varied microbial communities on ISS. Since these

investigations began, around a dozen crops have been grown in Veggie, conceivably introducing additional seed-borne and plant-associated microbes into the ISS microbiome. Studies support this in that many of the microbes associated with tomatoes and plant-supporting materials in this study were isolated and identified in previous Veggie experiments (Bunchek et al., 2024; Hummerick et al., 2021; Khodadad et al., 2020). Additionally, further similarities within the 21 genera and known members of the tomato root microbiome include members of the phyla Bacillota and Bacteroidota, which are observed in high abundances in tomato roots and rhizosphere. While the members of these phyla are known to colonize both tomato rhizospheres and roots, evidence of colonization of all tomato plant parts by a rhizosphere-specific microbiome member is given by the increase of the genus *Edaphobacter,* a member of the phylum Acidobacteriota (now recognized a*s* Acidobacteria), primarily under blue-rich light treatment in flight samples. Members of Acidobacteria are well-documented to be inhabitants of the tomato rhizosphere as opposed to other tomato plant parts, even roots (Cheng et al., 2022; Naumova et al., 2022). Our results reveal that the genus *Edaphobacter* made up 13.6% relative abundance on tomato fruit aboard the ISS. Thus, this observation may offer insight as to how typical soil-borne microorganisms colonize tomato plant parts in the microgravity environment. Of the shared genera between flight and ground, *Herbaspirillum* was the only genus that exhibited a decreased abundance in flight samples compared to ground samples. *Herbaspirillum* is known to confer multiple advantages to its plant host by promoting plant growth despite drought and high-salt stress environments (Cortés-Patiño et al., 2021). The coincidence of a decreased abundance of a drought-stress commensal microorganism and the presence of high-water saturation conditions for plants aboard the ISS may serve as an indicator of potential

524 microbiome shifts of tomato plants experiencing high water stress (da Piedade Melo et al., 2017;
525 Khodadad et al., 2020).
526
527 Comparisons isolating samples between red-rich and blue-rich light treatments serve to identify
528 broad changes in the microbiome community because of light treatments. In general, more
529 genera showed decreased abundance under red-rich light treatment in ground samples as opposed
530 to flight samples with one-third of genera having decreased abundance under red-rich light
531 treatment in flight while nearly all genera, except for *Heliimonas* and *Brevibacillus*, had
532 decreased abundance under red-rich light treatment in ground samples. Only one genus, *Devosi*a,
533 was shared between both light treatments with decreased abundance under red-rich light
534 treatment for both flight and ground samples.
535
536 The general decreased abundance observed amongst genera between red-rich to blue-rich light
537 treatment in ground samples would suggest that blue-rich light treatment was more favorable for
538 microbial growth. A confounding factor is the higher humidity at the base of the ground control
539 blue-rich Veggie unit which could also influence the microbial growth especially on the
540 materials at the base such as the wicking material. With the amount of watering and the obvious
541 excess in flight, it could be assumed that the rhizosphere was saturated resulting in the
542 production of adventitious roots indicating similar moisture content. However, decreases in
543 abundance remained less than 4-fold for most genera, indicating that shifts in the microbiome
544 community as a result of light treatment were not as significant as the differences in microbiome
545 community between flight and ground conditions. *Brevibacillus* exhibited a 2-fold increase in
546 abundance under red-rich light treatment in ground samples. *Brevibacillus* has been uniquely

identified to be antagonistic against *Fusarium oxysporum* infection of tomato plant species as well as providing plant growth promoting effects (Chandel and Deepika, 2009). Further research exploring if commensal plant microorganisms, (e.g. antifungal), are benefited by red-rich light treatment would be of interest.

More significant observations were made between red-rich and blue-rich light treatments within flight samples. For flight samples, it appears that red-rich light treatment favored growth of beneficial microorganisms for tomato plants given that the genera *Novosphingobium, Paenibacillus*, and *Sphingomonas* were all increased in abundance under the red-rich light treatment. These three genera have documented commensal roles to tomato plants as plant growth promoters and inhibitors of *Fusarium* infection (Chandel and Deepika, 2009). Additionally, plant pathogens *Rhodococcus* and *Curtobacterium* were decreased in abundance under red-rich light treatment in flight samples.

Light is conducted throughout plant tissues, thus exposing resident plant epiphytic and endophytic bacteria and fungi. Microbes have evolved to respond to red and blue light wavelengths through a variety of mechanisms including sensing red wavelengths by bacterial and fungal phytochromes triggering gene expression. (Beattie et al., 2018). For example, *Azospirillum brasilense* responds to red light by regulating carotenoid synthesis through the expression of bacteriophytochromes, providing protection from exposure to UV and oxidative stress (Kumar et al., 2012). Phytochromes have also been identified in fungi and influence growth, cell development and virulence (Hu et al., 2014, Wang et al., 2022). Some bacteria, including plant pathogens and symbionts have both red- and blue-light-sensing proteins, and

include members of the genera *Pseudomonas*, *Methylobacterium* and *Sphingomonas*, while *Burkholderia* has only red-light sensing proteins (Mandalari, 2013; Losi & Gärtner, 2021). Understanding the relationship between light and plant microbial response is complicated by the influence of other environmental conditions such as temperature, water availability, and interactions with the plant itself (Beattie et al., 2018).

Notably, *Ralstonia* and *Puia* both exhibited higher abundance under red-rich light treatment in flight samples and higher abundance under blue-rich light treatment in ground samples (Fig. 6 & Fig. 7). This may be due to multiple variables. First, *Ralstonia* was more abundant in chambers with elevated humidity, when comparing flight to flight or ground to ground (Fig. 6 & 7). However, it was still more abundant in both the chambers on ISS regardless of humidity when comparing flight to ground samples. Islam and Toyota (2004) demonstrated that *Ralstonia solanacearum* persisted in soil treatments with higher moisture content. Second, water supplied aboard the ISS contains an established microbial community. The genus *Ralstonia* is commonly found in the water system on board the ISS (Benardini et al., 2005; Bruce et al., 2005; Mijnendonckx et al., 2013). The assumption of *Ralstonia's* introduction to the tomato plants through the water supply aboard the ISS is corroborated by the highest relative abundance of *Ralstonia* (50.3%) being observed in the flight plant water samples. In contrast, the dominant genus in the ground water samples was "*Candidatus Obscuribacter*", which is the name given to a taxon that is well-defined, but unculturable (Soo et al., 2014). Like *Ralstonia's* abundance in the flight samples, "*Candidatus Obscuribacter*" was found on the roots and other non-plant parts of ground samples, with water being the primary source. This observation may serve then as an indication of the significant impact water supply has on the developing tomato microbiome in

593 flight and ground conditions.

594

595 Fungal genera sequenced in this study were like those of previous Veggie experiments on board
596 the ISS (Bunchek et al., 2024; Hummerick et al., 2021; Khodadad et al., 2020). While we were
597 able to identify *Penicillium*, *Aspergillus*, *Trichoderma*, and *Fusarium*, we did not recover any
598 sequences or isolates associated with *Alternaria*. This is surprising considering several ground
599 studies on tomatoes have found *Alternaria*, which in some instances can be pathogenic (Adhikari
600 et al., 2021; Habib et al., 2021; Saleem and El-Shahir, 2022). We were also able to identify
601 *Blumeria* sequences from ground blue-rich tomato fruit samples. Although tomato plants are not
602 typically susceptible to *Blumeria*, tomatoes can exhibit a hypersensitive necrosis response to
603 *Blumeria* as a perceived pathogen (Sameshima et al., 2004; Suzuki et al., 2017), however no
604 such phenotype was observed in our plants. Crops grown on ISS intended for consumption must
605 first and foremost be safe for the crew to eat. Our analysis of the microbial communities of
606 tomatoes grown in Veggie indicate microbiologically safe fruit, as no human pathogens were
607 cultured or detected by sequencing. Most of the scientific literature on the effect of light
608 spectrum on plant associated microorganisms has focused on plant pathogens and resistance with
609 very few reports on the effect on beneficial microbes. Essential to crop health is a robust
610 microbial community that will interact with the plant to promote growth and limit pathogen
611 invasion under challenging environmental conditions like those grown in Veggie on the ISS.
612 This is a consideration when defining horticultural practices such as the lighting treatments
613 chosen for this experiment. Our results suggest that different lighting wavelengths may alter the
614 plant microbial communities and select for certain microorganisms that may be beneficial to the

plants. Understanding the interaction between environmental conditions and a desired engineered microbiome will advance our goal of robust space crop production.

