

Gravitational and Space Research

Balancing Nutrient Content and Nitrate Levels in Space Agriculture: Investigating LED Light and CO₂ Effects on Space-Grown Leafy Green Vegetables

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Abstract

Sustainable cultivation of nutrient-rich vegetables is crucial for long-term space missions. Leafy greens such as red romaine lettuce and hybrid leafy Asian green are promising candidates due to their compact growth and high nutritional value. However, these crops can accumulate excessive nitrate and iron (Fe), potentially posing health risks in space. Nitrate (NO₃⁻) may convert to carcinogenic nitrosamines when consumed with processed foods under acidic gastric conditions, while excess Fe contributes to oxidative stress and bone loss. Ideal crop characteristics prioritize high potassium, magnesium, and calcium (Ca) concentrations while minimizing Fe accumulation to support astronaut cardiovascular and skeletal health.

This study hypothesizes that elevated light intensity and CO₂ enrichment could reduce NO₃⁻ accumulation while preserving favorable nutrient profiles. Using NASA's Growing Beyond Earth (GBE) 2023 research protocol, seeds were first exposed to neutron radiation via Californium-252 and then cultivated under controlled LED lighting and elevated CO₂ conditions to simulate spaceflight growing environments. Neutron radiation had no statistically significant effect on edible biomass or morphology ($p > 0.05$). CO₂ enrichment significantly reduced NO₃⁻ content, partially supporting the hypothesis, but led to nutrient trade-offs, including increased Fe levels and reduced magnesium and Ca. Contrary to expectations, higher light intensity increased nitrate accumulation. A NASA-aligned crop suitability ranking identified low light intensity (230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) and CO₂ enrichment (~1000 ppm) as the optimal conditions for enhancing space crop quality while mitigating dietary risks. These findings may inform NASA's space agriculture strategies for sustainable food production on long-duration missions and offer insights for controlled environment agriculture in resource-limited settings on Earth.

Keywords

space agriculture • nitrate metabolism • nutrient profiles • LED lighting • CO₂ enrichment • radiation effects

Introduction

Astronauts depend heavily on prepackaged foods during space missions. While these rations are calorie-dense and shelf-stable, their nutritional value degrades over time, particularly in vitamins A, C, B1, and B6, which can drop to inadequate levels within one to three years (Cooper et al., 2017; Johnson et al., 2021). Therefore, supplementing space diets with fresh crops grown *in situ* is essential for sustaining astronaut health on long-duration missions. Leafy greens, such as lettuce and pak choi, are ideal candidates due to their compact growth and high concentrations of vitamin C and antioxidants that support immune function and mitigate radiation damage (Massa et al., 2015).

In addition to vitamin content, spaceflight-induced physiological changes require specific mineral profiles to support muscle function, cardiovascular regulation, and skeletal integrity—all of which are compromised in microgravity. Potassium (K), magnesium (Mg), and calcium (Ca) are essential for these

functions, while excessive iron (Fe) intake has been linked to oxidative stress and accelerated bone loss in space (Yang et al., 2018; Chen et al., 2019). Since prepackaged space diets are already high in Fe, concerns about Fe overload are significant (Smith and Zwart, 2020). Thus, ideal space crops should be rich in K, Mg, and Ca while minimizing Fe accumulation (Massa et al., 2015; Fritsche et al., 2024).

NO₃⁻ content, though often overlooked, is another critical consideration. When consumed in moderation, dietary NO₃⁻—abundant in leafy greens—can be converted in the human body to nitric oxide, which promotes vascular health, enhances oxygen delivery, and modulates immune responses. These benefits are especially relevant during spaceflight, where astronauts are prone to cardiovascular deconditioning and early immune suppression (Hord et al., 2009; Yang et al., 2017; NASA Human Research Program, 2024). However, under acidic gastric conditions, NO₃⁻-derived nitrites (NO₂⁻) can react

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with NO_2^- -cured meats or cheeses to form carcinogenic nitrosamines (Smith et al., 2009; Karwowska & Kononiuk, 2020; Zhang et al., 2020) (Fig. 1). Although NASA has shifted toward safer food preservation methods such as freeze-drying, thermostabilization, and irradiation (Butler, 2014; Wu and Douglas, 2024a, 2024b), some commercially prepared entrees may still contain undisclosed NO_3^- -based additives. Given altered digestion and oxidative stress in space, reducing NO_3^- accumulation in crops is a prudent strategy to mitigate dietary risk and enhance crew safety.

This study tested the hypothesis that manipulating light intensity and CO_2 levels can reduce NO_3^- accumulation in leafy greens while maintaining a favorable nutrient profile. Recent studies suggest that high light intensity enhances NO_3^- assimilation (Gómez and Jiménez, 2020), while elevated CO_2 may suppress NO_3^- uptake by altering plant metabolism (Bloom and Burger, 2010; Igarashi et al., 2021). Building on these insights, this study followed the 2023 GBE research protocol and incorporated multiple environmental variables—light, CO_2 enrichment, and neutron radiation via Californium-252—to simulate space conditions. The experiments also utilized a custom 3D-printed CO_2 delivery system to replicate CO_2 conditions on the International Space Stations (ISS) through a cost-effective setup. While NO_3^- reduction was the primary focus, nutrient composition—particularly levels of Fe, K, Mg, and Ca—was also analyzed to identify potential trade-offs. A comprehensive evaluation metric modeled after NASA's Space Crop Readiness Levels (CRLs) framework was developed to quantify the combined effects of these variables. This metric integrated germination rates, edible biomass, plant volume, and nutrient composition to evaluate the overall suitability of each treatment and enable robust comparisons across conditions, identifying optimal environmental parameters for spaceflight and Earth-based controlled-environment agriculture.

Methods and Materials

Growth Materials and Setup

All seeds, growth media (arcilite, Turface ProLeague from Profile Products, LLC, Buffalo Grove, IL), 4" nursery pots, plant trays, wicking sheets, and 18-6-8 T180 controlled-release fertilizer (Nutricote from Florikan E.S.A., LLC, Sarasota, FL) and growth chambers were provided by the GBE program (<https://fairchildgarden.org/science-and-education/science/gbe/>, Fairchild Tropical Botanic Garden, Miami, FL). Experiments adhered to the GBE 2023 research protocol to simulate ISS conditions. Plants were cultivated in GBE growth chambers (16" W × 13" D × 17.9" H) designed to mirror the key features of the Vegetable Production System (Veggie)

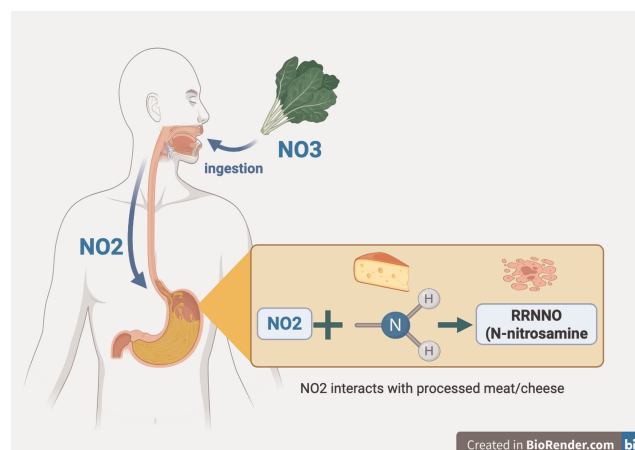


Figure 1. Schematic representation of the metabolic conversion of dietary NO_3^- into NO_2^- and the potential formation of carcinogenic nitrosamines under acidic gastric conditions when nitrate-rich vegetables are consumed alongside NO_2^- -containing preserved foods.

and Advanced Plant Habitat (APH) on the ISS. The Veggie is an open growth chamber designed as a “salad machine” to establish baseline salad performance under 1 *g* since 2014 (Massa et al., 2016; Khodadad et al., 2020), whereas APH is a closed chamber with advanced environmental sensors (Massa et al., 2016; Fritsche et al., 2024).

