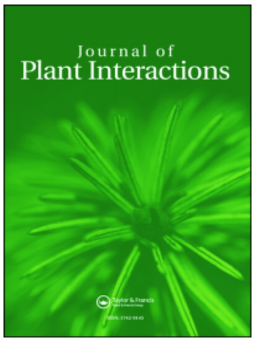

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Effects of elevated and super-elevated carbon dioxide on salad crops for space

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ABSTRACT

Space habitats typically have elevated CO_2 and NASA is considering growing leafy greens in space to supplement astronauts' diets. Dragoon' and 'Outredgeous' lettuce, 'Amara' mustard, 'Extra Dwarf' pak choi, shungiku, 'Red Russian' kale, 'Toscano' kale, and 'Barese' Swiss chard were grown for 4 weeks at 400, 1500, 3000, and 6000 ppm CO_2. Shoot fresh mass at 28 days was greater for one of more elevated CO_2 levels for all species/cultivars except 'Toscano' kale. Fresh mass varied by species/cultivar, with pak choi and 'Dragoon' lettuce showing the greatest yields. Super-elevated CO_2 (6000 ppm) reduced shoot mass for both lettuce cvs. compared to 3000 ppm. Elevated CO_2 increased K levels for most species/cultivars but decreased Mg for some species/cultivars. CO_2 affected Vitamin B1 and Vitamin C content but had no effect on Vitamin K. 'Toscano' and 'Red Russian' kale, and Amara mustard had the highest mineral and vitamin content.

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KEYWORDS
Life support; bioregenerative; CO_2; space; leafy greens

1. Introduction

The possibility of using plants to provide oxygen (O_2) and food during space travel has been discussed and studied for nearly 70 years (Myers 1954). The concept is based on the process of photosynthesis, which uses CO_2 as an input to the carbon reduction cycle (Calvin cycle) driven by light (photosynthetically active radiation – PAR) in the 400–700 nm waveband (Galston 1992). In addition to CO_2 and light, the plants would require a controlled environment with adequate atmosphere composition and pressure, acceptable temperature (~10–35°C) and humidity (~40–85%), adequate supply of water, and mineral nutrients (Marschner 1995; Mitchell 2022).

Studies of crops for life support by NASA in the 1980s and 90s focused largely on agronomic staple crops, such as wheat, soybean, potato, peanut, and sweet potato (Wheeler 2017). In the past 10–20 years, more attention has been given to fresh vegetables and fruits, which can be grown in small plantings on early missions to supplement the crew's diet (Kliss et al. 2000; Richards et al. 2004, 2006; Massa et al. 2015). Fresh food crops typically have a short shelf life but have a high impact on improving diet acceptability and adding supplemental nutrients (Cooper et al. 2017; Massa et al. 2015, 2017; Johnson et al. 2021). Numerous studies have documented the dietary value of leafy greens, which are a good source of nutrients such as K, Ca, Fe, Vitamin C, Vitamin K, and more (Hemmige Natesh et al. 2017; Kumar et al. 2020), and NASA has sponsored ground-based testing of leafy crops such as spinach, lettuce, chard, green onion, beet, pak choi, mizuna, and Chinese cabbage, as well as dwarf tomato, dwarf pepper, and strawberry (Knight and Mitchell 1988; Gilrain et al. 1999; Subbarao et al. 1999; Goins and Yorio 2000; Richards et al. 2004; Hummerick et al. 2010; Massa et al. 2013, 2015; Graham and Wheeler 2017; Mickens et al. 2018, 2019; Spencer et al. 2019, 2020). These crops were grown in controlled environments typically at low to moderate photosynthetic photon flux density (PPFD) levels using just one CO_2 level.

CO_2 from human exhalation can accumulate in space craft and space habitats; concentrations are controlled with physico-chemical systems such as zeolite beds (Knox et al. 2015). Throughout the life of the International Space Station, the cabin CO_2 has ranged from about 1500 to 7000 ppm (0.2 to 0.7 kPa) (Law et al. 2014), while levels on the Russian Mir Station sometimes reached 10,000 ppm (Monje et al. 2000). The effects of CO_2 on plants have been widely studied and the literature is extensive; a common observation is that elevated CO_2 increases photosynthesis and yields for C_3 plants, while decreasing transpiration (Kimball 1983; Drake et al. 1996; Leakey et al. 2012; Bhargava and Mitra 2020). As a result, some controlled environment systems, such as greenhouses, plant factories, and vertical farms enrich CO_2 concentrations to increase crop yields (Porter and Grodzinski 1985; Mitchell 2022). But there appears to be little advantage for exceeding CO_2 levels much greater than 1000–1500 ppm, as C_3 photosynthetic rates and yields typically saturate in this range (Knight and Mitchell 1988; Wheeler et al. 1994; Erwin and Gesick 2017). In some cases, going to super-elevated CO_2 levels (e.g. > 5000 ppm) can cause negative effects on growth and peculiar stomatal responses in some species (Wheeler et al. 1993; 1999; Bugbee et al. 1994; Reuveni and Bugbee 1997; Wang et al. 2015). NASA-sponsored research with lettuce by Knight and Mitchell (1988) showed that elevating CO_2 from 350 to 1000 ppm increased shoot fresh and dry mass of cv. Waldmann's Green, but that elevating it further to 2000 ppm had no additional benefit. Related studies by Richards et al. (2004) showed that elevating CO_2

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

from 400 to 1200 ppm increased fresh and dry mass of lettuce cv. Flandria at three different daily light integrals (DLIs): 8.6, 17.2, and 26 mol photons m^{-2} d^{-1}. Similar results were noted for green onions cv. Kinka and radish cv. Cherry Bomb II (Richards et al. 2004). McKeehen et al. (1996) grew lettuce cv. Waldmann's Green and radish cv. Giant White Globe at 400, 1000, 5000, and 10,000 ppm CO_2 and reported decreased leaf nitrate, increased starch, and increased ash content with elevated CO_2. Other studies with radish at elevated (1000 ppm) and super-elevated (5000 and 10,000 ppm) CO_2 showed no effects on growth for cvs. Cherry Belle and Giant White Globe, but decreased shoot fresh and dry mass for cv. Early Scarlet Globe at 5000 and 10,000 ppm compared to 1000 ppm CO_2 (Mackowiak et al. 1994), suggesting CO_2 effects can vary among genotypes of the same species. Meta-analysis of CO_2 studies with multiple vegetable crops by Dong et al. (2018) indicated that elevated CO_2 increased the concentration of fructose, glucose, total soluble sugar, total antioxidant capacity, total phenols, total flavonoids, ascorbic acid, and calcium, but decreased the concentration of protein, nitrate, magnesium, iron, and zinc. Ssamchoo and Romaine lettuce grew better in elevated CO_2 when compared to a non-CO_2 enriched getting (Noh and Jeong 2021). Greenhouses studies with basil cv. Cardinal, lettuce cv. Auvona, and Swiss chard cv. Magenta Sunset showed increased plant height and width, along with increased shoot fresh and dry mass when grown at 800 ppm versus 410 ppm (Singh et al. 2020).

In this work, our goal was to test candidate leafy green crops in controlled environments that mimic conditions that might be encountered in spacecraft settings, like the International Space Station (ISS). We examined how elevated (1500 and 3000 ppm) and super-elevated (6000 ppm) CO_2 would affect crop growth and nutritional value when compared to current ambient levels near 400 ppm.

2. Materials and methods

Plants were grown in two walk-in environmental control chambers (Percival PGW-48, Perry, Iowa) at NASA's Kennedy Space Center, Florida, US in 2017–2018. The chamber air temperature and relative humidity (RH) were maintained at 23 ± 0.4°C and 50 ± 3% for all tests. Environmental set points were chosen to simulate conditions like the cabin air on the International Space Station. Lighting was provided by six Heliospectra LED grow lights (RX30, Göteborg, Sweden) in each chamber. The RX30 LED fixtures provided near UV (350–400 nm, $\lambda_{max} \approx 385$ nm) ~5 μmol m^{-2} s^{-1}; blue (400–500 nm, $\lambda_{max} \approx 450$ nm) = 22%; green (500–600 nm from white LEDs) = 27%; red (600–700 nm, $\lambda_{max} \approx 630$ and 660 nm) = 51%. The photosynthetic photon flux density (PPFD) at canopy level averaged about 320 μmol m^{-2} s^{-1} with a 16 h light, 8 h dark photoperiod, providing a daily light integral (DLI) of 18.4 mol m^{-2} d^{-1}. Canopy level PPFD was adjusted weekly to maintain uniform lighting for plants of different heights.

CO_2 concentration was managed by adding pure CO_2 to the chambers and monitored using the manufacturer's infrared gas analyzer (Percival). For maintaining the 400 ppm 'ambient' treatment, a CO_2 scrubbing system using NaOH-coated pellets was used. The scrubber was located inside the chambers and would activate when CO_2 exceeded the upper limit. This was necessary due to external CO_2 levels in the room around the chambers ranging from 600 to 800 ppm. Two chambers were used simultaneously for testing, allowing the 400 and 1500 ppm CO_2 concentrations to be tested at the same time. This was followed by replanting the chambers and then controlling to the 3000 and 6000 ppm levels. Following this, all four treatments were repeated using a similar sequence but switching chambers from the initial test to offset potential chamber effects. Since this testing was carried out in Florida near sea level with a total atmospheric pressure of 101 kPa, these CO_2 concentrations correspond to partial pressures of 0.04, 0.15, 0.30, and 0.60 kPa.