Author Disclosure Statement

No competing financial interests exist.

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Authors contributions

ARD, CLK, LES, RCM, RMW, CAM, GLD, GDM – Contributed to the Experimental Design; CJS, MEH, CLK, ARD, JMS, Data Analysis; CJS, MEH, CLK, CJM-Contributed to the Manuscript Development; all authors reviewed the manuscript for accuracy.

Data Accessibility

The sequencing data associated with this manuscript are stored at Genelab under the name V05-microbiome-16S/ITS OSD-766; doi 10.26030/ywjn-5e23

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Table 1. Relative humidity and temperature data collected every 15 m with HOBO® data loggers inside the Veggie units and ambient ISS data collected every minute over the duration of the experiment. HOBO data (Veggie) are grouped according to day (lights on) and night (lights off) status with (n) number of data points. Ambient ISS flight and ground conditions (nAmbient=145,433). Ground conditions for Veggie day (nVeggie = 3793) and night (nVeggie = 4624). Flight conditions for Veggie day (nVeggie = 5697) and night (nVeggie = 4416). HOBO data readings for one hour period following day-to-night and night-to-day transitions were omitted due to condition restabilization. Standard deviation is in parentheses.

	Ground		**Flight**	
CO_2 *Ambient ISS* (µmol/mol)	2002.3 (150.7)		1946 (10.1)	
Relative Humidity (%) *Ambient ISS*	37.6 (2.3)		37.6 (2.1)	
Veggie (HOBO)	Red-rich	Blue-rich	Red-rich	Blue-rich
Day	82.5 (8.6)	92.6 (11.3)	97.1 (7.9)	88.2 (7.6)
Night	82.4 (9.3)	95.0 (11.7)	98.6 (6.3)	89.5 (6.1)
Temperature (°C) *Ambient ISS*	23.0 (0.4)		23.0 (0.4)	
Veggie (HOBO)	Red-rich	Blue-rich	Red-rich	Blue-rich
Day	21.3 (1.2)	22.2 (1.7)	24.3 (0.8)	24.1 (1.2)
Night	21.5 (0.8)	22.5 (0.6)	23.4 (1.6)	22.8 (1.7)

Table 2. Light settings in ground and flight Veggie units.

Red-Rich					**Blue-Rich**				
Flight			**Ground**		**Flight**			**Ground**	
Locker 1	S/N001		S/N003		Locker 3	S/N010		S/N006	
Color	Setting	PAR*	Setting	PAR*	Color	Setting	PAR*	Setting	PAR*
Red	**260.00**	228.13	**220.00**	225.11	Red	**150.00**	125.43	**120.00**	126.85
Blue	**40.00**	29.09	**30.00**	27.93	Blue	**150.00**	125.36	**130.00**	127.84
Green	**on (30)**	22.50	**on (30)**	25.06	Green	**on (30)**	26.58	**on (30)**	26.26
	Sum	279.72	Sum	278.09		Sum	277.37	Sum	280.96

*PAR is estimated average per Veggie.

Table 3. Water volume (mL) used over the duration of VEG-05 tomato growth experiment.

	Red-Rich Flight	Red-Rich Ground	Blue-Rich Flight	Blue-Rich Ground
Total Number of Plant Pillows*	5	6	4	6
Total Water (mL)	15,505	31,697	15,565	30,805
Water to Plant Pillows* (mL)	6,080	20,897	5,775	20,005
Water to Root Mat Reservoir (mL)	9,000	10,800	9,200	10,800

*Only plant pillows containing plants that grew to maturity were counted

Table 4. Cultured Isolates. R=red-rich, B=blue-rich, W=water sample. Blanks indicate no isolation in either ground or flight samples. Fungi are in bold.

Bacteria- Flight	Bacteria-Ground
Bacillus safensis/pumilus, (R,B) *B. megaterium (B)*	*Bacillus safensis/pumilus (R, B), B. cereus, B. atropheus/subtilis B*
Paenibacillus tundrae (R, B) P. macerans (R)	*Paenibacillus xylanilyticus (B), P. provencis*
Microbacterium spp. *(R, **B**)*	*Microbacterium* spp. *(R, B, W)*
Rhizobium radiobacter (R, B, W)	*Rhizobium rhizogenes (R, B, W)*
Burkholderia contaminans (R, B, W)	*Burkholderia pyrrocinnia/cepacia (R, B, W)*
Curtobacterium flaccumfaciens (R, B,W)	*Curtobacterium pusillum (B)*
Dyadobacter spp. *(R)*	
Brevibacillus spp. *(R, B)*	
Cellulomonas hominis (R, B)	
Methylobacterium exotorquens (R, B)	
Methylorubrum spp. *(R, B)*	
Pantoea agglomerans (R, B)	
Rhodococcus fascians (R, B)	

	Arthrobacter globiformis strain NV W13(R, B)
	Fictibacillus arsenicus (R, B)
	Leifsonia poae (R, B, W)
	Neisseria (R)
	Sphingobacterium thalpophilum (R, B, W)
	Sphingomonas spp. *(R)*
	Staphylococcus saprophyticus (R, B)
	Streptococcus vestibularis (R, B)
	Streptomyces (R, B)
Fusarium solani	***Fusarium* spp., *F. oxysporum***
***Paecilomyces* spp.**	***Paecilomyces* spp.**
Penicillium* spp. , *P. citrinum, P. decumbans	***Penicillium* spp.**
	***Aspergillus* spp., *A. ustus,* A. versicolor**
	Purpureocillium lilacinum

Table 5. Average number of reads for each sample type for 16S rRNA gene and ITS region sequencing samples.

Sample Type	*16S*	*ITS*
Fruit	113	52
Leaf	301	70
Root	17,808	398
Adv Root	10,363	1,509
Substrate	22,591	1,609
Swab	10,297	3,040
Water	17,615	731
Wick	27,376	5,089

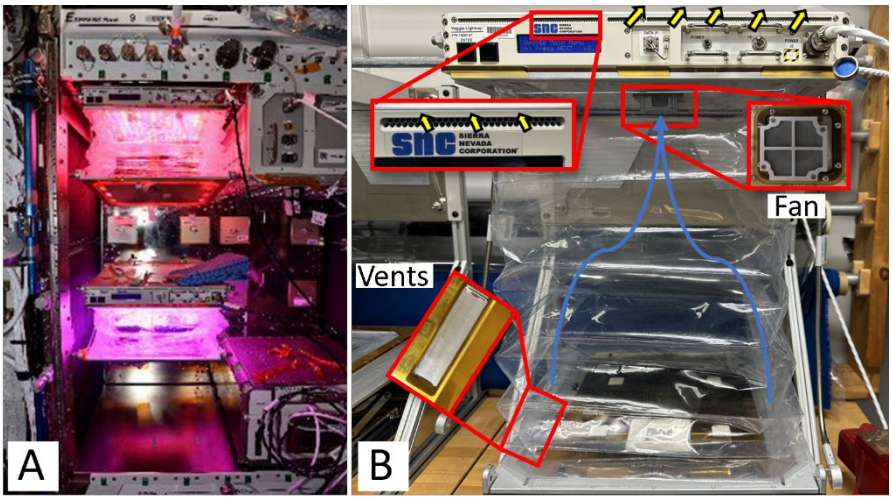


Figure 1. A) ISS installed Veggie units after tomato growth initiation. Lockers containing Red-rich lighting treatment (top) and blue-rich lighting treatment (bottom). B) Airflow capabilities of Veggie unit. Vents (bottom left corner labeled "Vents") in the bottom pull air (blue arrows) in from the cabin environment using a fan embedded into the LED light array (top-middle labeled "Fan"). This air is then exhausted at the front of the unit (yellow arrows), which vents to the cabin.