The GBE growth chambers used in this study had four reflective side panels to simulate APH's enclosed, controlled system, and six plant pots arranged to simulate six plant pillows used in the Veggie system (Massa et al., 2017). Each chamber also included an LED lighting panel, (a quantum board with red, green, blue, and white LEDs from Horticulture Lighting Group, LLC, Westerville, OH, custom order by GBE), an LED controller, a centrally mounted circulation fan running continuously (24/7), and environmental sensors including a digital hygrometer, a CO_2 monitor, a kilowatt meter, and an Apogee quantum meter for photosynthetic photon flux density (PPFD) measurement (Fig. 2). Each nursery pot was filled with approximately 450 mL of arcilite and supplemented with 3.3 grams of Nutricote controlled-release fertilizer (18-6-8 T180).

For each trial, one cultivar was grown per chamber for 28 days under a 12-hour photoperiod. Six cultivars were tested: red romaine lettuce (*Lactuca sativa* cv. Outredgeous), mizuna (*Brassica rapa* var. *nipposinica*), mustard (JS) (*Brassica carinata* cv. Amara), hybrid leafy Asian green (*Brassica rapa* var. *chinensis* cv. Rosie F1), Scarlet Frills mustard greens (*Brassica juncea*), and Garnet Giant mustard greens (*Brassica juncea*) (Table 1). Two seeds of the same cultivar were planted per pot, and seedlings were thinned to one per pot upon the appearance of the first true leaf. Each chamber contained

Table 1. Cultivars Tested in This Study. Seed selections were based on the Growing Beyond Earth (GBE) seed list from 2015–2022 and research protocol 2023. Cultivars labeled with “IR” indicate neutron-irradiated seed groups.

Species	Common name	Cultivar /Variety	Manufacturer (GBE ID)
<i>Lactuca sativa</i>	Red Romaine lettuce	‘Outredgeous’	Johnny’s Seeds (GBE 1, GBE 1IR)
<i>Brassica rapa</i> var. <i>nipposinica</i>	Mizuna	‘Mizuna’	Johnny’s Seeds (GBE 24, GBE24IR)
<i>Brassica rapa</i> var. <i>chinensis</i>	Pak Choi	‘Extra dwarf’	Kitazawa Seeds Co. (GBE 56, GBE56IR)
<i>Brassica carinata</i>	Mustard (JS)	‘Amara’	Johnny’s Seeds (GBE 113, GBE113IR)
<i>Brassica rapa</i> var. <i>chinensis</i>	Hybrid Leafy Asian Green/ Pak Choi	‘Rosie F1’	Johnny’s Seeds (GBE 120, GBE120IR)
<i>Brassica juncea</i>	Mustard Green	‘Scarlet Frills’	Johnny’s Seeds (GBE 195, GBE195IR)
<i>Brassica juncea</i>	Mustard Green	‘Garnet Giant’	Johnny’s Seeds (GBE 196, GBE196IR)



Figure 3. Neutron Radiation Facility at Colorado State University. **(A)** Rodent radiation experiment setup, showing neutron radiation source in the center. **(B)** Plant seed radiation setup, illustrating the positioning of seed packets for uniform neutron exposure. Both images are from Dr. Alexander Meyers' 2023 presentation, used with permission.

level, averaging approximately 500 ppm (500 ± 20 ppm) over a 28-day growing period. Elevated CO₂ treatments (~1000 ppm, ~1250 ppm, and ~1500 ppm) were established by adding ~500 g dry ice per application, repeated 9 to 15 times over during the experiment. CO₂ concentrations were monitored using a meter placed just in front of each growth chamber. When chamber temperature dropped to 16°C, a seedling heat map was placed under the plant tray to maintain adequate warmth. Table 2 summarizes the experimental design, including the photoperiods and the CO₂ treatment design alongside radiation and light intensity treatments.

Estimation of Internal CO₂ Concentration

Because CO₂ meters were placed outside the semi-enclosed chambers—each with gaps between panels and a 2-inch open

base—internal CO₂ levels could not be measured directly. To estimate internal concentrations, we modeled daily gas accumulation over 28 days based on dry ice input, chamber volume (~ 60.9 L), and average temperature and relative humidity. Calculations used the ideal gas law, with a correction for water vapor pressure. A full description of the modeling procedure and worked example are provided in Supplemental Methods (see Supplemental materials). To account for CO₂ loss via diffusion and plant uptake, we applied a daily loss rate of $12.5\% \pm 2.5\%$, consistent with NASA studies reporting 5~10% daily losses in closed systems (Wheeler et al. 1991, 2014) and reflecting the less sealed design of the GBE chambers. Table 3 outlines the modeled average daily internal CO₂ concentrations for each experimental group.

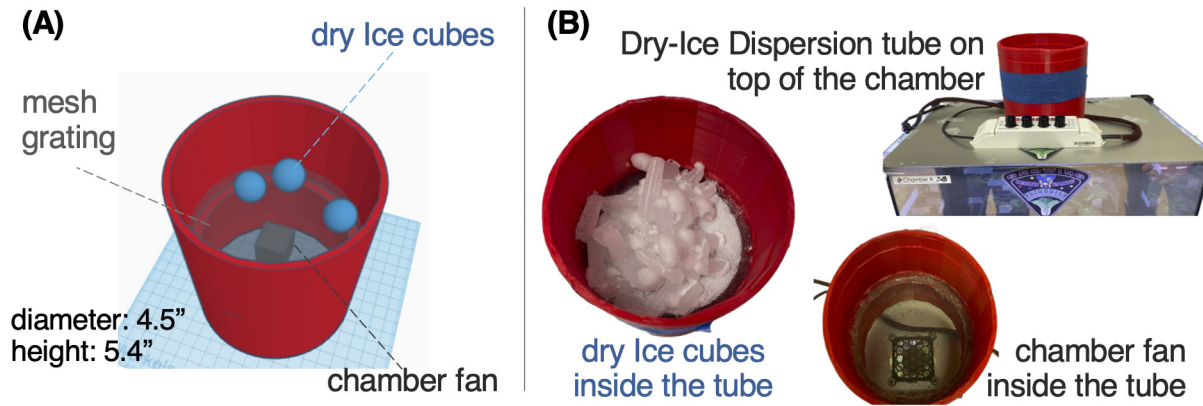


Figure 4. Custom-designed 3D-printed dry-ice CO₂ dispersion tube setup. **(A)** A computer-assisted design (CAD) drawing for the system and **(B)** a real-life image of the system.

Table 2. Experimental Design outlining control and experimental variables.

Conditions	Control	Experimental
Photoperiod	12/12	12/12
Seeds	Non-irradiated	Irradiated
Light Intensity	230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ LED (R25G0B25W180)	305 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ LED (R25G0B25W255)
CO ₂ (Light Intensity Trials)	~ 500 ppm	~ 500 ppm
CO ₂ (CO ₂ Trials)	~ 500 ppm	Exp. 1: ~1000 ppm (+ 2500g dry ice) Exp. 2: ~1250 ppm (+ 5000g dry ice) Exp. 3: ~1500 ppm (+ 7500g dry ice)

Table 3. Summary of CO₂ estimation parameters and modeled internal concentrations for each experimental chamber.

Trial	Dry Ice (g)	Temp (°C)	RH (%)	CO ₂ (ppm)
Exp. 1	7725.4	16.3 ± 0.7	36.2 ± 1.2	1490 ± 188
Exp. 2	4801.3	17.8 ± 0.5	38.3 ± 0.9	1192 ± 68
Exp. 3	3997.0	20.3 ± 0.8	37.0 ± 1.0	1160 ± 145

Note: Values represent total dry ice application (Dry ice, g), average chamber temperature (temp, °C), chamber humidity (RH, %), and estimated average internal CO₂ levels (CO₂, ppm) over the 28-day growing period, with standard error of the mean (± SEM).