Plant species/cultivars tested included, 'Dragoon' lettuce (*Lactuca sativa* L.), 'Extra Dwarf' pak choi (*Brassica rapa* L. subsp. *chinensis* (L.) Hanelt), shungiku (*Glebionis coronaria* (L.) Cass. ex Spach; no cultivar name from seed supplier), 'Barese' Swiss chard (*Beta vulgaris* L. subsp. *vulgaris*), 'Red Russian' kale (*Brassica oleracea* L. *var. viridis* L.), 'Toscano' kale (*Brassica oleracea* L. var. *palmifolia* DC.), 'Amara' mustard (*Brassica carinata A. Braun*) no cultivar name from seed supplier, and 'Outredgeous' lettuce (*Lactuca sativa* L.). All seeds except 'Extra Dwarf' pak choi were purchased from Johnny's Selected Seed (Winslow, Maine, USA); 'Extra Dwarf' pak choi was purchased from Kitazawa Seed (Salt Lake City, Utah, USA). Seeds of each plant type were sown in 12 square plastic pots (9 cm tall, 10.2 cm wide) containing a mixture of 70% professional growing mix (Sun Gro, Agawam, MA), and 30% arcillite (Turface MVP, PROFILE Products LLC; Buffalo Grove, IL). Pots were started side by side with pots for each species/cultivar covering an area of about 0.12 m^2 (Figures 1 and 2). Four seeds were sown in moistened media in each pot. Pots were automatically irrigated to excess several times daily with Peter's 13-2-13

Figure 1. Leafy vegetable crops grown in a controlled environment chamber to study CO_2 effects. White tubes are irrigation drip lines. All plants were grown in 10-cm square pots and watered to excess several times daily with a complete nutrient solution.

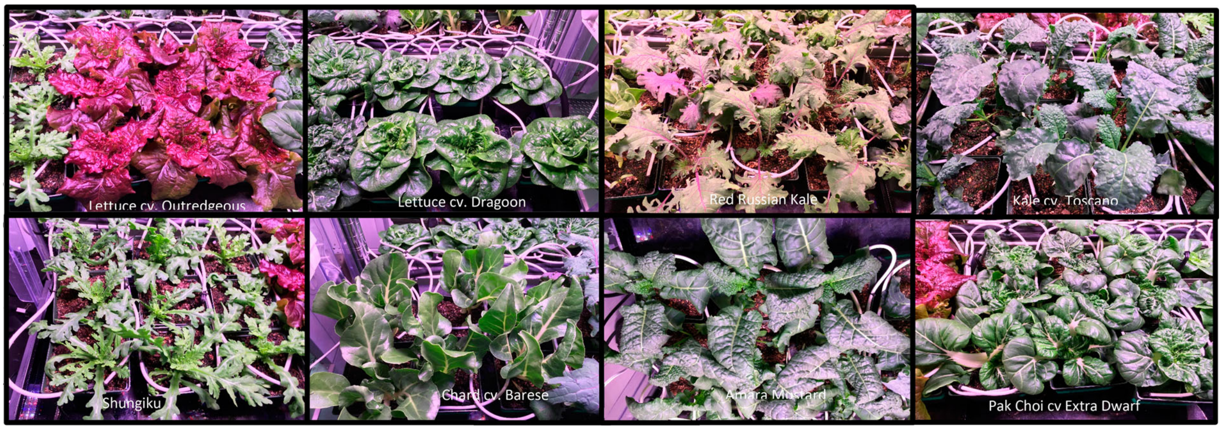

Figure 2. Photos of different leafy green species and cultivars used for the testing. Photos taken ca. 25 days age.

(Everris, Geldermalsen, The Netherlands) nutrient solution, with a 1200 µS cm^{-1} electrical conductivity. All pots were covered with transparent plastic and misted with deionized (DI) water for the first 3 days to promote germination. At 7 days after planting (DAP), seedlings were thinned to one per pot to obtain the most uniform plants.

2.1. Whole plant measurements

Harvests occurred at 14, 21, and 28 days after planting (DAP). Plant height and shoot canopy cover were measured just prior to harvest. Shoot canopy diameter was estimated by looking down from above the plant and measuring the width in two directions offset by 90°. Three plants from each of the eight species/cultivar and each CO_2 treatment were harvested and shoot fresh weights recorded at 14 and 21 days, and 6 plants from each were harvested at 28 days. Following fresh mass (FM) measurements, shoot materials were placed in a drying oven and dried for at least 72 h at 70°C, except for 28-day plants, where dry mass was calculated from subsamples from each species/cultivar and CO_2 treatment based on their % moisture. Following harvests at 14 and 21 DAP, plant pots were randomly rotated within the growing area to reduce position effects.

2.2. SPAD chlorophyll estimates

Prior to fresh mass measurements at the 28-day harvest, three SPAD readings were taken from upper canopy leaves for each plant to non-destructively estimate chlorophyll content (portable SPAD-502DL meter; Konica Minolta Sensing, Osaka, Japan) and calculate an average SPAD per plant. Chlorophyll estimates were expressed directly as SPAD readings.

2.3. Proximate, elemental, and vitamin analysis

Immediately following harvest, approximately 100 g of fresh leaf tissue from each species/cultivar and each CO_2 treatment were placed in zip lock plastic bags and packed in dry ice and sent to a commercial laboratory (Eurofins Nutritional Analysis Center, Des Moines, IA, USA) for analysis of proximate composition (ash, carbohydrate, fat, moisture, protein), elemental content (calcium, iron, magnesium, phosphorous, potassium), and vitamins B1, C, and K. The commercial lab took each sample group and divided it into three subsamples for the analyses. Elements except N were analyzed by Inductively Coupled Spectrometry-ICP (AOAC 984.27, 927.02, 985.01, 965.17 mod.). Nitrogen was analyzed by combustion and subsequently used to estimate protein content by multiplying N by 4.39, which accounts for non-protein forms of N in the tissue, such as nitrate (AOAC 990.03; AOAC 992.15; Fujihara et al. 2001). Percent moisture was analyzed by vacuum oven (AOAC 925.09), ash by combustion / muffle furnace (AOAC 942.05), crude fat by acid hydrolysis (AOAC 954.02), and carbohydrate calculated by difference (CFR 21-calc.). Vitamin levels were analyzed as follows: Vitamin B1-Thiamine HCl (AOAC 942.23 mod., Most Matrices), Vitamin C-Ascorbic Acid (AOAC 967.22 mod.), and Vitamin K1 (Phylloquinone) by an internal Eurofins Scientific Inc. method.

2.4. Statistical analysis

Response variables (shoot biomass, shoot height, shoot diameter, shoot proximate composition, and shoot elemental and vitamin content) were analyzed using linear mixed model methodology as implemented in SAS® PROC MIXED (SAS/STAT 15.2; SAS Institute, Cary, NC). CO_2 concentration, crop type, and their interaction were treated as fixed effect. Experimental repeat nested within CO_2 level was treated as a random effect. We checked the residuals graphically for signs of non-homogeneity of variances based on suggestions by Kozak and Piepho (2018). If there was a problem, we grouped residual variances into homogeneous groups using the AICc penalized fit statistic to avoid overfitting. Least squares means were compared using the Least Significant Difference (t-test) approach without any adjustment for multiple comparisons based on the recommendations made by Milliken and Johnson (2009) and Saville (2015).

3. Results and discussion

3.1. SPAD measurements

SPAD values are commonly used to estimate chlorophyll content of leaves and a comparison of SPAD readings taken at 28 days is shown in Figure 3 and Table 1. ‘Barese’ Swiss chard, ‘Dragoon’ lettuce, ‘Red Russian’ kale, shungiku, and ‘Toscano’ kale all showed a general trend of increasing SPAD values with increased CO_2, suggesting actual leaf

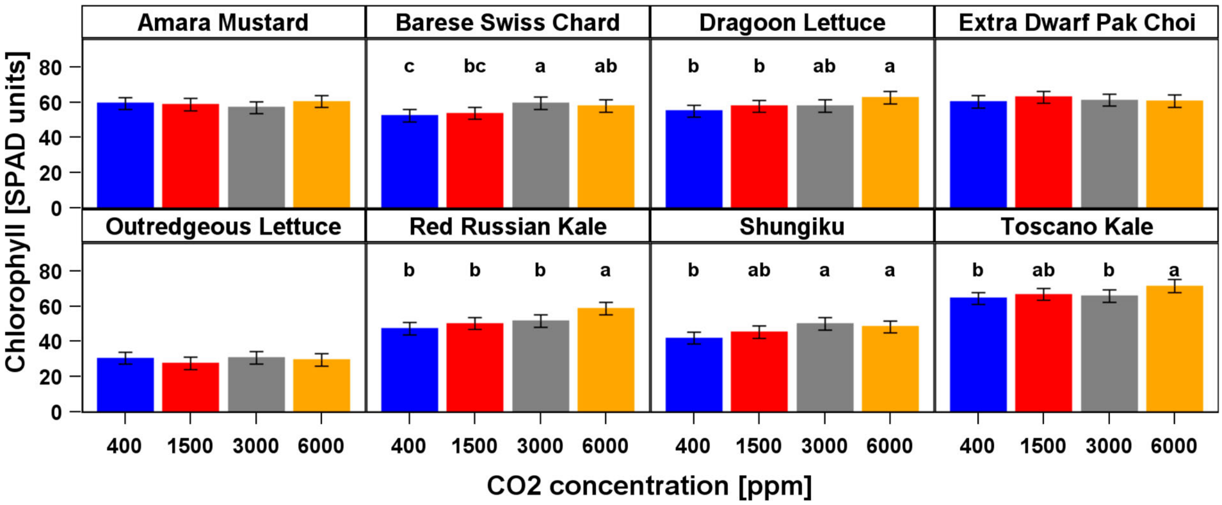

Figure 3. Relative chlorophyll content [SPAD units] from upper canopy leaves measured at final harvest 28 days after planting. Error bars represent 95% confidence intervals and letters indicate difference in response to CO_2 based on a LSD $_{0.05}$. Lettering was omitted in the absence of any statistically meaningful difference among CO_2 levels.

chlorophyll content increased with increased CO_2. In contrast, 'Amara' mustard, 'Extra Dwarf' pak choi, and 'Outredgeous' lettuce showed no significant effect of CO_2 on SPAD value, indicating CO_2 effects on SPAD levels varied by species/cultivar. Leaf SPAD and chlorophyll have often been reported to decrease with elevated CO_2 (Idso et al. 1996; Soares et al. 2022), but in some situations, such as open field free-air CO_2 enrichment (FACE) studies, CO_2 was shown to increase SPAD values, as observed in our study (Pinter et al. 1994).