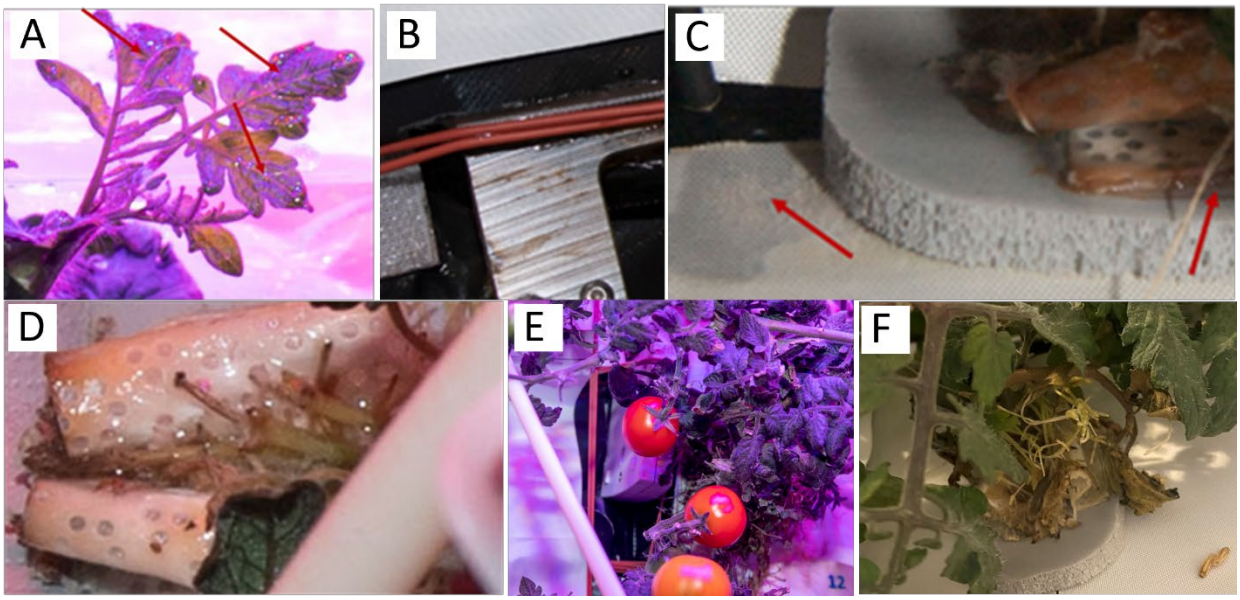


Figure 2. Visible water accumulation on tomato plants (Solanum lycopersicum cv. Red Robin) on ISS. A) leaves B) bungee cords, C) wick and root mat material, D) adventitious roots growing from the tomato stem, E) Placement of HOBO in Veggie Production Unit (red arrow). Notice it is between the pillows and on its side, F) Ground plant for comparison showing similar growth to flight plants.

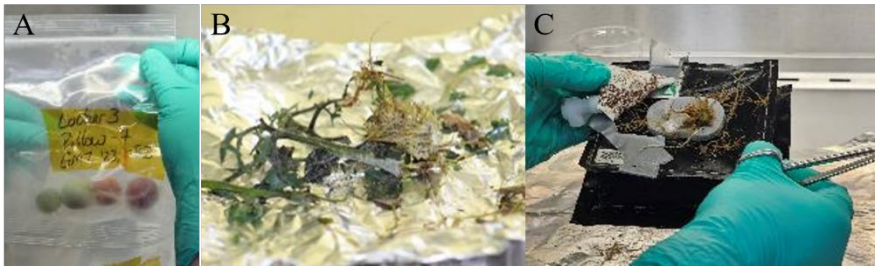


Figure 3. Sample processing at Kennedy Space Center. A) Tomato fruit from blue-rich treatment day 83 harvest, B) stem, leaves, and adventitious roots (red-rich) C) pillow, root material and substrate in pillow.

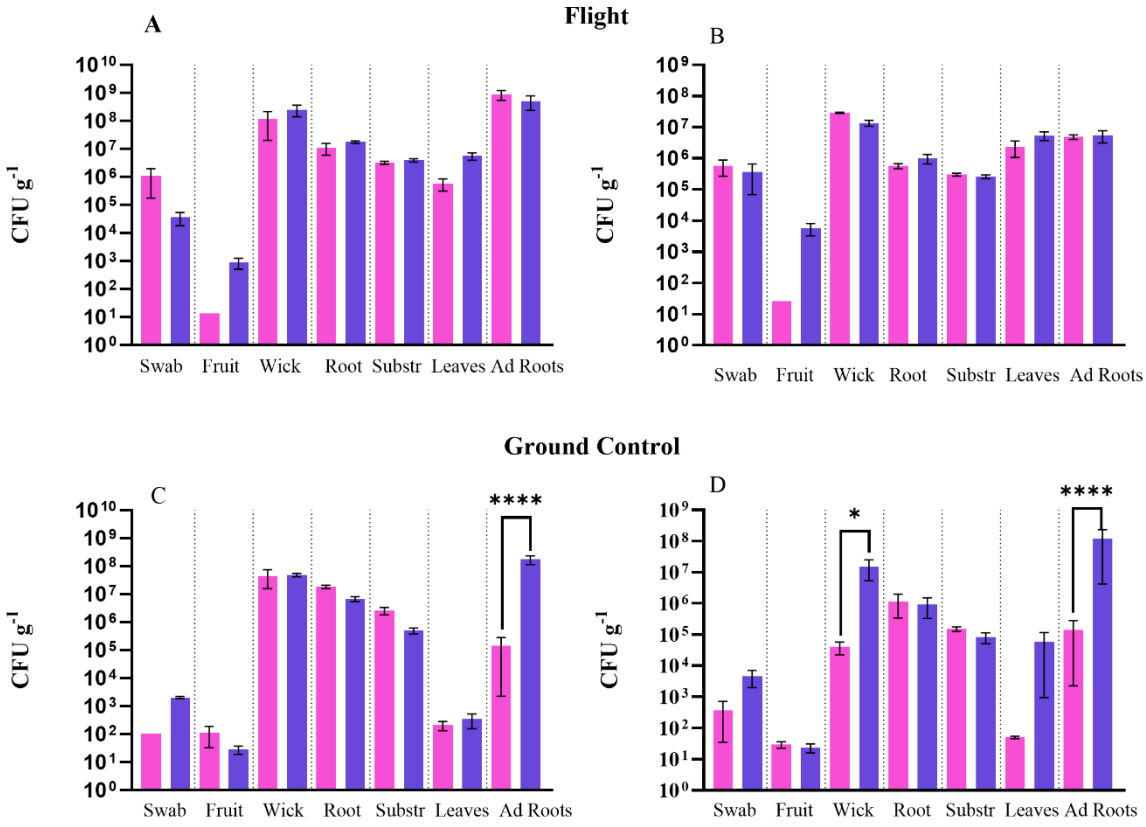


Figure 4. Bacterial aerobic plate counts (A and C) and fungal counts (B and D) (CFU/gram, except for swabs, which was CFU/swab) from ISS flight (A and B) and ground control experiments (C and D). Error bars are standard errors of the mean. Per treatment within ground control and ISS flight: Swabs n=3, fruit flight red-rich n=1, blue-rich n=4, fruit ground red rich n=16, blue rich n=14, wick and roots, n=2, Substrate (Substr) n=6, leaves flight red-rich n=5, blue-rich n=4, leaves ground n=6, Adventitious Roots (Ad Roots) flight n=6, ground n=4, leaves. Significance between pairs of same sample type either ground or flight is indicted by brackets *P<0.01, ****P<0.001. Pink bars indicate exposure to red-rich light treatment and purple bars represent exposure to the blue-rich light treatment.