Data Collection

Plant growth data (plant dimensions and observations) and environmental data (chamber temperature, chamber humidity, and total water usage) were monitored daily during weekdays and recorded weekly. Table 4 summarizes the environmental data, including the average temperatures, relative humidity,

water consumption, and CO₂ across all trials, along with the associated standard errors of the means (SEM). Following harvest, various growth parameters and nutrient concentrations of plant leaf tissue were measured to assess the effects of radiation, light intensity, and CO₂ treatments on plant growth and nutrient composition. Radiation effects were evaluated by germination rates, edible biomass, and plant volume of six plant types with data sourced from 85 GBE schools ($n = 6$ to $n = 102$ per plant type). Light intensity experiments focused on analyzing NO₃⁻ and nutrient content in red romaine lettuce cultivated by Dow High Space Farmers (DHSF), whereas CO₂ treatment effects were evaluated using hybrid leafy Asian green from Jefferson Middle School (JMS). Guided by the nutrient prioritization outlined in the Introduction, analyses targeted K, Mg, Ca, Fe, and NO₃⁻ levels. At harvest, plant leaf tissue was collected from each treatment group and submitted to a certified commercial laboratory (A&L Great Lakes Laboratories, Fairview, IL) for nutrient composition analysis. The laboratory quantified K, Mg, Ca, Fe, and NO₃⁻ concentrations using standard Association of Official Analytical Chemists (AOAC) protocols for plant nutrient tissue analysis. Nutrient values were used to generate normalized, weighted scores as described in the Spaceflight Suitability Matrix.

Statistical Analysis

Statistical analyses were conducted using a custom Python 3.9 workflow to assess treatment effects and validate school-based findings against GBE data sets. Welch's t-tests were used to compare local and GBE-wide results, and two-way ANOVA and Tukey's HSD tested the effects of radiation, light intensity, and CO₂ enrichment on plant growth and nutrient composition. Pearson correlation and linear regression were used to evaluate relationships with NO₃⁻ levels. Cumulative sample sizes used in statistical comparisons are reported

Table 4. Environmental Data: Average environmental growth conditions measured across all trials. Chamber temperature (Temp °C), relative humidity (RH, %), total water usage (Water, mL), and external CO2 (ppm) were recorded on weekdays; values are reported as mean ± standard error of the mean (SEM).

Trials	Temp (°C)	RH (%)	Water (mL)	CO2 (ppm)
86 GBE Schools (6 Testing Cultivars)	23.8 ± 0.4	42.9 ± 1.3	5502.7 ± 339.9	N/A
Light Intensity Trial: Control	21.9 ± 0.2	35.2 ± 2.0	4708.3 ± 809.3	505.6 ± 6.1
Light Intensity Trial: Exp.	26.4 ± 0.4	28.0 ± 1.2	4408.3 ± 391.4	517.8 ± 16.0
CO ₂ Trial: Control	23.8 ± 0.2	33.6 ± 1.9	4700 ± 195.8	500 ± 20.0
CO ₂ Trial: Exp. 1	16.3 ± 0.7	36.2 ± 1.2	9700.0	1135.4 ± 39.4
CO ₂ Trial: Exp. 2	17.8 ± 0.5	38.3 ± 0.9	6450.0	1215.0
CO ₂ Trial: Exp. 3	20.3 ± 0.8	37.0 ± 1.0	5000.0	1215.0

Table 5. Spaceflight Suitability Matrix.

Traits	Weight	Factor (Category)	Justification with References
Germination (%)	1.5	Plant growth (Establishment)	Ensures successful crop establishment under variable spaceflight conditions (Massa et al., 2015).
Edible Biomass (g)	2	Plant Growth (Yield)	Maximizes harvestable mass per unit area in bioregenerative systems (Darby et al., 2024; Massa et al., 2015).
Plant Volume (cm³)	1.5 (inverted)	Plant Growth (Morphology)	Compact morphology supports limited-volume growth in spacecraft systems (Massa et al., 2015).
K (mg/g)	2	Nutritional (beneficial)	Essential for nerve function and cardiovascular regulation under microgravity (Darby et al., 2024).
Mg (mg/g)	1.5	Nutritional (beneficial)	Supports enzyme activity and chlorophyll production; however, this support may decline under elevated CO ₂ levels (Massa et al., 2015).
Ca (mg/g)	1	Nutritional (beneficial)	Protects bone health, which is compromised in microgravity (Darby et al., 2024; Massa et al., 2015).
Fe (µg/g)	1.5 (inverted)	Nutritional (risk)	Excess Fe contributes to the risk of oxidative stress and bone loss (Darby et al., 2024; Smith & Zwart, 2020).
Nitrate (NO ₃ ⁻) (mg/g)	1.5 (inverted)	Nutritional (risk)	High nitrate intake may form carcinogenic nitrosamines in space diets (Darby et al., 2024; Karwowska & Kononiuk, 2020).

Note: Weighted trait matrix modeled after NASA's CRLs criteria. Traits are organized by weight and factor (category) by relevance to space crop performance.

in figure captions. Graphical outputs included bar plots with standard error of the mean (SEM), along with statistical annotations such as *p*-values and grouping letters (e.g., A, B, C) at a significance threshold of *p* < 0.05.

Spaceflight Suitability Matrix

To evaluate spaceflight suitability across CO₂ and radiation treatments, a composite scoring system was developed using trait-specific weights adapted from NASA's CRLs criteria (Massa et al., 2015; Fritsche et al., 2024). Table 5 outlines the assigned weights and physiological justifications for each trait. Plant-growth-related factors included germination rate (x1.5), edible biomass (x2), plant volume (x1.5, inverted), while nutritional factors included K (x2), Mg (x1.5), Ca (x1), Fe (x1.5, inverted), and NO₃⁻ (x1.5, inverted). All values were normalized to a 0–1 scale using min–max scaling. For traits where lower values are considered more favorable—such as plant volume,

Fe, and NO₃⁻—normalized scores were inverted by subtracting each from 1. These normalized values were then multiplied by their respective weight, and the resulting weighted scores were summed to yield a composite score for each treatment. This scoring approach was first applied to nutrient-only traits (Table 6) and then extended to include growth and morphological parameters in the comprehensive treatment evaluations presented in Tables 7 and 8 (see Results section).

Results

Effects of Radiation on Plant Growth and Morphology

Neutron radiation did not significantly impact plant morphology, edible biomass, or plant volume across all six tested cultivars grown under 230 µmol·m⁻²·s⁻¹ PPFD conditions. Visual comparison of red romaine lettuce grown

Table 6. Nutrient Ranking Matrix for Hybrid Leafy Asian Green Plant Leaf Tissue under Varying CO₂ and Radiation Treatments.

Treatment	K	Mg	Ca	Fe	Sum	Ranking
500 ppm CO ₂ non-IRR	0.00	0.32	1.00	0.00	1.32	8
1000 ppm CO ₂ non-IRR	1.79	0.00	0.45	0.21	2.45	5
1250 ppm CO ₂ non-IRR	2.00	1.21	0.00	0.95	4.16	2
1500 ppm CO ₂ non-IRR	1.93	1.35	0.17	0.65	4.10	3
500 ppm CO ₂ IRR	0.01	0.27	0.97	0.46	1.71	7
1000 ppm CO ₂ IRR	1.92	1.50	0.12	1.50	5.04	1
1250 ppm CO ₂ IRR	1.15	1.24	0.03	0.49	2.91	4
1500 ppm CO ₂ IRR	1.42	0.00	0.48	0.03	1.93	6

Note: Composite scores based on normalized and weighted nutrient values (K, Mg, Ca, and Fe), using the trait weights listed in Table 5. Nutrient values were derived from leaf tissue collected at harvest. All values were normalized on a 0-1 scale; weights were then applied to generate scores.

Table 7. Normalized, Weighted Rankings of Red Romaine Lettuce Grown Under Different Light Intensity and Radiation Treatments.

	230 PPFD NonIRR	230 PPFD IRR	305 PPFD NonIRR	305 PPFD IRR
Germination ×1.5	0.17	1.50	0.00	0.00
Edible Biomass ×2	0.00	0.39	1.27	2.00
Volume ×1.5 (inverted)	1.26	1.06	1.50	0.00
K ×2	0.55	2.00	0.00	0.61
Mg ×1.5	0.75	1.50	0.75	0.00
Ca ×1	0.00	1.00	1.00	0.75
Fe ×1.5 (inverted)	1.49	1.50	0.00	1.34
NO ₃ ⁻ ×1.5 (Inverted)	1.50	1.00	1.38	0.00
Composite score (sum)	5.71	9.95	5.89	4.70
Ranking	3	1	2	4

Note: Rankings are based on plant growth factors (germination, edible biomass, and volume) and nutritional factors (K, Mg, Ca, Fe, and NO₃⁻) listed in Table 5. Nutrient values were derived from leaf tissue collected at harvest. All values were normalized on a 0-1 scale; weights were then applied to generate scores.

showed no observable differences in leaf count, coloration, or structure between irradiated and nonirradiated plants [Fig. 5(A)]. Statistical analysis confirmed these observations. Two-way ANOVA revealed no significant differences in edible biomass or plant volume across all six tested plant types that grew under 230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD [Fig. 5(B) and Fig. 5(C)]. Among plant types, mizuna exhibited the most significant variability in biomass and volume, with extreme outliers in nonirradiated groups. Hybrid leafy Asian green and Scarlet Frills mustard green showed the lowest median biomass, while red romaine, hybrid leafy Asian green, and mustard (JS) maintained compact growth profiles. Considering space agriculture suitability, irradiated hybrid leafy Asian green and red romaine lettuce ranked among the top performers, offering an optimal balance of high edible biomass and compact morphology [Fig. 5(D)].