3.2. Whole plant data

A time course graph of shoot fresh mass yields for all species/cultivars and all CO_2 levels is shown in Figure 4. The greatest yields were from 'Extra Dwarf' pak choi (up to 76 g per plant) followed by 'Dragoon' lettuce (up to 70 g per plant). Interestingly, 'Extra Dwarf' pak choi and 'Dragoon' lettuce were two of the highest performing cultivars selected by middle and high school students conducting citizen science crop research at hundreds of schools in the US through the Growing Beyond Earth ® Program managed by Fairchild Tropical Botanic Garden. In this program students tested crops in ground-based Veggie analog chambers in widely varying classroom environments. The lowest yields were from 'Toscano' kale (up to 29 g per plant) and shungiku (up to 39 g / plant). All eight species/cultivars showed continued growth up to the last harvest, suggesting that these species/cultivars could be harvested later if given adequate spacing (Prince and Bartok 1978). Some of these species/cultivars might be prone to flowering if harvesting is delayed; for example, we have seen that 'Extra Dwarf' pak choi can begin to flower under similar conditions when allowed to grow for 35 days. The time course harvest results show the versatility of leafy greens as controlled environment crops, where they can be harvested across various stages of growth as microgreens, baby greens, teen greens, or more full-sized greens. We have even tested the potential for multiple harvests from the same plants (cut-and-come-again approach) with mizuna mustard grown in the Veggie plant chamber on the International Space Station, where plants grew for 56 days and leaves were harvested approximately every 10 days beginning at 28 days (Massa et al., unpublished; Bunchek et al. 2021). Similar repetitive harvesting was performed by ISS astronauts in 2021 with 'Extra Dwarf' pak choi and 'Amara' mustard (Romeyn et al., unpublished).

Except for 'Toscano' kale, in general, elevated CO_2, i.e. $CO_2 \geq 1500$ ppm, increased fresh mass yields compared to 400 ppm, and this was especially noticeable for Swiss chard and the two lettuce cultivars tested (Figure 4). Lettuce cv. Dragoon averaged 42 g FM/plant under 400 ppm CO_2 at 28 days, and 70 g / plant at 3000 ppm – approximately a 60% increase in yield (Figure 4). 'Outredgeous' lettuce averaged 38 g /plant under 400 ppm and 59 g / plant at 3000 ppm, a 55% increase. By comparison, 'Toscano' kale was 24 g FM / plant at both 400 and 3000 ppm CO_2. Clearly the effect of CO_2 varied by species/cultivar in this study, but the evidence for the positive effect of elevated CO_2 on the growth of C_3 plants is extensive (Kimball 1983; Drake et al. 1996; Bhargava and Mitra 2020.) and is consistent with other studies with leafy greens (Singh et al. 2020). Yet there are some exceptions to elevated CO_2 promoting growth: NASA funded testing with Chinese cabbage (*Brassica rapa* L. subsp. *chinensis* (Lour.) Hanelt) cv. Tokyo Bekana showed reduced growth at elevated CO_2 (900 and 1350 ppm) compared to 450 ppm (Burgner et al. 2020) and field studies often show reduced CO_2 enhancement compared to controlled environment studies (Leakey et al. 2012). The lack of CO_2 enhanced growth for Chinese cabbage could have been interaction between the LED lighting and elevated CO_2 (Burgner et al. 2020), and some lettuce studies have reported that elevated CO_2 should be accompanied by a higher PPFD since stomatal aperture is widened by higher light intensity therefore accommodating the increased gas exchange (Franks and Beerling 2009; Esmaili et al. 2020). In addition, increased carboxylation rates by Rubisco at elevated CO_2 might not always translate into increased biomass if sufficient energy (ATP) is not available for other important metabolic functions (Kirschbaum 2011), which may explain the lack of CO_2 enhanced growth for some species. An equally important question for plant growth in confined space habitats are the effects of 'super-elevated' CO_2, where concentrations can be ≥ 3000 ppm (Salisbury et al. 1997; Monje et al. 2000; Law et al. 2014). Shoot fresh mass of 'Dragoon' lettuce

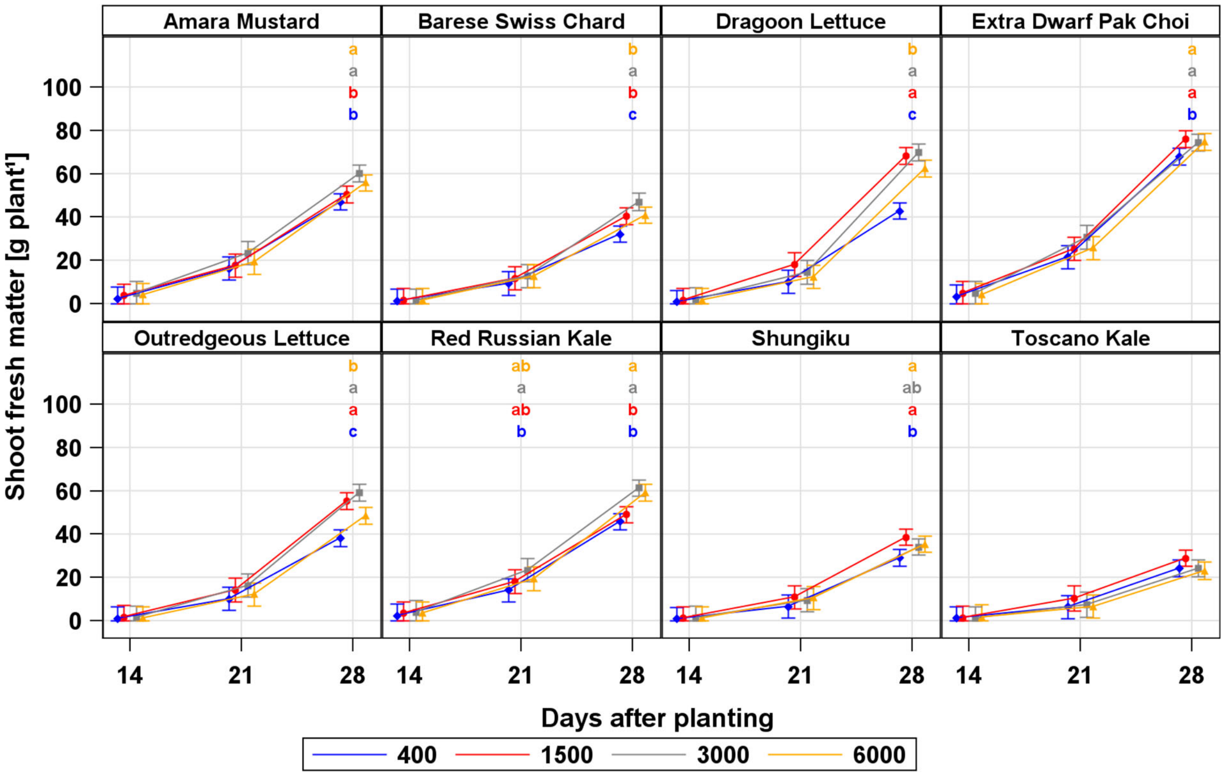

Figure 4. Time course graphs for shoot fresh mass for all species / cultivars at four CO_2 concentrations. All harvests occurred at 14, 21, and 28 days after planting although data points are offset slightly on the graph for ease of comparison. Error bars represent 95% confidence intervals and letters indicate difference in response to CO_2 based on LSD $_{0.05}$. Lettering was omitted in the absence of any statistically meaningful difference among CO_2 levels.

was reduced at 6000 ppm compared to 3000 ppm CO_2 (Figure 4) and shoot dry mass of ‘Outredgeous’ lettuce was reduced at 6000 compared to 3000 ppm (Figure 5). There are other reports of super-elevated CO_2 induced injury or negative effects on some plants (Van Berkel 1984; Ehret and Jolliffe 1985; Wheeler et al. 1993; Reuveni and Bugbee 1997). In prior studies with lettuce, we have seen reduced growth in lettuce cv. Waldmann’s Green at 10,000 ppm