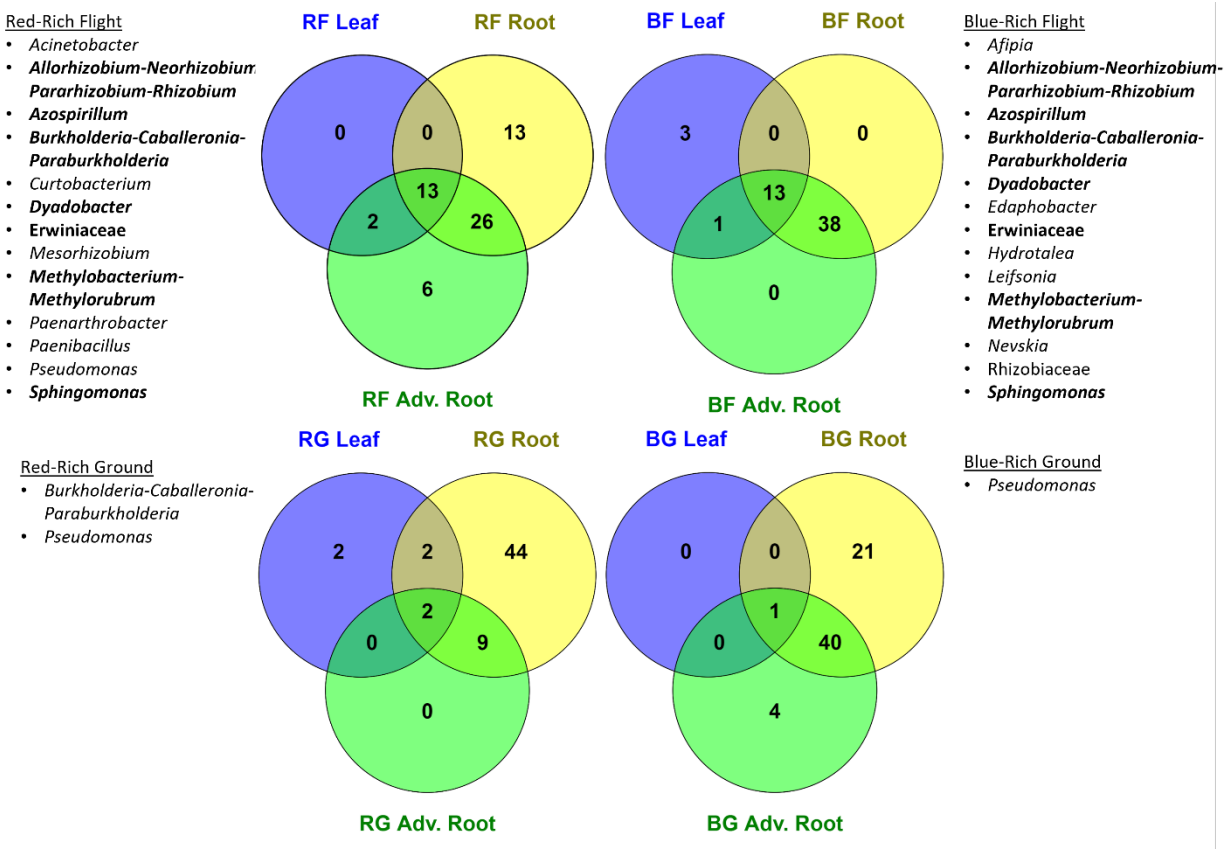


Figure 5. Venn diagrams showing tomato Leaf, Root, and Adventitious Root (Adv. Root) for red-rich and blue-rich light treatments for ISS flight and ground controls. The list of bacteria are genera common across all non-fruit plant parts (fruits were not used in this comparison due low microbial diversity). Bold names indicate genera that are common between red-rich and blue-rich light treatments for flight samples. RF = Red-rich flight, BF = Blue-rich flight, RG = Red-rich ground, BG = Blue-rich ground.