Representative Sample Analysis: DHSF and JMS vs. GBE Schools

Independent two-sample t-tests confirmed that germination rates, edible biomass, and plant volume from DHSF and JMS did not differ significantly from the broader GBE data sets (all $p > 0.05$). As shown in Fig. 6(A) and Fig. 6(B), DHSF and JMS results fell within the variability observed across GBE schools, validating the use of their data in subsequent analyses of light and CO₂ treatment effects.

Effects of Light Intensity on Plant Growth and Morphology

Similar to the effects observed with neutron radiation, variations in light intensity (230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ vs. 305 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) did not significantly influence germination rates, edible biomass, or plant volume across treatment groups [$p > 0.05$, two-way ANOVA; Figs. 7(A)–(C)]. Irradiated

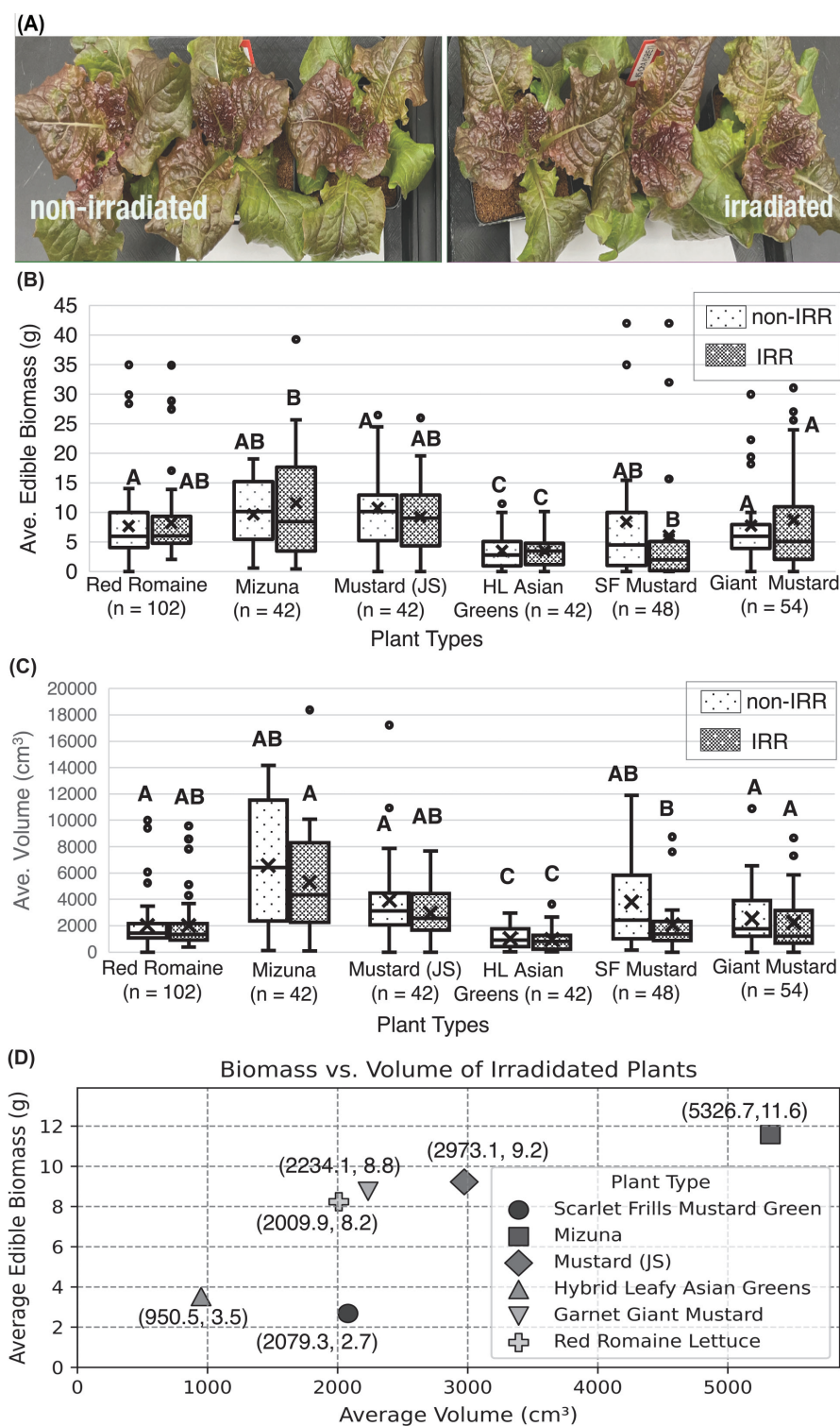


Figure 5. (A) Red romaine lettuce morphology under $230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD treatment. (B) Boxplot analyses of edible biomass across six cultivars. Data are presented as mean $\pm$ SEM, with varying sample sizes per cultivar ($n = 42\text{--}102$). Letters indicate statistically significant differences across plant types. (C) Boxplot analyses of plant volume across six cultivars. Data are presented as mean $\pm$ SEM, with varying sample sizes per cultivar ($n = 42\text{--}102$). Letters indicate statistically significant differences across plant types. (D) A scatter plot of average edible biomass versus plant volume for irradiated plants across six testing cultivars.

Table 8. Normalized, Weighted Rankings of Hybrid Leafy Asian Green Grown Under Varying CO₂ and Radiation Treatments.

	500 N	1000 N	1250 N	1500 N	500 I	1000 I	1250 I	1500 I
Germination	1.43	0.00	1.50	1.50	1.50	0.74	1.50	1.12
Edible Biomass	0.11	1.63	2.00	0.23	0.05	0.21	1.86	0.00
Volume	1.40	0.36	0.44	0.44	1.50	1.46	0.00	1.37
K	0.00	0.22	2.00	1.93	0.01	1.92	1.15	1.42
Mg	0.32	1.50	1.21	1.35	0.26	1.50	1.24	0.00
Ca	1.00	0.55	0.00	0.17	0.97	0.12	0.03	0.48
Fe	0.00	1.21	0.95	0.65	0.46	1.50	0.49	0.03
NO₃⁻	n/a	0.00	0.45	0.00	n/a	0.62	0.95	1.40
Sum (Excl. NO₃⁻)	4.26	5.47	8.09	6.27	4.76	7.45	6.27	4.42
Sum (Incl. NO₃⁻)	n/a	5.47	8.54	6.27	n/a	8.07	7.22	5.83
Ranking (Excl. NO₃⁻)	8	5	1	3	6	2	3	7
Ranking (Incl. NO₃⁻)		6	1	4		2	3	5

Note: Rankings are based on plant growth and nutrient factors (see Table 5), with nutrient values derived from harvested leaf tissue. All values were normalized (0–1 scale) and weighted before scoring. Composite scores are shown both with and without NO₃⁻ due to limited data in some treatments. Column abbreviations indicate CO₂ concentration in ppm and irradiation condition: **N** = Non-IRR (nonirradiated), **I** = IRR (irradiated). For example, 500N = ~500 ppm CO₂, nonirradiated.

and nonirradiated plants displayed comparable growth metrics under elevated light conditions, indicating that neither increased light intensity nor its interaction with neutron radiation substantially impacted overall plant development. However, elevated light intensity (305 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) did induce distinct pigmentation changes in red romaine lettuce, specifically, an increase in purple pigmentation. Although not part of the original experimental objectives, pigmentation frequency was recorded on-site during plant care and later verified using standardized photographs, which were analyzed with ImageJ software (NIH), with four independent observers cross-checking the results. Irradiated plants exhibited nearly double the pigmentation frequency compared to nonirradiated controls, a relationship confirmed by chi-square analysis ($\chi^2 = 15.91$, $p = 0.00118$), suggesting that anthocyanin expression was enhanced under combined high light intensity and radiation exposure (Massa et al., 2015) [Fig. 7(D) and Fig. 8].