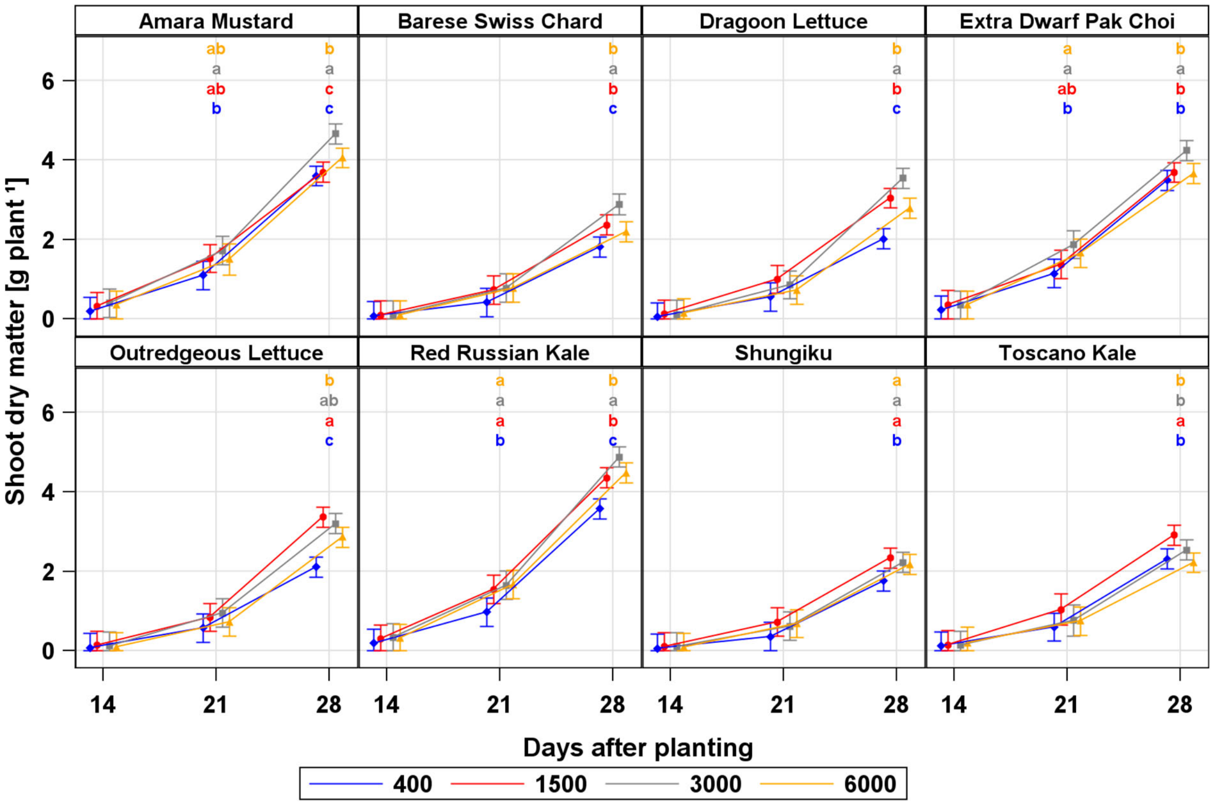

Figure 5. Time course graphs for shoot dry mass for all species / cultivars at four CO_2 concentrations. All harvests occurred at 14, 21, and 28 days after planting although data points are offset slightly on the graph for ease of comparison. Error bars represent 95% confidence intervals and letters indicate difference in response to CO_2 based on LSD $_{0.05}$. Lettering was omitted in the absence of any statistically meaningful difference among CO_2 levels.

CO_2 compared to 1500 and 5000 ppm (unpublished) and a similar effect was noted with several radish (*Raphanus sativus* L.) cultivars (Mackowiak et al. 1994).

Related time-course dry mass harvest data are shown in Figure 5. As with fresh mass, elevated CO_2 levels produced more biomass than the 400 ppm control plants, but the optimal level of elevated CO_2 varied among species/cultivars (Figure 5). Red Russian kale also showed significant difference in dry mass among CO_2 treatments at 21 days, as did 'Extra Dwarf' pak choi (Figure 5), but Extra Dwarf pak choi did not show any difference in shoot fresh mass at 21 days (Figure 4). The percent moisture (and hence % dry mass) showed a significant response to CO_2, but the differences among species/cultivars were more pronounced (Table 1). Average percent dry mass for shoots for all the CO_2 treatments in descending order were as follows: 'Toscano' kale, 10.1%; 'Red Russian' kale, 8.1%; 'Amara' mustard, 7.5%; shungiku, 6.2%; 'Barese' Swiss chard, 5.7%; 'Outredgeous' lettuce, 5.7%; 'Extra Dwarf' pak choi, 5.1%; 'Dragoon' lettuce, 4.6% (Table 1). In this study we focused on CO_2 effects for different leafy green crops, but numerous studies have shown interacting effects between light and CO_2 and further testing to gather more complete response surface data for light and CO_2 is needed, both for space and for earth (Wheeler et al. 1991; Drake et al. 1996; Richards et al. 2004; Perez-Lopez et al., 2015; Wang et al. 2015; Poorter et al. 2021).

A time-course comparison of shoot heights for all species/cultivars after 28 days showed that the crops broke into two general groups, with the taller group including 'Amara' mustard (up to 20 cm) 'Toscano' kale (up to 20 cm), 'Red Russian' kale (up to 19 cm), and 'Barese' Swiss chard (up to 16 cm) at 28 DAP (Figure 6). The shorter species/cultivars included 'Outredgeous' lettuce (up to 13 cm), shungiku (up to 10 cm), 'Dragoon' lettuce (up to 9 cm), and 'Extra Dwarf' pak choi (up to 8 cm) at 28 DAP. Except for 'Extra Dwarf' pak choi, all species/cultivars seemed to be increasing in height up to 28 days. 'Extra Dwarf' pak choi appeared to reach its maximum height at near 21 days (Figure 6). CO_2 had significant effects on plant heights for some species/cultivars, with the tallest plants at 28 DAP for 'Outredgeous' and 'Dragoon' lettuce, 'Red Russian' kale, 'Amara' mustard, and 'Barese' Swiss chard occurring at 3000 ppm CO_2 (Figure 6). As a point of comparison, the growing heights for NASA's Advanced Plant Habitat (APH) and Veggie plant chambers on the International Space Station are about 45 cm (Massa et al. 2016), so all the plants tested in these studies should be able to grow for 28 days in these chambers. A newer plant chamber that NASA is planning to build, called Ohalo III, would only have 36 cm of vertical growth dimension, not including the rooting system components. Hence short growing crops will be especially important for Ohalo III testing on the ISS and future Mars transit missions, where growth volume will be limited and shorter crops will be needed to allow adequate air circulation in the chamber (Kitaya et al. 2003). Elevated CO_2 has been shown to increase shoot heights in some field and greenhouse crops (e.g. Singh et al. 2020; Lamichane et al. 2021) but there are limited data for CO_2 effects on plant height for completely controlled environments.

Table 1. Analysis of variance for different measures collected at 28 days after planting (final harvest).

Measurement	CO_2	Species/Cultivar	CO_2 x Species/Cultivar
SPAD	< 0.0001	< 0.0001	0.0181
Moisture	0.0271	< 0.0001	0.2680
Ash	0.0477	< 0.0001	0.3557
Protein[a]	0.0008	< 0.0001	0.5717
Crude Fat	0.2293	0.0004	0.1408
Carbohydrate	0.1642	0.0003	0.6890
Calcium	0.1965	< 0.0001	0.6931
Iron	0.5161	0.0714	0.4422
Magnesium	0.0740	0.0000	0.0050
Phosphorus	0.3067	0.0001	0.7170
Potassium	0.0027	< 0.0001	0.5954
Vitamin C	0.0685	0.0095	0.0012
Vitamin B1	0.0812	0.0004	0.1885
Vitamin K1	0.0685	0.0095	0.0012

[a] Estimated based on total N content X 4.39 (Fujihara et al. 2001).

Shoot diameters as measured by maximum distance between leaf tips and viewed from above increased with age (Figure 7). By 28 DAP, 'Red Russian' kale and 'Amara' mustard shoot diameters were near 30 cm, while 'Extra Dwarf' pak choi and 'Dragoon' lettuce were approximately 17–18 cm. There were clear differences for shoot diameters among plant species/cultivars and CO_2 treatments, with the greatest diameters at 28 DAP for 'Amara' mustard at 400 and 3000 ppm; 'Barese' Swiss chard at 1500, 3000, 6000 ppm; 'Outredgeous' at 400, 1500, and 3000 ppm; shungiku at 1500 ppm; and Toscano 400, 1500, and 3000 ppm (Figure 7). Greenhouse studies of leafy greens showed that elevated CO_2 increased shoot heights and diameters for lettuce, Swiss chard, and basil (Singh et al. 2020). Because of area and volume limitations for growing plants in space, plants with small shoot heights and diameters are desirable. Volume-efficient crop production was a primary design concern for NASA's Biomass Production Chamber, which used vertically stacked hydroponic shelves and electric lamp banks, as well as the selection of dwarf or semi-dwarf cultivars (Wheeler et al. 1996b; Wheeler 2017). In addition, efficient management of available growing area and volume (height) will be needed to optimize crop productivity for space systems (Berkovich et al. 2009; Hanford et al. 2018), and this remains an active area of research and testing for NASA. This could include automated spacing systems to reduce wasted growing area and or volume (Prince and Bartok 1978).

Among the goals of this research were achieving high productivity along with selection of crops that are safe and nutritious for the crew. These types of criteria will help advance the 'crop readiness level' for candidate species/cultivars (Romeyn et al. 2019). Some of these same goals are drivers for terrestrial controlled environment agriculture, especially for leafy greens in settings such as greenhouses, plant factories, and vertical farms (Kitaya 2019; Sharath Kumar and Heuvelink 2020; Wong et al. 2020; Mitchell 2022).