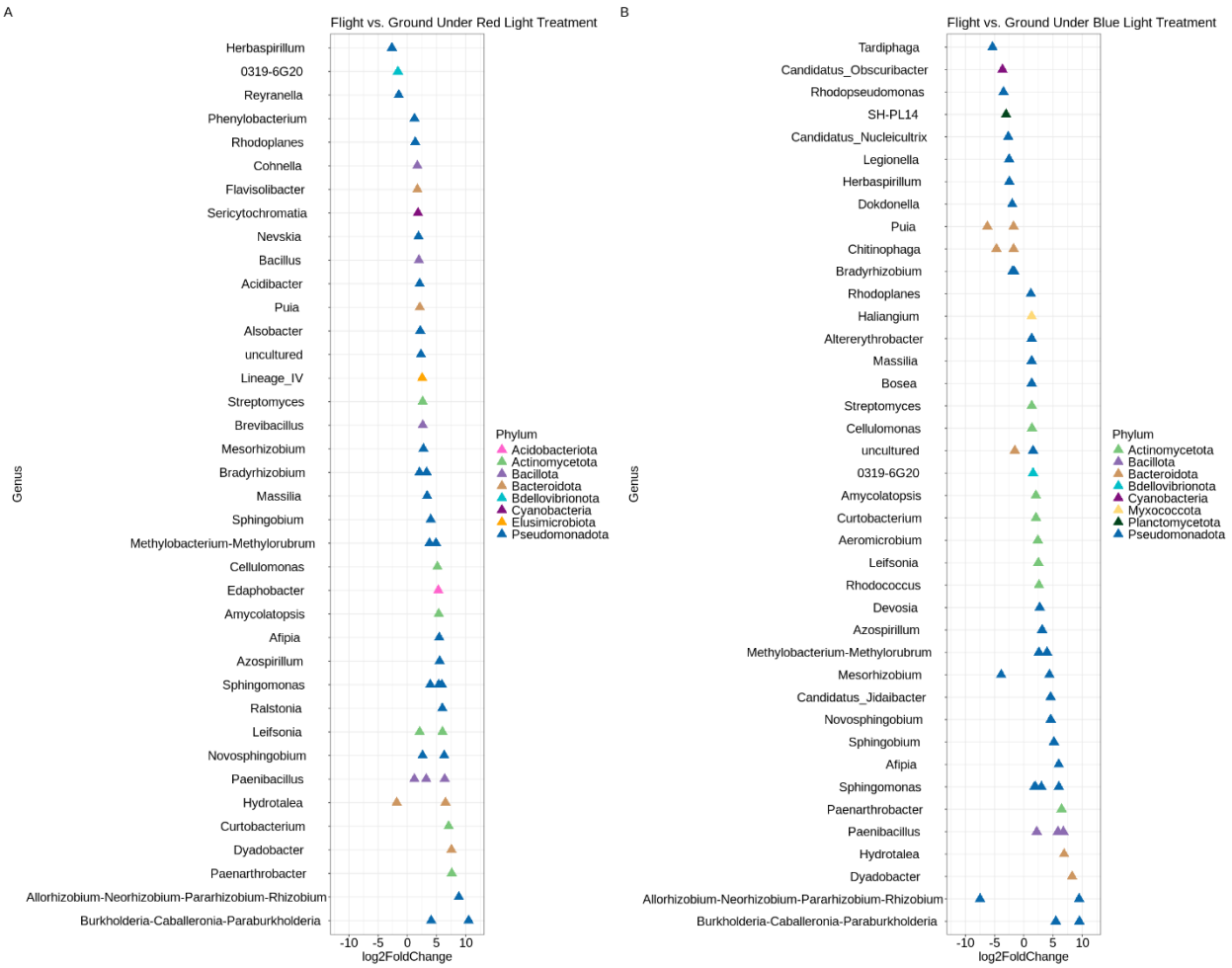


Figure 6. Differential abundance plots showing relative change of bacteria genera between flight and ground samples. A.) Comparison of changes in relative genera abundance between red-rich flight samples and red-rich ground samples. B.) Comparison of changes in relative genera abundance between blue-rich flight samples and blue-rich ground samples. Note: Differential abundance plots contain all samples from each treatment, including plant and non-plant samples. Each plot point represents a genus, colored by phylum. Multiple plot points per genus represents multiple species detected within that genus. Plots display significant differential abundance determined by $P < 0.05$.

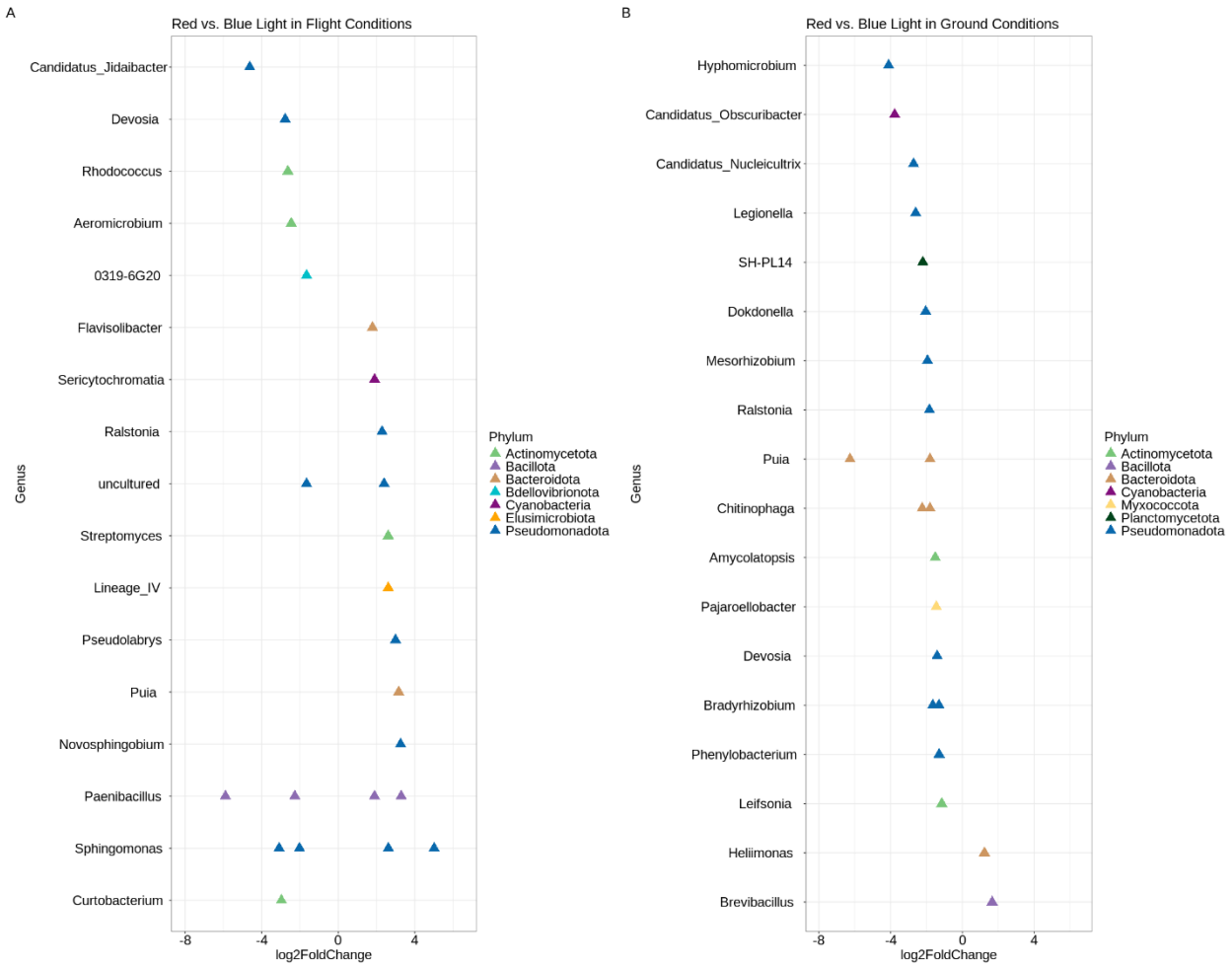


Figure 7. Differential abundance plots showing relative change of bacteria genera between light treatments on flight and ground. A) Comparison of changes in relative genera abundance between flight red-rich and blue-rich samples. B) Comparison of changes in relative genera abundance between ground red-rich and blue-rich samples. Note: Differential abundance plots contain all samples from each treatment, including plant and non-plant samples. Each plot point represents a genus, colored by phylum. Multiple plot points per genus represents multiple species detected within that genus. Plots display significant differential abundance determined by $P < 0.05$.