Effects of CO₂ Enrichment on Plant Growth

CO₂ enrichment significantly influenced the growth of hybrid leafy Asian green, with the most pronounced effects observed at ~1250 ppm. Under ~1000 ppm CO₂, nonirradiated seeds exhibited a reduced germination rate (33%) compared to their irradiated counterparts (66%). This trend reversed at ~1500 ppm, where nonirradiated seeds achieved 100% germination [Fig. 9(A)]. For edible biomass, plants grown under ~1250 ppm achieved the highest yields (15.9 g for nonirradiated; 15.1 g for irradiated), significantly outperforming those grown under ~1000 ppm and ~1500 ppm CO₂ levels [Fig. 9(B)].

Plant volume followed a similar trend, with the largest mean volumes also observed under ~1250 ppm CO₂ [Fig. 9(C)]. Across all treatments, ~1250 ppm CO₂ treatment consistently enhanced germination, biomass, and volume compared to ~500 ppm (control) and ~1000 ppm. While seed irradiation improved germination at ~1000 ppm, it slightly reduced biomass production at ~1250 ppm.

Effects of LED Light Intensity on Nutrient Composition

Californium-252 neutron radiation did not significantly impact red romaine lettuce nutrient composition. As shown in Fig. 10, K levels remained relatively stable between 230 and 305 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD treatments, with a slight decrease observed in irradiated plants at higher light intensity (76.9 mg/g). Mg and Ca concentrations were minimally affected. However, Fe concentrations increased significantly under elevated light conditions, reaching 164.6 $\mu\text{g/g}$ in nonirradiated plants. These results suggest that while light intensity had limited effects on macronutrients, it may enhance Fe uptake, posing potential concerns for astronaut health.

Effects of CO₂ on Nutrient Composition

CO₂ enrichment influenced nutrient composition in both irradiated and nonirradiated hybrid leafy Asian green [Fig. 11(A) and Fig. 11(B)]. Although data for the ~500-ppm group were unavailable, NO₃⁻ concentrations decreased as CO₂ levels increased, reaching the lowest values at ~1500 ppm. K and Mg peaked at ~1000 ppm but declined at higher CO₂ concentrations, especially in irradiated plants. Ca remained consistently higher in nonirradiated plants, while Fe

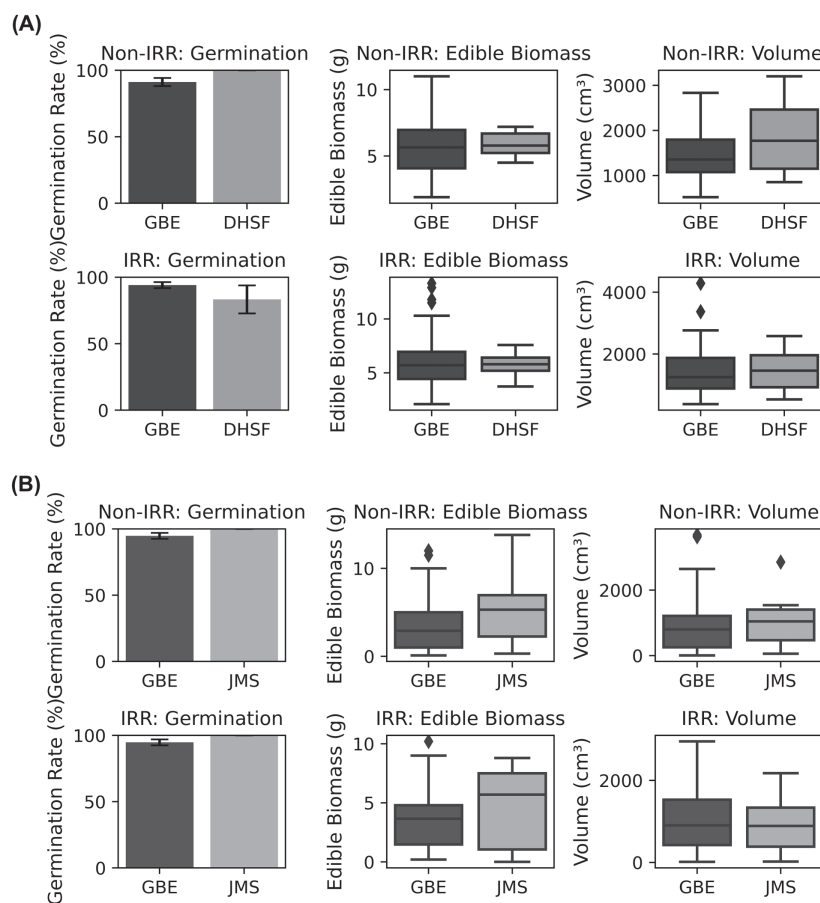


Figure 6. (A) Comparison of germination rates, edible biomass, and plant volume between GBE schools (n = 102) and DHSF (n = 6) for irradiated and nonirradiated red romaine lettuce. Data are presented as mean ± SEM.

(B) Comparison of germination rates, edible biomass, and plant volume between GBE schools (n = 42) and JMS (n = 6) for irradiated and nonirradiated hybrid leafy Asian green. Data are presented as mean ± SEM.

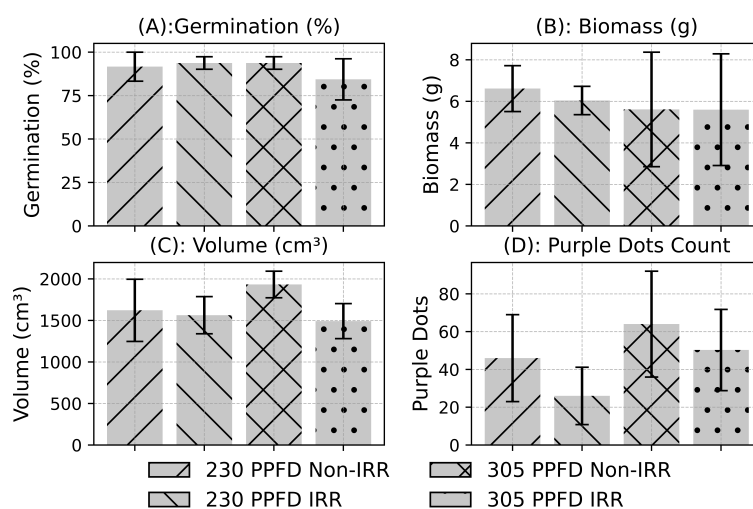


Figure 7. Effects of light intensity (230 vs. 305 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) and neutron irradiation on red romaine lettuce's germination **(A)**, biomass **(B)**, volume **(C)**, and purple dot count **(D)**. No significant differences were detected across treatments. Data are mean ± SEM (n = 6 per treatment).



Figure 8: A representative image illustrates the purple pigmentation occurrence of red romaine lettuce.

concentrations increased across all CO₂ levels, with irradiated plants showing greater accumulation. These findings indicate that radiation amplifies CO₂-driven nutrient imbalances, accelerating Mg and Ca declines while promoting Fe uptake.

Nutrient Ranking Matrix for Combined Effects of CO₂ and Radiation

To assess nutrient balance across CO₂ and radiation treatments, composite scores were calculated for hybrid leafy Asian green using normalized and weighted scoring framework described in the Spaceflight Suitability Matrix in the Methods section (Table 5). As shown in Table 6, the irradiated group at ~1000 ppm CO₂ achieved the most favorable nutrient profile (sum = 5.04), and the ~1250 ppm nonirradiated group ranked second (sum = 4.16). In contrast, the ~500 ppm CO₂ treatments scored lowest for both nonirradiated and irradiated groups (sum = 1.32–1.71). The ~1500 ppm irradiated group exhibited the second-lowest score (sum = 1.93), due to elevated Fe and reduced Mg levels. These results indicate the ~1000 ppm irradiated group optimally maintains nutrient stability under simulated spaceflight conditions.