3.3. Proximate composition

Analysis of the leafy green species and cultivars grown at different CO_2 concentrations for moisture content and proximate (ash, protein, fat, and carbohydrate) composition are shown in Tables 1 and 2. Not surprisingly, the leafy greens had a relatively high moisture content (~90-96%) and hence lower total content of protein, fat, and carbohydrate on a fresh mass basis when compared to some agronomic crops like wheat, soybean, and potato grown in controlled environments (Wheeler et al. 1996a). All measures of proximate composition were significantly different among species

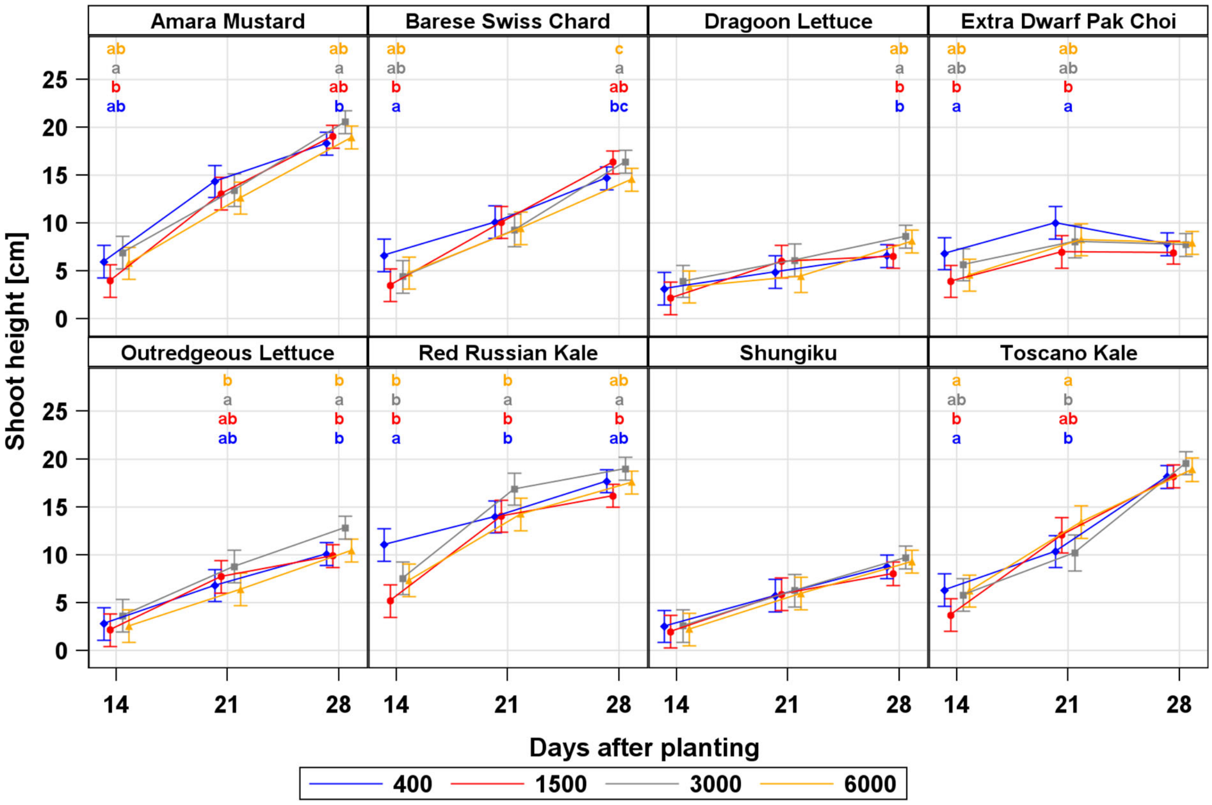

Figure 6. Time course graphs for shoot height for all species / cultivars at four CO_2 concentrations. All harvests occurred at 14, 21, and 28 days after planting although data points are offset slightly on the graph for ease of comparison. Error bars represent 95% confidence intervals and letters indicate difference in response to CO_2 based on LSD $_{0.05}$. Lettering was omitted in the absence of any statistically meaningful difference among CO_2 levels.

/ cultivars, with CO_2 showing significant effects on moisture, ash, and protein. Ash content of the shoot materials ranged from ∼1-2% on a fresh mass basis, carbohydrate from less than 1% ('Extra Dwarf' pak choi) to about 3% ('Toscano' kale and 'Red Russian' kale), fat from about 0.4% ('Dragoon' lettuce) to about 1.0% ('Toscano' kale), and protein from about 1.2% ('Dragoon' lettuce) to about 3.3% ('Toscano' kale) (Table 2). Protein was calculated based on total N content of the tissue X 4.39 (Fujihara et al. 2001). Estimates of dietary energy content (kcal/g) are often included in proximate analysis, but we only had a total carbohydrate estimate, which did not discern fiber and non-fiber constituents making accurate estimates of dietary calorie content difficult. But leafy greens are typically low in calories (Kumar et al. 2020), hence any CO_2 effect might not be meaningful in terms of the overall diet, especially for early space mission where they would only be used for supplemental fresh foods.

Prior NASA sponsored studies showed that ash levels increased in lettuce cv. Waldmann's Green and radish cv. Giant White Globe with increased CO_2, while protein levels tended to decline in radish roots (McKeehen et al. 1996). Studies with tomatoes (*Solanum lycopersicum* L. cvs. Reimann Philipp and Red Robin) showed no significant changes in proximate composition of tomato fruits when plants were grown at 400, 1200, 5000, and 10,000 ppm CO_2, but statistical analyses were not included in those studies (Wheeler et al. 1997). Meta-analysis for a range of crop types showed that elevated CO_2 reduced protein content for fruit, stem, and root vegetables but had no effect on protein levels for leafy vegetables (Dong et al. 2018). Reduced shoot nitrogen content in some crops (e.g. grains) with elevated CO_2 could be related to down-regulation of Rubisco protein at elevated CO_2 or from the effects of reduced photorespiration on nitrate assimilation (Loladze 2014; Dong et al. 2018; Bhargava and Mitra 2020; Ziska 2022). Protein levels of eight different leafy greens in our study showed increases with increased CO_2 (Table 2). This contrast in findings could be related to the different species or cultivars, to overall range of CO_2 tested, or the horticultural approaches used. For example, CO_2 in our study ranged 400–6000 ppm, versus the ambient CO_2, > 200 and ≤ 450 ppm, and elevated CO_2 being 540–1200 ppm in the review by Dong et al. (2018), which included field studies that might not have used regular watering with a complete nutrient solution, as we did. Overall, 'Toscano' kale and 'Red Russian' kale stood out as having higher protein, carbohydrate, and fat per unit weight, which may be related to the lower percent moisture in these two species at the 28 DAP harvest (Table 2).

3.4. Elemental analysis

Analyses of the different leafy crops grown at different CO_2 concentrations for elemental (Ca, Fe, Mg, P, K) composition are shown in Tables 1 and 3. Potassium content increased significantly with increased CO_2, with shungiku and 'Barese' Swiss chard showing the highest levels. Magnesium content showed both a significant CO_2 and CO_2 X crop interaction effects, with Mg levels declining for 'Extra Dwarf' pak choi and 'Red Russian' kale at elevated CO_2 and being lowest at 1000 ppm. In contrast, Mg increased with elevated CO_2 for 'Barese' Swiss and shungiku (Table 3). Overall, 'Toscano' kale had the highest Mg levels but showed no change with CO_2. Prior controlled environment studies with bean (*Phaseolus vulgaris* L.) plants at 1200 ppm CO_2 also showed decreased concentrations of Mg, along with N, P, and Ca,