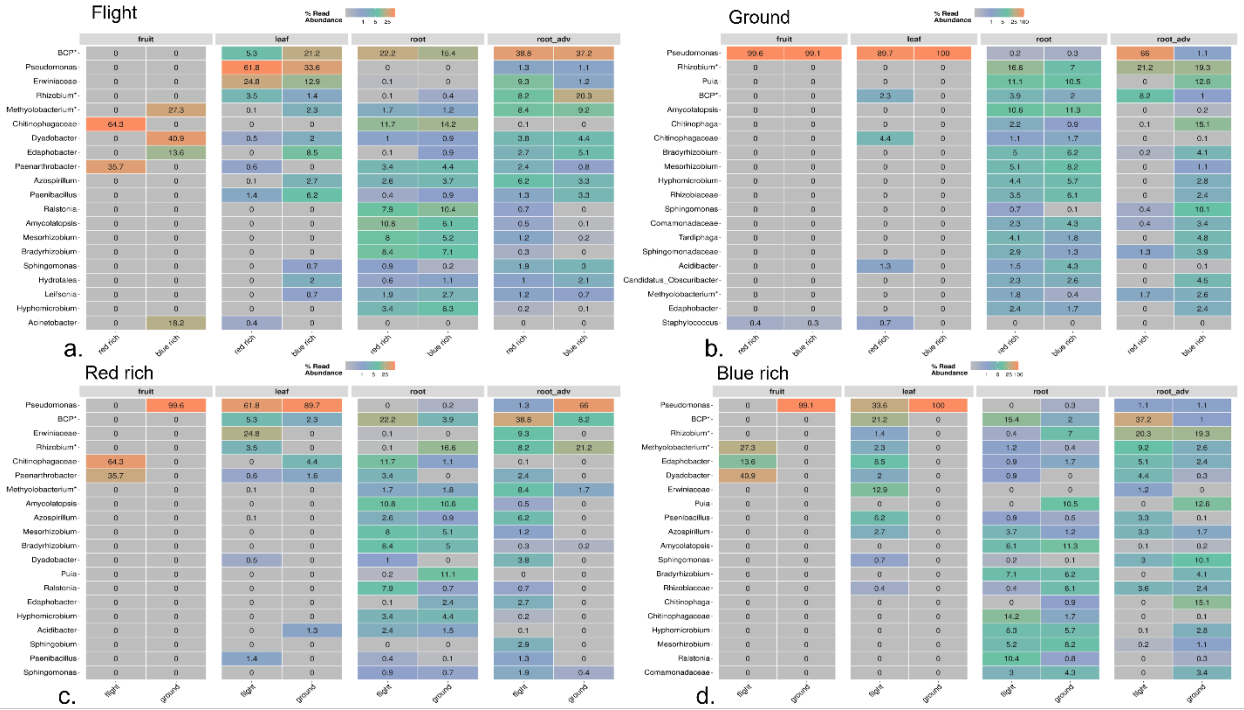


Figure 8. Heatmaps of top 20 bacteria genera on tomato fruit, leaf, root and adventitious root samples using 16S rRNA sequencing. The numbers indicate the percent read abundance of each genus with orange indicating a high abundance and blue indicating a low abundance a.) Flight red-rich plant samples and flight blue-rich plant samples. b.) Ground red-rich plant samples and ground blue-rich plant samples. c.) Flight and ground red-rich plant samples. d.) Flight and ground blue-rich plant samples. *The following genera names were shortened for figure formatting: Methylobacterium/Methylorubrum = Methyloobacterium, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium = Rhizobium, and Burkholderia-Caballeronia-Paraburkholderia = BCP.

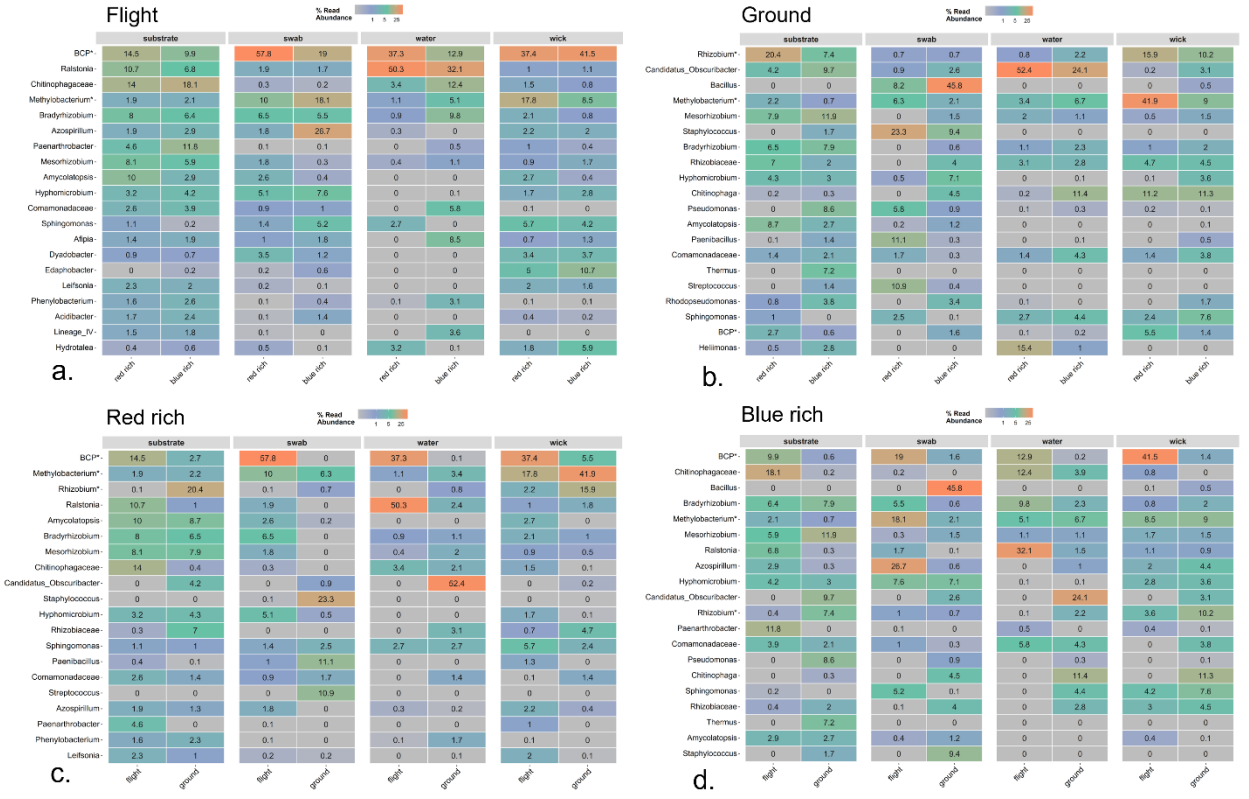
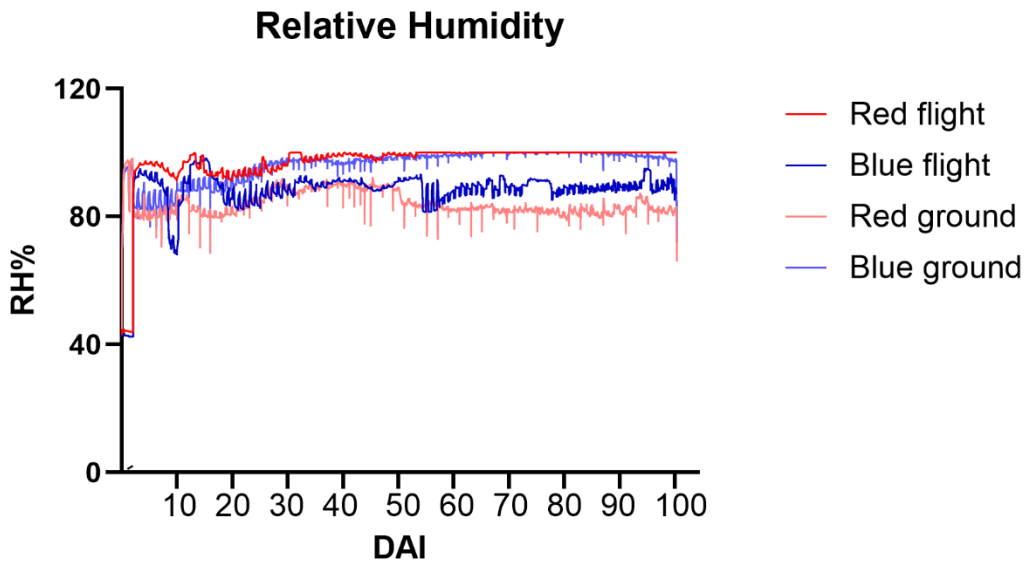
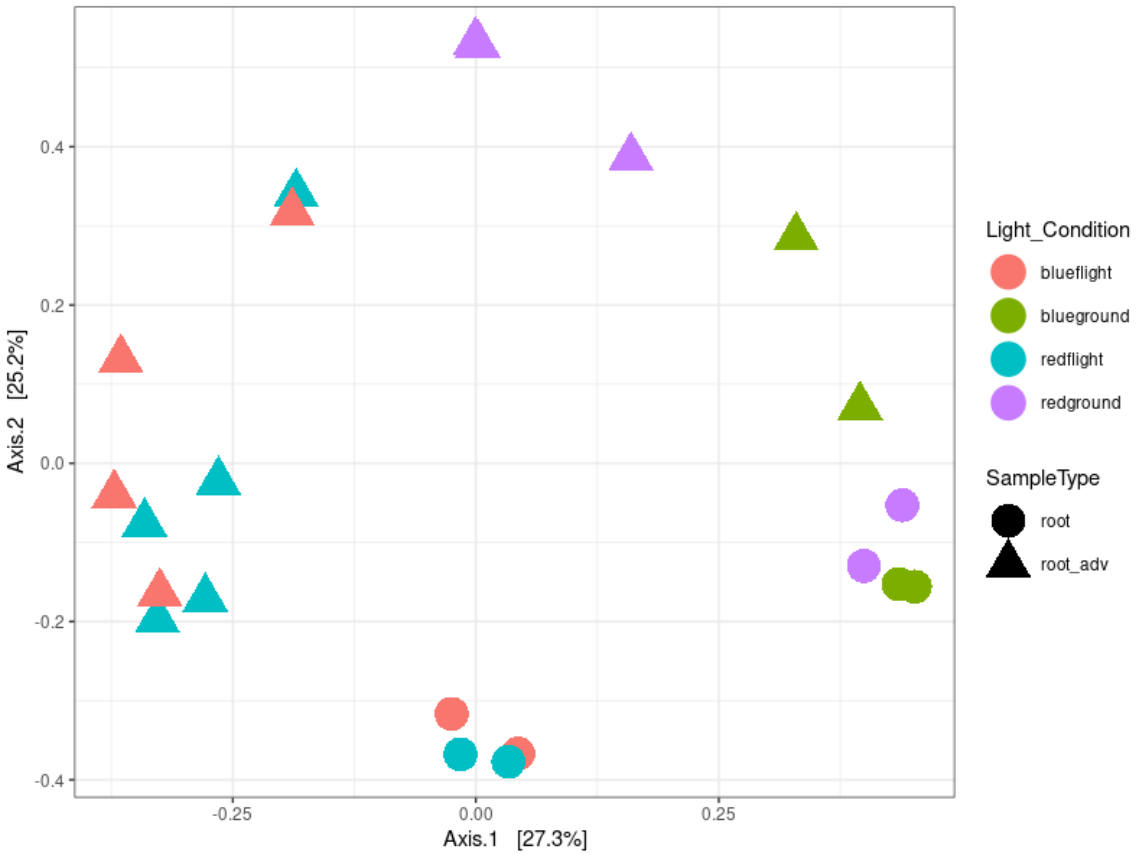