NO₃⁻ Content Analysis

Light intensity and CO₂ levels significantly influenced NO₃⁻ metabolism in space-grown crops. Red romaine lettuce grown under elevated light (305 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) accumulated more NO₃⁻ than those under 230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD, with irradiated plants showing a steeper trendline (0.0107 versus 0.0013), suggesting radiation may accelerate

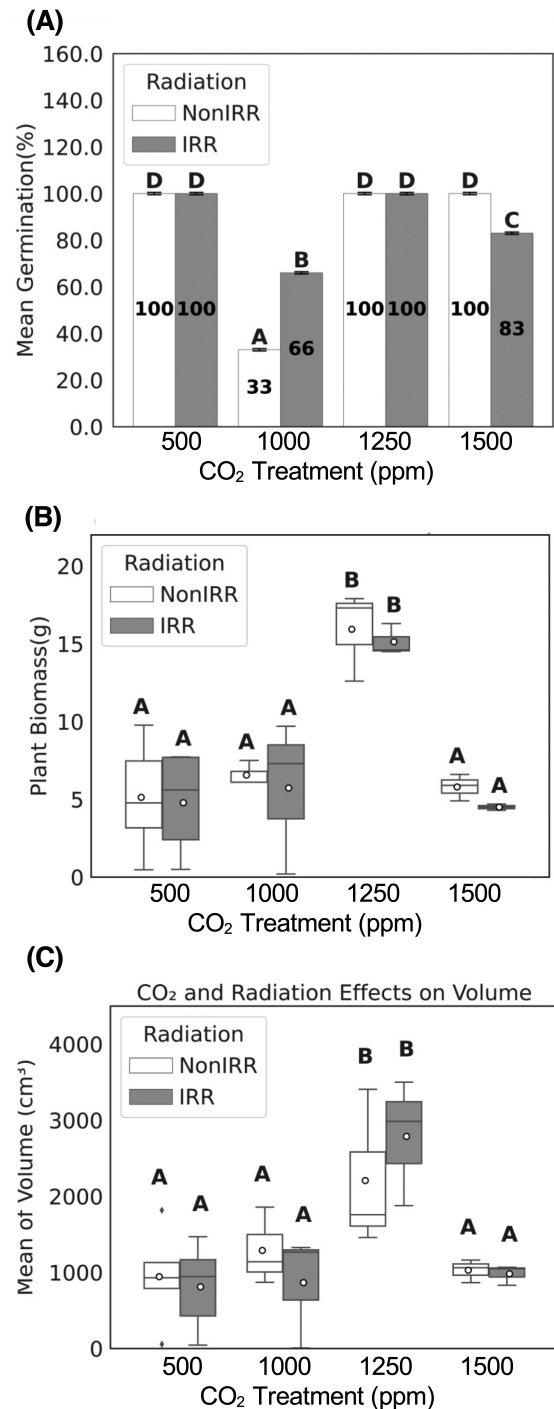


Figure 9: Effects of CO₂ enrichment and seed irradiation on harvested hybrid leafy Asian green. (A) Germination rate (%), (B) edible biomass (g), and (C) plant volume (cm³) are shown for four CO₂ levels: ~500 ppm (control, n = 15 per radiation group), ~1000 ppm, (n = 3 per group), ~1250 ppm (n = 3 per group), and ~1500 ppm (n = 3 per group), with each group, including both nonirradiated (Non-IRR) and irradiated (IRR) plants. Data are presented as mean $\pm$ SEM.

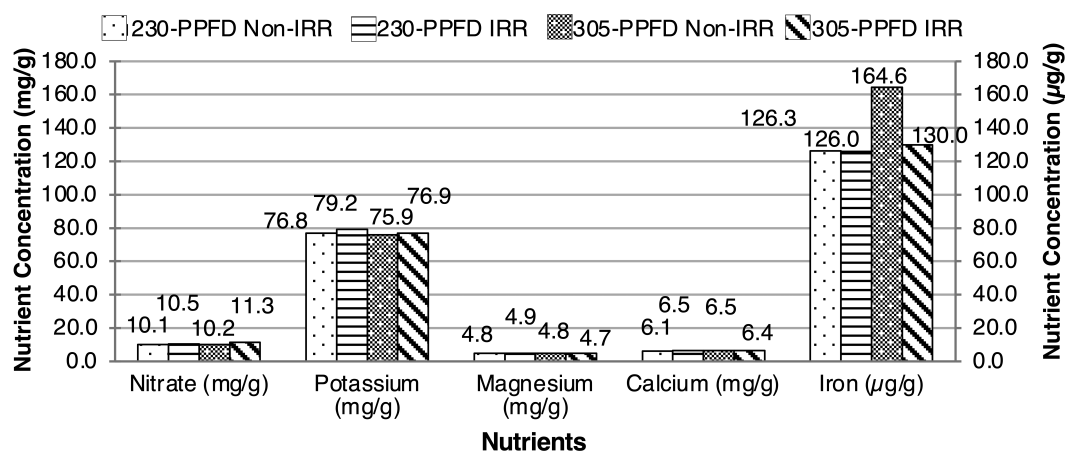


Figure 10. Nutrient composition analysis of harvested red romaine lettuce plant leaf tissue under two light intensities. Data are mean $\pm$ SEM (n = 6 per treatment).

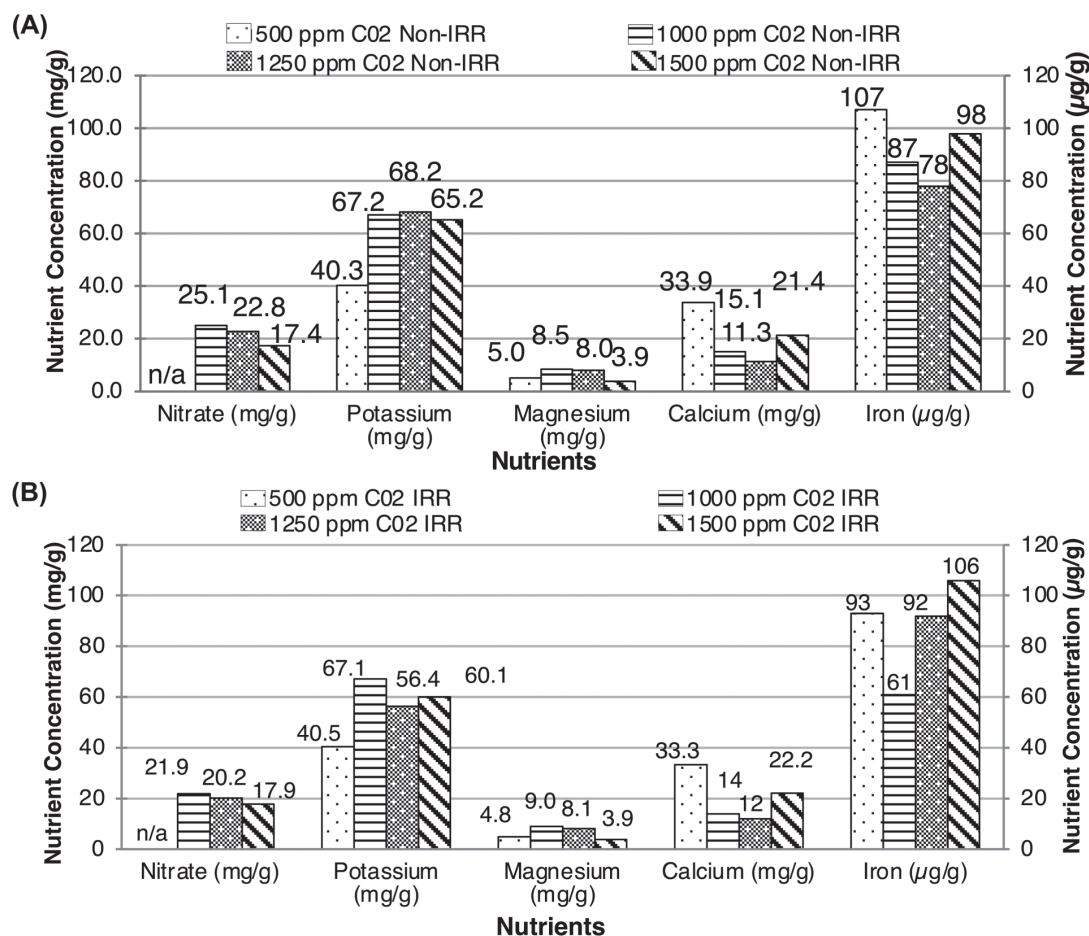


Figure 11. (A) Nutrient composition analysis of harvested nonirradiated (Non-IRR) hybrid leafy Asian green plant leaf tissue under varying CO₂ levels. Data are mean $\pm$ SEM (n = 3 per treatment).