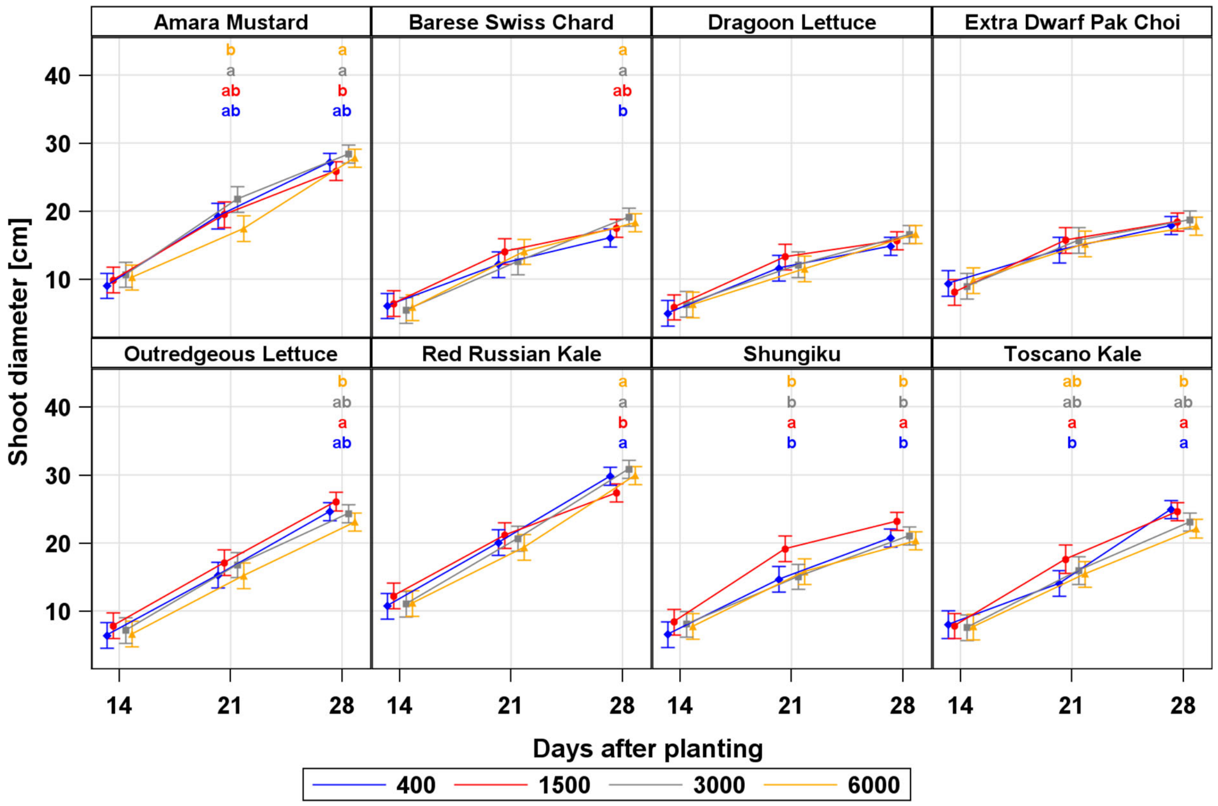

Figure 7. Time course graphs for shoot diameter for all species / cultivars at four CO_2 concentrations. All harvests occurred at 14, 21, and 28 days after planting although data points are offset slightly on the graph for ease of comparison. Error bars represent 95% confidence intervals and letters indicate difference in response to CO_2 based on LSD $_{0.05}$. Lettering was omitted in the absence of any statistically meaningful difference among CO_2 levels.

Table 2. Least squares means ± SE for proximate composition of 28-day-old leafy green shoots grown at four CO_2 concentrations.

Crop	CO_2 (ppm)	Moisture and volatiles (%)	Ash (%)	Carbohydrates (%)	Crude fat (%)	Protein (%)
'Amara' Mustard	400	92.3 ± 0.05a	1.59 ± 0.037a	2.27 ± 0.134a	0.59 ± 0.051a	2.24 ± 0.058b
	1500	92.7 ± 0.85a	1.72 ± 0.104a	1.76 ± 0.825a	0.68 ± 0.051a	2.23 ± 0.058b
	3000	92.3 ± 0.19a	1.68 ± 0.037a	2.02 ± 0.134a	0.55 ± 0.063a	2.46 ± 0.188ab
	6000	92.7 ± 0.65a	1.75 ± 0.075a	1.30 ± 1.045a	0.73 ± 0.120a	2.44 ± 0.058a
'Barese' Swiss Chard	400	94.3 ± 0.05a	1.73 ± 0.037a	1.41 ± 0.134a	0.37 ± 0.014a	1.50 ± 0.058b
	1500	94.2 ± 0.75a	1.60 ± 0.104a	1.71 ± 0.508a	0.38 ± 0.051a	1.52 ± 0.058b
	3000	94.0 ± 0.33a	1.85 ± 0.140a	1.11 ± 0.319a	0.39 ± 0.014a	1.89 ± 0.058a
	6000	94.6 ± 1.00a	1.69 ± 0.175a	0.98 ± 0.734a	0.37 ± 0.014a	1.85 ± 0.131a
'Dragoon' Lettuce	400	95.3 ± 0.05b	1.07 ± 0.037a	1.42 ± 0.134a	0.46 ± 0.051a	1.23 ± 0.058a
	1500	95.5 ± 0.05a	0.89 ± 0.118a	1.44 ± 0.134a	0.37 ± 0.034a	1.23 ± 0.131a
	3000	95.0 ± 0.19b	1.08 ± 0.037a	1.77 ± 0.459a	0.41 ± 0.014a	1.26 ± 0.131a
	6000	95.5 ± 0.40ab	1.09 ± 0.037a	0.99 ± 0.734a	0.40 ± 0.014a	1.38 ± 0.188a
'Outredgeous' Lettuce	400	94.5 ± 0.33ab	1.28 ± 0.075a	1.81 ± 0.134a	0.44 ± 0.063ab	1.63 ± 0.058a
	1500	93.9 ± 0.50ab	1.41 ± 0.280a	2.00 ± 0.134a	0.43 ± 0.014b	1.65 ± 0.058a
	3000	94.6 ± 0.05a	1.34 ± 0.037a	1.43 ± 0.319a	0.46 ± 0.014ab	1.89 ± 0.270a
	6000	94.1 ± 0.19b	1.40 ± 0.037a	1.71 ± 0.459a	0.48 ± 0.014a	1.89 ± 0.058a
'Extra Dwarf' Pak Choi	400	94.9 ± 0.19a	1.18 ± 0.037a	1.24 ± 0.134a	0.41 ± 0.051a	1.38 ± 0.131b
	1500	95.2 ± 0.19a	1.14 ± 0.075a	0.89 ± 0.134a	0.47 ± 0.034a	1.59 ± 0.270b
	3000	94.3 ± 0.05b	1.27 ± 0.075a	1.32 ± 0.459a	0.42 ± 0.014a	1.52 ± 0.188ab
	6000	95.1 ± 0.05a	1.21 ± 0.147a	0.51 ± 0.319a	0.43 ± 0.034a	1.62 ± 0.188a
'Red Russian' Kale	400	92.2 ± 0.05a	1.67 ± 0.037a	2.57 ± 0.134a	0.55 ± 0.014c	2.11 ± 0.058a
	1500	91.2 ± 0.33a	1.60 ± 0.104a	3.60 ± 0.459a	0.67 ± 0.014a	2.09 ± 0.131a
	3000	92.1 ± 0.19a	1.66 ± 0.037a	2.44 ± 0.319a	0.60 ± 0.070abc	2.28 ± 0.131a
	6000	92.4 ± 0.70a	1.62 ± 0.118a	2.33 ± 0.459a	0.62 ± 0.014b	2.13 ± 0.058a
Shungiku	400	93.9 ± 0.05a	1.65 ± 0.075a	1.26 ± 0.134b	0.44 ± 0.014a	1.89 ± 0.058a
	1500	94.0 ± 0.45ab	1.34 ± 0.140a	2.11 ± 0.134a	0.47 ± 0.034a	1.50 ± 0.306a
	3000	93.5 ± 0.19b	1.67 ± 0.037a	1.19 ± 0.508ab	0.48 ± 0.014a	2.22 ± 0.188a
	6000	93.8 ± 0.80ab	1.76 ± 0.104a	1.00 ± 0.734ab	0.45 ± 0.014a	2.35 ± 0.270a
'Toscano' Kale	400	90.5 ± 0.19a	1.82 ± 0.037b	3.15 ± 0.134a	0.81 ± 0.034b	2.61 ± 0.131b
	1500	89.9 ± 0.19ab	1.92 ± 0.037b	3.00 ± 0.134a	0.98 ± 0.014a	2.92 ± 0.058b
	3000	89.6 ± 0.19b	2.13 ± 0.037a	2.75 ± 0.319a	0.94 ± 0.051ab	3.25 ± 0.306ab
	6000	89.3 ± 0.75ab	2.16 ± 0.075a	2.72 ± 0.734a	1.05 ± 0.170ab	3.32 ± 0.131a

Note: Means within species/cultivar groups followed by the same letter are not statistically meaningfully different based on a $LSD_{0.05}$.

but total quantities of these elements in the plant tissue were not affected, and the authors suggested this could have been a dilution effect by increased carbohydrate production at elevated CO_2 (Porter and Grodzinski 1989). Studies by NASA showed increased Ca levels in tomato fruits with elevated CO_2 (Wheeler et al. 1997), but no significant effects on Ca levels were noted for the leafy greens grown in our study (Table 3). Organs like tomato fruit do not transpire much

Table 3. Least squares means on a fresh mass basis ± SE for elemental composition of 28-day-old leafy green plant shoots grown at four CO_2 concentrations.