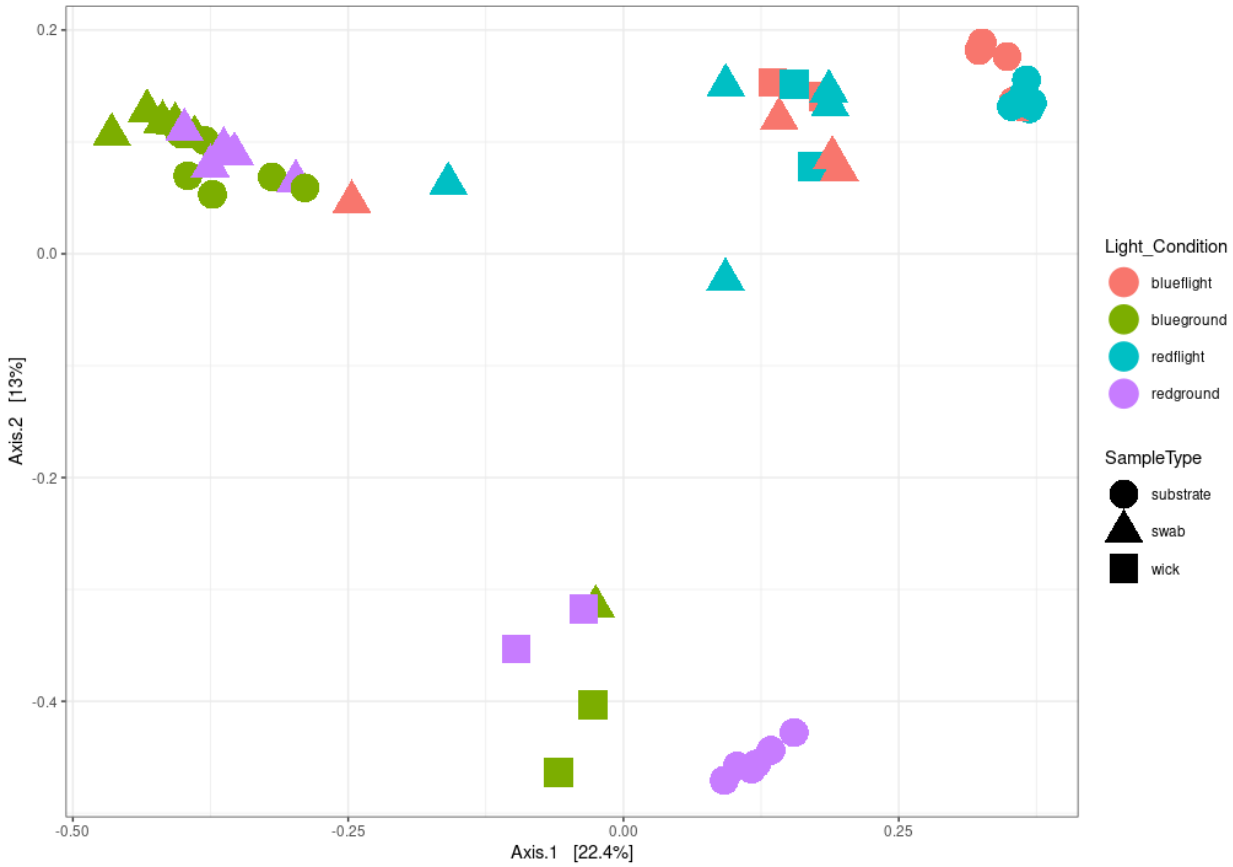
Figure 9. Heatmaps of top 20 bacteria genera on pillow substrate, swabs, water and wick samples using 16S rRNA gene sequencing. The numbers indicate the percent read abundance of each genus with orange indicating a high abundance and blue indicating a low abundance. a.) Flight red-rich non-plant samples and flight blue-rich non-plant samples. b.) Ground red-rich non-plant samples and ground blue-rich non-plant samples. c.) Flight and ground red-rich non-plant samples. d.) Flight and ground blue-rich non-plant samples. *The following genera names were shortened for figure formatting: Methylobacterium-Methylorubrum = Methyloobacterium, Allorhizobium-NeorhizobiumPararhizobium-Rhizobium = Rhizobium, and Burkholderia-Caballeronia-Paraburkholderia = BCP.