(B) Nutrient composition analysis of harvested irradiated (IRR) hybrid leafy Asian green plant leaf tissue under varying CO₂ levels. Data are mean $\pm$ SEM (n = 3 per treatment).

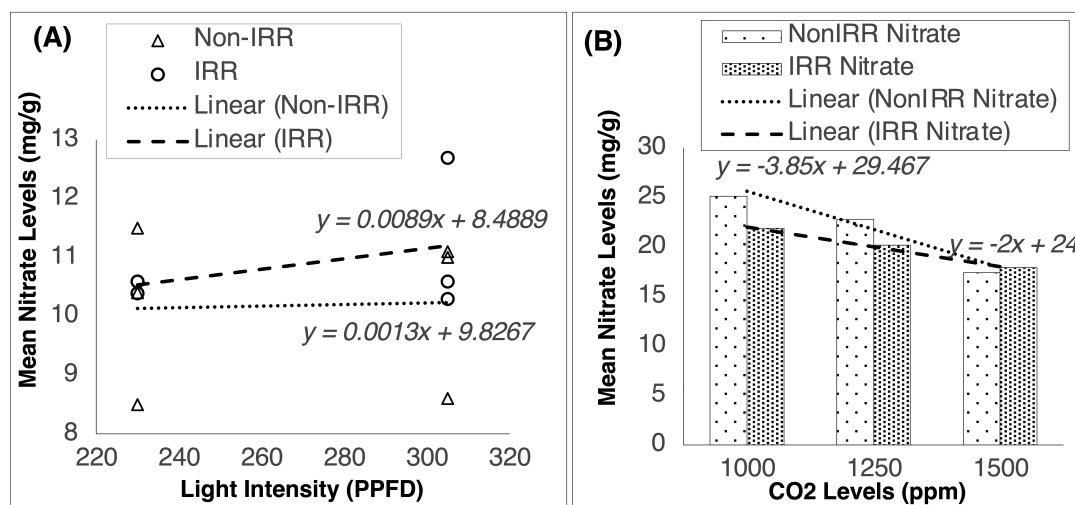


Figure 12. (A) Effects of light intensity on NO_3^- concentrations of harvest nonirradiated (Non-IRR) and irradiated (IRR) red romaine lettuce plant leaf tissue. Data are mean $\pm$ SEM ($n = 6$ per treatment). **(B)** Effects of CO_2 on NO_3^- concentrations of harvested (Non-IRR) and irradiated (IRR) hybrid Asian leafy green plant leaf tissue. Data are mean $\pm$ SEM ($n = 3$ per treatment).

NO_3^- accumulation [Fig. 12(A)]. While a moderate positive correlation ($R = 0.503$) was observed, it did not reach statistical significance ($p = 0.309$), indicating that more research is needed to confirm this trend. Conversely, CO_2 enrichment significantly reduced NO_3^- levels in hybrid leafy Asian green, with a strong negative correlation ($R = -0.845$, $p = 0.049$) [Fig. 12(B)]. This reduction was more pronounced in irradiated plants (slope = -4.35) than in nonirradiated ones (slope = -2.0), highlighting CO_2 as an effective strategy to mitigate NO_3^- accumulation in space-grown vegetables. These findings suggest that optimizing light and CO_2 conditions can improve astronaut dietary safety during long-duration missions.

Discussion

This study evaluated how light intensity, CO_2 enrichment, and neutron radiation affect NO_3^- accumulation, nutrient composition, and growth in leafy greens to optimize cultivation for space missions. The hypothesis that increased light and CO_2 would reduce NO_3^- levels was only partially supported. While CO_2 enrichment effectively lowered NO_3^- content, higher light intensity ($305 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) slightly increased it, suggesting the effects of these environmental factors on leafy greens are cultivar-specific.

The NO_3^- -reducing effect of CO_2 enrichment observed in this study is consistent with previous findings (Taub, 2010; Igarashi et al., 2021), demonstrating its potential to mitigate NO_3^- accumulation in space-grown vegetables. However, trade-offs accompanied this benefit, including reductions in Ca and Mg concentrations and increases in Fe levels. Neutron

radiation had minimal impact on biomass and volume but appeared to intensify these nutrient imbalances and induced purple pigmentation in red romaine, likely due to anthocyanin upregulation (Massa et al., 2015).

To integrate growth performance, nutrient composition, and NO_3^- accumulation across treatments, composite scores were calculated using the Space Agriculture Suitability Matrix described in the Methods section. Table 7 presents composite scores for red romaine lettuce grown under two light intensities with and without neutron irradiation. The irradiated group grown under $230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD achieved the best overall performance (sum = 9.95). In comparison, $305 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD with irradiation produced the least favorable outcomes (sum = 4.70), revealing significant nutrient imbalances and NO_3^- buildup. Table 8 summarizes composite scores for hybrid leafy Asian green under varying CO_2 enrichment and radiation treatments. The ~ 1250 ppm CO_2 treatment (nonirradiated) performed best (8.54 with NO_3^- included; 8.09 excluding NO_3^-). Among irradiated groups, the ~ 1000 ppm CO_2 treatment (irradiated) was the best (8.07 with NO_3^- ; 7.45 excluding NO_3^-). In contrast, higher CO_2 levels (~ 1500 ppm) disrupted key nutrients in irradiated and nonirradiated plants, particularly Mg and Ca.

These results suggest that $230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD with ~ 1000 ppm CO_2 (irradiated) optimizes crop quality and nutrient profiles for space agriculture, while $305 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD with 1250 ppm CO_2 (nonirradiated) is more suitable for Earth-based controlled-environment agriculture, where maximizing yield and nutrient content is prioritizing over strict dietary constraints. The low Ca and low Fe concentrations observed in the ~ 1250 ppm CO_2 treatment may reflect a

biomass dilution effect, wherein increased tissue mass leads to a broader distribution of nutrients, and consequently, reduced concentrations per unit mass (Wheeler et al., 2024). This interpretation aligned with prior findings in high-yield crop cultivars and highlights the trade-offs between biomass production and nutrient density. However, the observed inverse trend—where higher CO₂ reduces NO₃⁻ but elevates Fe—underscores the complexity of nutrient responses and the need for innovative strategies to balance these competing outcomes.

Astronaut Relevance and Applications

Red romaine lettuce and hybrid leafy Asian green emerged as promising candidates for space agriculture, demonstrating compact growth and favorable nutrient profiles aligned with NASA's dietary targets (Massa et al., 2015; Fritsche et al., 2024). NO₃⁻ levels in irradiated red romaine were well below World Health Organization limits—requiring over 8.37 cups/day to exceed the threshold—and were further reduced by CO₂ enrichment. NO₃⁻ may also support cardiovascular health in space, though risks remain if combined with NO₂⁻-preserved foods. More concerning were elevated Fe levels under high CO₂ and light conditions, given the already Fe-rich space diet and its link to oxidative stress and bone loss. These findings underscore the need for precise control of growth conditions to make leafy greens safe for regular astronaut consumption.

Limitations and Future Directions

The 28-day growth cycle may not fully capture long-term plant responses, highlighting the need for extended trials and horticultural practices like cut-and-come-again harvesting. Future studies should incorporate additional ISS-relevant radiation types, such as protons and galactic cosmic ray analogs, to better simulate the full range of space radiation exposure. While this study identified optimal conditions for light intensity and CO₂ enrichment, several limitations warrant consideration. All CO₂ treatments were conducted under 230 μmol·m⁻²·s⁻¹ PPFD, and independent trials are needed to explore potential interactions with higher light intensities. While dry-ice sublimation effectively enriches CO₂, continuous delivery systems should be explored for ISS analogs. Cultivar selection and optimized fertilizers may address nutrient imbalances observed at elevated CO₂ levels. Nutrient analysis was conducted on a limited number of replicates, particularly for CO₂ treatments (*n* = 3). Increasing the sample size and including the 500 ppm CO₂ control group would strengthen statistical power and provide a more complete picture of enrichment effects. Finally, while neutron radiation had a minimal impact on overall growth, it appeared to interact with CO₂, amplifying nutrient imbalances and increasing anthocyanin pigmentation in

red romaine lettuce under higher light conditions. These subtle effects warrant further investigation, along with early-stage observations such as cotyledon morphology, to detect developmental radiation sensitivity that is not visible in mature tissues.