Crop	CO_2 (ppm)	Calcium (%)	Iron (%)	Magnesium (%)	Phosphorus (%)	Potassium (%)
'Amara'	400	0.176 ± 0.0021a	0.0020 ± 0.00125ab	0.049 ± 0.0009b	0.056 ± 0.0011a	0.532 ± 0.0240b
Mustard	1500	0.165 ± 0.0094ab	0.0009 ± 0.00000a	0.051 ± 0.0009ab	0.054 ± 0.0011ab	0.587 ± 0.0405ab
	3000	0.168 ± 0.0021b	0.0007 ± 0.00005b	0.050 ± 0.0009ab	0.051 ± 0.0011b	0.583 ± 0.0105ab
	6000	0.167 ± 0.0081ab	0.0008 ± 0.00010ab	0.053 ± 0.0009a	0.055 ± 0.0031ab	0.595 ± 0.0105a
'Barese'	400	0.073 ± 0.0021a	0.0006 ± 0.00000a	0.046 ± 0.0009b	0.041 ± 0.0031ab	0.646 ± 0.0405a
Swiss Chard	1500	0.075 ± 0.0093a	0.0007 ± 0.00015a	0.059 ± 0.0009a	0.044 ± 0.0011a	0.693 ± 0.0105a
	3000	0.073 ± 0.0081a	0.0008 ± 0.00015a	0.062 ± 0.0023a	0.041 ± 0.0044ab	0.769 ± 0.0540a
	6000	0.062 ± 0.0094a	0.0007 ± 0.00010a	0.059 ± 0.0023a	0.041 ± 0.0011b	0.709 ± 0.0105a
'Dragoon'	400	0.060 ± 0.0081a	0.0020 ± 0.00100a	0.020 ± 0.0009ab	0.046 ± 0.0031a	0.399 ± 0.0240ab
Lettuce	1500	0.058 ± 0.0063a	0.0009 ± 0.00005a	0.019 ± 0.0009b	0.039 ± 0.0011b	0.399 ± 0.0105b
	3000	0.056 ± 0.0021a	0.0011 ± 0.00015a	0.020 ± 0.0009ab	0.040 ± 0.0011ab	0.413 ± 0.0105ab
	6000	0.061 ± 0.0081a	0.0009 ± 0.00020a	0.023 ± 0.0009a	0.042 ± 0.0031ab	0.441 ± 0.0105a
'Outredgeous'	400	0.072 ± 0.0063a	0.0009 ± 0.00000a	0.025 ± 0.0035a	0.054 ± 0.0044a	0.482 ± 0.0405a
Lettuce	1500	0.061 ± 0.0094a	0.0024 ± 0.00155a	0.029 ± 0.0040a	0.053 ± 0.0115a	0.599 ± 0.0900a
	3000	0.059 ± 0.0021a	0.0021 ± 0.00115a	0.027 ± 0.0009a	0.046 ± 0.0011a	0.524 ± 0.0240a
	6000	0.069 ± 0.0093a	0.0014 ± 0.00020a	0.029 ± 0.0009a	0.049 ± 0.0011a	0.557 ± 0.0105a
'Extra Dwarf'	400	0.170 ± 0.0021a	0.0006 ± 0.00000b	0.046 ± 0.0009a	0.045 ± 0.0011a	0.367 ± 0.0105a
Pak Choi	1500	0.149 ± 0.0063b	0.0006 ± 0.00010abc	0.043 ± 0.0009bc	0.044 ± 0.0031ab	0.392 ± 0.0105a
	3000	0.158 ± 0.0081ab	0.0006 ± 0.00000a	0.045 ± 0.0009ab	0.044 ± 0.0011ab	0.399 ± 0.0240a
	6000	0.127 ± 0.0168ab	0.0006 ± 0.00000c	0.038 ± 0.0023c	0.042 ± 0.0011b	0.402 ± 0.0240a
'Red Russian'	400	0.212 ± 0.0021a	0.0009 ± 0.00000a	0.056 ± 0.0009a	0.058 ± 0.0011a	0.536 ± 0.0105a
Kale	1500	0.173 ± 0.0265a	0.0006 ± 0.00000c	0.053 ± 0.0065a	0.051 ± 0.0044a	0.541 ± 0.0240a
	3000	0.191 ± 0.0168a	0.0007 ± 0.00000b	0.052 ± 0.0023a	0.053 ± 0.0031a	0.557 ± 0.0105a
	6000	0.184 ± 0.0198a	0.0007 ± 0.00010abc	0.054 ± 0.0023a	0.054 ± 0.0057a	0.582 ± 0.0340a
Shungiku	400	0.088 ± 0.0081a	0.0031 ± 0.00120a	0.028 ± 0.0023a	0.062 ± 0.0044a	0.626 ± 0.0105b
	1500	0.068 ± 0.0135a	0.0013 ± 0.00020a	0.026 ± 0.0023a	0.051 ± 0.0080a	0.557 ± 0.0655ab
	3000	0.079 ± 0.0063a	0.0017 ± 0.00050a	0.030 ± 0.0009a	0.061 ± 0.0011a	0.701 ± 0.0105a
	6000	0.083 ± 0.0105a	0.0010 ± 0.00020a	0.031 ± 0.0023a	0.065 ± 0.0057a	0.719 ± 0.0105a
'Toscano'	400	0.279 ± 0.0198a	0.0010 ± 0.00005ab	0.092 ± 0.0030a	0.074 ± 0.0011a	0.499 ± 0.0105b
Kale	1500	0.271 ± 0.0145a	0.0009 ± 0.00005b	0.095 ± 0.0045a	0.067 ± 0.0031b	0.567 ± 0.0105a
	3000	0.311 ± 0.0081a	0.0012 ± 0.00015a	0.097 ± 0.0009a	0.081 ± 0.0090ab	0.603 ± 0.0340ab
	6000	0.304 ± 0.0250a	0.0012 ± 0.00015ab	0.098 ± 0.0030a	0.077 ± 0.0057ab	0.624 ± 0.0405a

Note: Means within species/cultivar groups followed by the same letter are not statistically meaningfully different based on a $LSD_{0.05}$.

Table 4. Least squares means on a fresh mass basis ± SE for Vitamin B1, Vitamin C and Vitamin K of 28-day-old leafy green shoot tissue.

Crop	CO_2 (ppm)	Vitamin B1 (mg/100 g)	Vitamin C (mg/100 g)	Vitamin K1 (µg/g)
'Amara'	400	0.070 ± 0.0022a	7.335 ± 2.8384a	2.220 ± 0.1542a
Mustard	1500	0.062 ± 0.0022b	12.125 ± 7.5750a	3.895 ± 2.7850a
	3000	0.052 ± 0.0022c	8.335 ± 4.0468a	1.915 ± 0.1542a
	6000	0.059 ± 0.0022bc	5.690 ± 2.8384a	1.440 ± 0.5518a
'Barese'	400	0.021 ± 0.0161a	0.220 ± 0.5120a	2.370 ± 0.7743a
Swiss Chard	1500	0.010 ± 0.0046a	0.709 ± 0.5120a	1.660 ± 0.3543a
	3000	0.023 ± 0.0046a	0.806 ± 0.5120a	1.320 ± 0.1542a
	6000	0.025 ± 0.0046a	0.564 ± 0.5120a	1.375 ± 0.1542a
'Dragoon'	400	0.037 ± 0.0022ab	1.308 ± 0.5120a	2.860 ± 0.1542a
Lettuce	1500	0.035 ± 0.0022b	0.616 ± 0.5120a	1.715 ± 0.3543b
	3000	0.044 ± 0.0022a	1.175 ± 0.5120a	1.935 ± 0.1542b
	6000	0.043 ± 0.0097ab	0.890 ± 0.5120a	1.410 ± 0.3543b
'Outredgeous'	400	0.046 ± 0.0022a	2.035 ± 0.5120a	1.905 ± 0.1542a
Lettuce	1500	0.061 ± 0.0139a	1.391 ± 0.5120a	1.080 ± 0.1542b
	3000	0.044 ± 0.0022a	1.374 ± 0.5120a	2.025 ± 0.7743ab
	6000	0.045 ± 0.0022a	0.810 ± 0.5120a	1.100 ± 0.3543ab
'Extra Dwarf'	400	0.042 ± 0.0022a	1.605 ± 0.5120a	3.270 ± 0.5518a
Pak Choi	1500	0.047 ± 0.0046a	2.095 ± 0.5120a	1.900 ± 1.2300a
	3000	0.038 ± 0.0022a	2.410 ± 1.3805a	1.985 ± 0.1542a
	6000	0.050 ± 0.0133a	2.428 ± 1.3805a	2.715 ± 1.6850a
'Red Russian'	400	0.046 ± 0.0022a	2.035 ± 0.5120a	1.905 ± 0.1542a
Kale	1500	0.061 ± 0.0139a	1.391 ± 0.5120a	1.080 ± 0.1542b
	3000	0.044 ± 0.0022a	1.374 ± 0.5120a	2.025 ± 0.7743ab
	6000	0.045 ± 0.0022a	0.810 ± 0.5120a	1.100 ± 0.3543ab
Shungiku	400	0.077 ± 0.0068a	3.040 ± 0.5120b	2.510 ± 0.3543a
	1500	0.057 ± 0.0068ab	10.285 ± 2.4150ab	2.320 ± 0.3543a
	3000	0.048 ± 0.0022b	9.290 ± 0.5120a	1.910 ± 0.5518a
	6000	0.071 ± 0.0068a	3.715 ± 0.5120b	1.825 ± 0.7743a
'Toscano'	400	0.070 ± 0.0068a	0.620 ± 0.5120a	1.770 ± 0.3543ab
Kale	1500	0.061 ± 0.0259a	0.220 ± 0.5120a	1.040 ± 0.1542b
	3000	0.063 ± 0.0022a	0.947 ± 0.5120a	1.895 ± 0.3543a
	6000	0.074 ± 0.0068a	0.425 ± 0.5120a	1.360 ± 0.5518ab

Notes: All data expressed on a fresh mass basis. Means within species/cultivar groups followed by the same letter are not statistically meaningfully different based on a $LSD_{0.05}$.

water in comparison to leaves, and Ca deficiencies are often associated with decreased transpiration (Barta and Tibbitts 1986), thus one might argue that transpiring tomato leaves at lower CO_2 might have redirected Ca away from tomato fruit in the earlier NASA study. Meta analyses of mineral composition from field grown plants showed that elevated

CO_2 generally decreased mineral nutrients around 8% (Loladze 2014), while greenhouse studies with leafy greens showed decreased leaf N, P, and Mg with increased CO_2, but changes in elemental levels were not consistent among species/cultivars (Singh et al. 2020). The effects of increased CO_2 on plant nutrient content and its effects on human nutrition have been an area of concern (Myers et al. 2014; Bhargava and Mitra 2020), yet for our study with leafy greens grown in a controlled environment and watered with nutrient solution, only Mg showed a decrease for some species/ cultivars, and other elements such as K and N (expressed as protein) showed increases with CO_2. Nitrate levels have been reported to decrease for many species, suggesting levels may vary depending on elevated CO_2 effects on nitrate uptake and/or nitrate reduction (McKeehen et al. 1996; Gruda 2005; Dong et al. 2018). Other hydroponic studies comparing soybeans at 400 and 800 ppm CO_2 grown with or without iron stress showed decreased content of P, K, Mn, Zn and Fe in leaves at 800 ppm and no iron stress, but increased K and N roots, and increased Mg in leaves at elevated CO_2 for plants grown under iron stress (Soares et al. 2022).