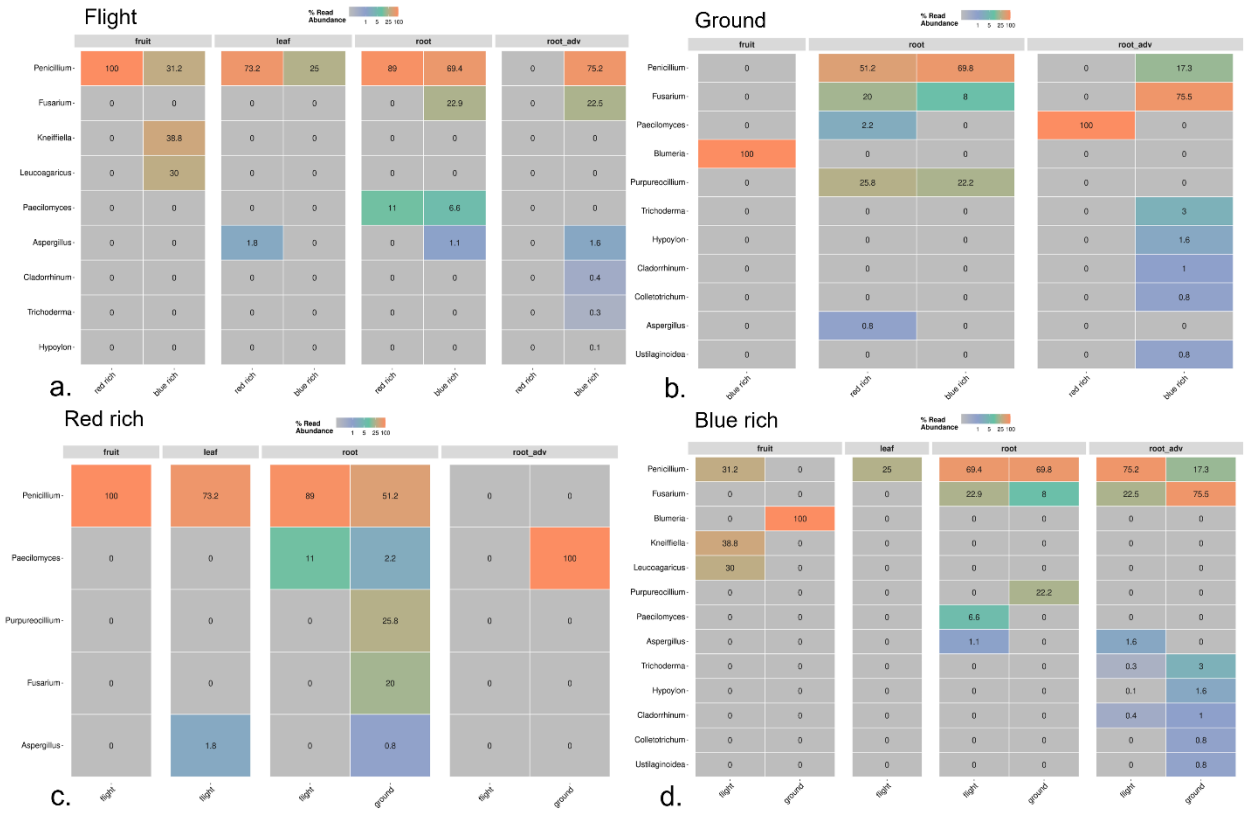
Supplemental Figure 1. Comparison of relative humidity (RH%) between Veggie chambers through time recorded at Days after Initiation (DAI) from HOBO® data loggers inside Veggie unit growth chambers for VEG-05.



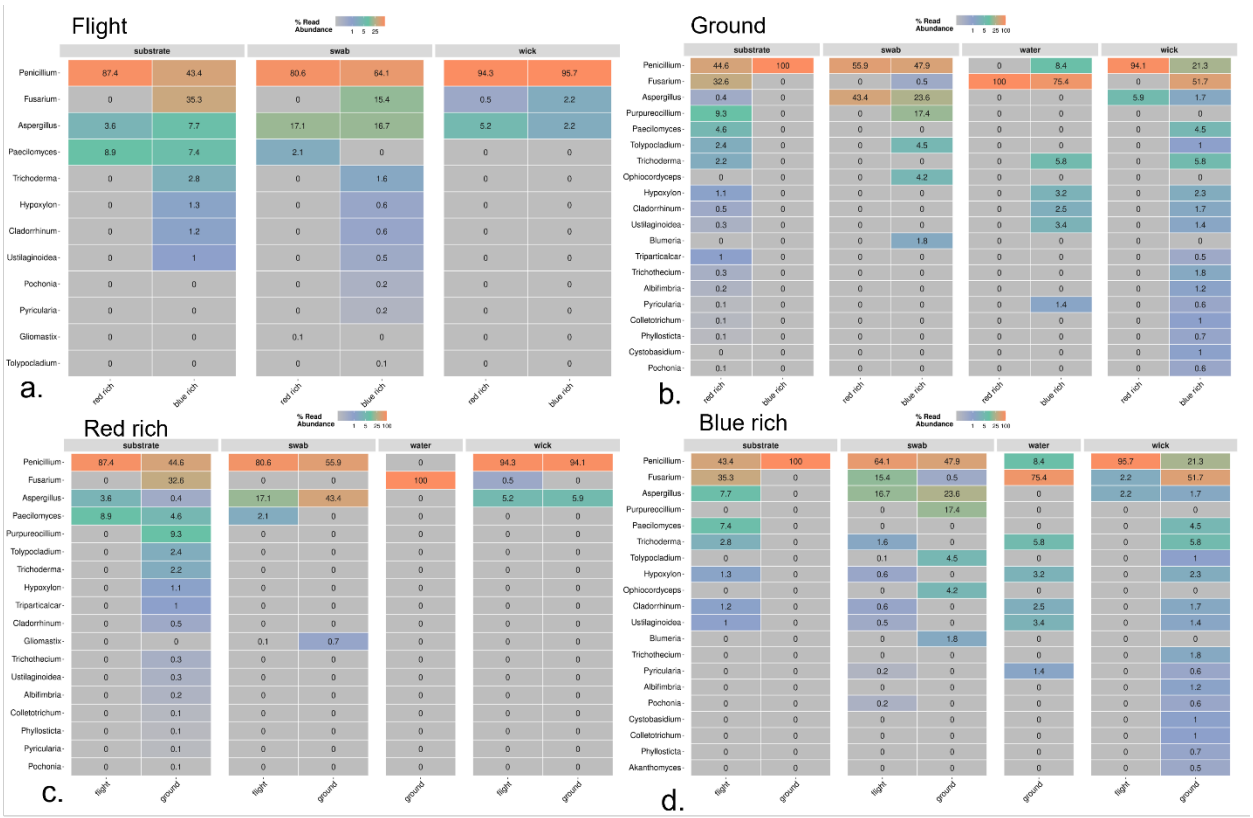
Supplemental Figure 2. Principle Coordinate Analysis (Bray-Curtis) showing bacteria 16S rRNA gene beta diversity of tomato roots, and adventitious roots in four chambers used in VEG-05 experiment. Notice, there is no overlap between chambers with higher humidity, flight red-rich samples (blue coloring) and ground blue-rich samples (green coloring).



Supplemental Figure 3. Principle Coordinate Analysis (Bray-Curtis) showing bacteria 16S rRNA gene beta diversity of substrate, swab, and wick samples in four chambers used in VEG-05 experiment. Notice, there is no overlap between chambers with higher humidity, flight red-rich samples (blue coloring) and ground blue-rich samples (green coloring)



Supplemental Figure 4. Heatmaps showing the top 20 (or less) fungal genera using the ITS region on tomato fruit, leaf, root, and adventitious root samples. The numbers indicate the percent read abundance each of each genus with orange indicating a high abundance and blue indicating a low abundance. a.) Flight red-rich plant samples and flight blue-rich plant samples. b.) ground red-rich plant samples and ground blue-rich plant samples. c.) Flight and ground red-rich plant samples. d.) Flight and ground blue-rich plant samples.



Supplemental Figure 5. Heatmaps showing the top 20 (or less) fungal genera using the ITS region on pillow substrate, swab, water and wick samples. The numbers indicate the percent read abundance of each genus with orange indicating a high abundance and blue indicating a low abundance. a.) Flight red-rich non-plant samples and flight blue-rich non-plant samples. b.) ground red-rich non-plant samples and ground blue-rich non-plant samples. c.) Flight and ground red-rich non-plant samples. d.) Flight and ground blue-rich non-plant samples.