Conclusions

This study evaluated the effects of light intensity and elevated CO₂ levels on NO₃⁻ accumulation and nutrient compositions in space-grown leafy greens, with simulations of space radiation exposure using Californium-252 neutron radiation. The findings demonstrate that *Outredgeous* red romaine lettuce and hybrid leafy Asian green are promising for space agriculture, offering a favorable balance of compact growth, high edible biomass, and nutrient profile suitability for astronaut health. While space-grown vegetables exhibited higher NO₃⁻ levels than their Earth-grown counterparts, CO₂ enrichment effectively reduced NO₃⁻ concentrations, mitigating potential health risks. However, trade-offs in nutrient composition, including fluctuations in Mg, Ca, and Fe levels, emphasize the need for carefully controlled growth conditions. By optimizing environmental parameters, space agricultural systems can ensure that crops meet key nutritional and structural requirements for sustainable food production in microgravity. Importantly, the results from DHSF and JMS were found to be representative of broader GBE data sets, reinforcing the generalizability of these findings to other student-conducted trials and cultivar assessments.

This research explores critical issues in gravitational and space science by addressing the intersection of plant physiology and environmental stressors in microgravity conditions. By examining how light intensity, CO₂ levels, and radiation affect the nutrient profiles and NO₃⁻ content of space-grown vegetables, the study contributes to the understanding of plant adaptation to extreme environments. It underscores the importance of selecting crops that maximize yield while maintaining compact growth and favorable nutrient profiles. The results provide actionable insights for improving controlled-environment agriculture for long-duration space missions and terrestrial applications in resource-limited settings. These contributions may support NASA's broader objectives for sustainable food systems on missions to the moon and Mars while informing Earth-based applications where food security and resource efficiency are critical.

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Growing Beyond Earth® (GBE) is a classroom-based citizen science project led by Fairchild Tropical Botanic Garden in Florida. Through GBE, teachers and students in grades 6–12 learn about NASA Science research and conduct experiments that help NASA advance research on growing plants in space. GBE includes a series of experiments conducted on Earth in a plant habitat that mimics key features of Veggie and APH, which are operated on at the ISS. The GBE participants follow rigorous research protocols designed by GBE and the NASA Science Mission Directorate in the fall and conduct their original research, approved by GBE, in the spring. Since its inception, over 40,000 GBE participants across 19 time zones have contributed hundreds of thousands of data points and tested 180 varieties of edible plants for NASA. JMS and H. H. Dow High School, home to DHSF, are GBE's first national partner schools in Michigan. Space farmers from Jefferson Middle and Dow High schools have won yearly top awards at the GBE student research symposium since 2018, reflecting their commitment to advancing space agriculture. This research was presented at the American Society for Gravitational and Space Research (ASGSR) 2024 Annual Meeting, where it won First Place in the High School Poster Section, showcasing the scientific rigor and innovative contributions of DHSF.

Authors' Contributions

All authors conducted the experiments and participated in data collection, data analysis, and manuscript drafting. MEH and LST edited the manuscript, and all authors reviewed, read, and approved the manuscript.

Disclosure Statement

The authors declare that there is no conflict of interest.

Consent to Participate

All listed authors have approved the manuscript before submission. The authors declare that there is no conflict of interest.

Consent for Publication

All listed authors have approved the submission of this manuscript to the journal *Gravitational and Space Research*.

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Supplemental Methods: CO₂ Estimation Model

To estimate internal CO₂ concentrations in the semi-enclosed chambers, we modeled daily gas accumulation over the 28-day growing period based on the amount of dry ice added, chamber dimensions, average temperature, and relative humidity. The modeling was performed in three steps:

Step 1. Estimating CO₂ Gas Volume from Dry Ice

We used the ideal gas law to convert grams of dry ice (solid CO₂) into the volume of gas it would release under typical chamber conditions,

$$PV = nRT,$$

where P is pressure (atm); V is volume (L); n is the number of moles of CO₂ (ppm); R is 0.0821 L · atm · mol⁻¹ · K⁻¹ (gas constant); T is temperature in Kelvin (°C + 273.15).

Dry ice mass was converted to moles using the molar mass of CO₂ (44.01 g/mol). The total gas volume was then calculated over the 28 days and divided by the chamber volume (60.9 L) to estimate the daily CO₂ input in ppm.

Step 2. Adjusting for Water Vapor Pressure

Because the chamber air contains water vapor, the pressure used in the gas law needs to be adjusted to account only for the dry air component. We calculated saturation vapor pressure using the Tetens equation,

$$e_s = 0.061078 \times \exp \left(\frac{17.27 \times T_c}{T_c + 237.3} \right),$$

where e_s = saturation vapor pressure (kPa); $\exp$ = exponential function, T_c = air temperature in degrees Celsius (°C).

To get the actual vapor pressure (P_{H_2O}), we multiplied by the chamber's relative humidity (RH),

$$P_{H_2O} = RH \times e_s$$

where e_s = saturation vapor pressure (kPa).

This value was subtracted from the total pressure (1 atm) to obtain the dry air pressure, which is used in the gas law.

Step 3. Modeling Accumulation with Loss

To simulate how CO₂ accumulates and dissipates in the chamber over the 28 days, we applied a simple iterative model,

$$CO_{2n} = CO_{2n-1} \times (1 - \text{loss}) + \text{daily input (ppm)},$$

where CO_{2n} is the modeled CO₂ concentration (in ppm) on day n, and the daily input is derived from the volume of CO₂

gas added that day. The daily input (in ppm) was estimated as the ratio of the daily CO₂ volume to the chamber volume, multiplied by 100 (the correction factor) to reflect realistic mixing within the chamber based on the external CO₂ readings, which ranged from 950 to 1350 ppm,

$$\text{daily input (ppm)} = (\text{daily CO}_2 \text{ volume} / \text{chamber volume}) \times 100.$$

Accumulation was modeled under $12.5 \pm 2.5\%$ daily loss rates to account for escape through structural gaps and plant uptake.

Outputs

The estimated daily internal CO₂ concentrations (ppm) for each trial are summarized in Table 2.

Worked Example

Consider Experimental Trial 1, which received a total of 7,725.4 g of dry ice over 28 days. The average temperature in the chamber was 16.3°C, and the relative humidity was 36.2%.

Step 1: Convert dry ice to moles:

$$n = 7725.4 \text{ g} / 44.01 \text{ g/mol} \approx 175.6 \text{ mol}$$

Step 2: Estimate water vapor pressure using the Tetens equation:

$$e_s = 0.061078 \times \exp [(17.27 \times 16.3) / (16.3 + 237.3)] \approx 1.85 \text{ kPa}$$

$$RH = 36.2\% \rightarrow P_{H_2O} = 0.362 \times 1.85 \text{ kPa} \approx 0.67 \text{ kPa} = 0.0066 \text{ atm}$$

Step 3(a): Calculate CO₂ gas volume using the ideal gas law ($P = 1 \text{ atm} - 0.0066$):

$$V = nRT / (P - P_{H_2O}) \\ V = (175.6 \text{ mol} \times 0.0821 \times 289.45 \text{ K}) / (1 - 0.0066) \approx 4175.9 \text{ L total}$$

Step 3(b):

$$\text{Daily addition} = 4175.9 \text{ L} / 28 \approx 149.1 \text{ L/day} \\ \text{Daily ppm} = (149.1 / 60.9) \times 100 \approx 244.9 \text{ ppm/day}$$

Step 3(c): Modeled accumulation with 15% daily loss over 28 days with an initial 500 ppm (control group):

$$CO_{2n} = CO_{2n-1} \times 0.85 + 244.9$$

The average CO₂ level was approximately 1500 ppm (as shown in Table 2).