3.5. Vitamin content

Analyses of vitamin contents of the different species and cultivars grown at different CO_2 concentrations are shown in Tables 1 and 4. Vitamin C and B1 content were significantly different among species / cultivars, with Vitamin C ranging from ~1 to 16 mg / 100 g fresh mass and Vitamin B1 from ~0.02 to 0.08 mg/100 g fresh mass. CO_2 had a significant effect on both Vitamin C and B1 content, but there was a significant interaction for CO_2 X crop for Vit C content (Tables 1 and 4). Vitamin C was highest in 'Toscano' kale and 'Amara' mustard and lowest in 'Barese' Swiss chard and shungiku (Table 4). Vitamin B1 was equally high in shungiku, 'Red Russian' kale, and 'Toscano' kale and lowest in 'Barese' Swiss chard. Related studies with kale showed that total glucosinolate content increased with elevated CO_2 but no vitamin analyses were reported in that study (Chowdhury et al. 2021). Vitamin K ranged from ~1 to 4 µg / g (Table 4) but these differences were not significant among species/cultivars or CO_2 treatments. Vitamin K was highest in 'Amara' mustard and lowest in 'Outredgeous' lettuce (Table 4). These three vitamins are important for space diets, as both Vitamin C and Vitamin B1 tend to degrade with time, while Vitamin K is slightly low in the packaged foods used for space (Cooper et al. 2012, 2017; Douglas et al. 2020). For long duration Mars missions, pre-deployment of foods prior to human arrival has been discussed, which would require a shelf life of up to 5 years (Cooper et al. 2017; Douglas et al. 2020). Hence, the ability to provide a supplemental source of fresh food such as leafy greens containing these vitamins and other important nutrients could reduce the risk of poor nutrition for Mars missions.

4. Conclusions

Our results suggest that 'Dragoon' lettuce and 'Extra Dwarf' pak choi would be good candidates for space food production systems, based on their high yields and small shoot growth. 'Toscano' kale, 'Red Russian' kale and 'Amara' mustard had better nutritional attributes but were among the taller crops tested in our study. These types of data will help us understand the 'crop readiness level' for the different species/cultivars and their eventual down selection for growing in volume-limited settings in space (Romeyn et al. 2019). 'Outredgeous' lettuce, 'Extra Dwarf' pak choi, 'Dragoon' lettuce, 'Red Russian' kale, and Amara mustard have already been tested in exploratory studies on the International Space Station, but further testing is needed, particularly with other water and nutrient delivery techniques envisioned for weightlessness (e.g. Wetzel et al. 2023). Our findings suggest that elevating CO_2 to 1500–3000 ppm should increase growth and yield for most of these leafy green species/cultivars, while for others, CO_2 at a super-elevated concentration of 6000 ppm could decrease yields, e.g. 'Outredgeous' and 'Dragoon' lettuce, when compared to 1500 or 3000 ppm. But equally noteworthy is that 6000 ppm did not have any negative effect on fresh or dry mass yields of the other six species. Protein (from total N) and K levels of the leafy greens increased with elevated CO_2 in our study, but related field studies generally show decreases in elemental content, including N, and hence protein, with increased CO_2 (Loladze 2014; Dong et al. 2018). Elevated CO_2 decreased Mg in our studies, as with some reports in the literature. Elevated CO_2 increased both Vitamin C and Vitamin B1 but had no effect on Vitamin K when analyzed for all the species/cultivars in our study. The results suggest that effects of elevated CO_2 on the nutritional content of plants can vary among species or cultivars and with horticultural approaches, and that crop and cultivar-specific testing with the envisioned horticultural approach may be needed to better understand the crop responses to CO_2.

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Disclosure statement

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LaShelle E. Spencer is a scientist with the Laboratory Support Services Contract (LASSO) at NASA's Kennedy Space Center (KSC). She serves as Lead Horticulturist, supporting Space Crop Production research. She received her BS in Horticulture and MS in Plant and Soil Science from Tuskegee University. Prior to joining the LASSO team, she conducted extensive research on hydroponic plant growth systems for space research at Tuskegee University. Following this, she served with the Ground Research team on the Life Science Services Contract at KSC, further supporting hydroponic plant research. She later honed her skills in analytical chemistry, micro and molecular biology by participating in over a dozen bioregenerative life support themed projects, which included plants, water, and waste recycling. She has been co-investigator for several spaceflight experiments including the PH-04 chile (chili) pepper study on the International Space Station (ISS). This was the longest, most complex plant growth study to date on the ISS.

Ruqayah H. Bhuiyan is a controlled environment grower / research investigator at Corteva Agriscience R&D facility in Indianapolis, Indiana, USA. She oversees the plant quality and growth of research material in greenhouses, growth chambers, and vertical controlled environments. Ruqayah assesses the plant physiological responses to external stimuli and develops growing procedures to facilitate research. Previously, Ruqayah held a position at Vision Greens in Ontario, Canada, where she worked as the head grower for a vertical farm producing lettuce, basil, and arugula. Ruqayah completed her master's degree in plant physiology at the University of Georgia, where she worked with Dr. Marc van Iersel on lighting systems in controlled environments.

Matthew A. Mickens is a Project Scientist at NASA's Kennedy Space Center (KSC). He received his BS and MS in Plant, Soil, and Environmental Science, and his Ph.D. in Energy and Environmental Systems with a concentration in Inorganic Chemistry from North Carolina A&T State University. Subsequently, he spent 3 years at KSC as a research fellow in the NASA Postdoctoral Program (NPP) where his primary expertise was studying plant growth and morphology responses to LED light recipes. He also spent 5 years in commercial CEA as Project Manager and Chief Science Officer for Elevate farms and oversaw the installation indoor hydroponic vertical farms and commenced their operations in Welland, Ontario, CA and Orange, NJ, USA. Upon returning to KSC, he now serves as the technical POC for advancing microgreens as a candidate space crop, heading the food safety team and task force for the future Ohalo III food production growth chamber for Mars transit vehicles, and managing KSC's primary processing area for all plant growth chamber research.

Jess M. Bunchek is a botanist (B.Sc., Purdue University), agronomist (M.Sc., The Pennsylvania State University), and current PhD candidate in space systems engineering at the University of Bremen (Germany) and the German Aerospace Center (DLR) Institute of Space Systems. While at NASA's Kennedy Space Center, she supported space crop production on the International Space Station, primarily with the Veggie plant growth chambers. From late 2020 to early 2022, Jess overwintered in Antarctica at the German Neumayer Station III as the EDEN ISS greenhouse operator, a collaborative project between the German Aerospace Center (DLR), NASA, and the Alfred Wegener Institute for Polar and Marine Research.

Edzard van Santen is a statistician/plant breeder and a full Professor in the University of Florida (UF) Agronomy Department since 2016, after a 29-year career as plant breeder and consulting statistician at Auburn University, USA. While at Auburn, he had an active applied breeding program in energy, forage, and turf grasses and also worked with cover crop species such as ball clover, crimson clover, and white lupin. He is currently the director of the UF/IFAS Statistical Consulting Unit, a service unit sponsored by the Senior VP for Agriculture and Natural Resource and part of the UF/IFAS research infrastructure. This Statistical Consulting Unit is charged with assisting graduate students, staff, and faculty with the design, conduct, and analysis of research studies in agronomy, animal sciences, entomology, food science, horticultural sciences, etc. He is currently also involved in the USDA funded Fostering Resilience and Ecosystem Services in Landscapes by Integrating Diverse Perennial Circular Systems (RESILIENCE CAP) grant as the lead statistician.

Gioia D. Massa is a plant scientist at NASA's Kennedy Space Center working on space crop production for the International Space Station and future exploration missions. Prior to joining NASA, she worked as a postdoctoral fellow at Purdue University and Kennedy Space Center, conducting research on crops for bioregenerative life support. She has studied a range of crops for future food production in space, focusing on their horticultural and environmental requirements, including responses to light spectral quality using light emitting diodes (LEDs). She led the science team for initial testing and validation of the Veggie plant growth chamber on the International Space Station (ISS) and has conducted comparative studies with leafy greens and tomatoes on the ISS. Her other research interests include nutrition, flavor, food safety and the psychological benefits of space-grown crops. Throughout her career, Gioia has been an avid supporter of student outreach.

Matthew W. Romeyn was a plant ecologist / molecular biologist at NASA's Kennedy Space Center (KSC). Matt was responsible for technology demonstration studies with the Veggie plant chamber on the International Space Station (ISS) and oversaw operations for KSC's controlled environment research laboratory. His research focused on microgreens as a candidate food crop, plant microbiome studies, and he served as NASA's lead for the PH-04 study with chile (chili) peppers grown in NASA's Advanced Plant Habitat on the ISS. This was the longest plant experiment every conducted in space. He was passionate about the development and implementation of a crop readiness plan and on-orbit gardening handbook with the goal that in the future, crews will be able to select and independently grow plants of their choosing while in space. Tragically, Matt died in an auto accident in 2022. He will always be missed by his many colleagues and friends at KSC and the space biology community.

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