Main text – 3,000-4,000 words.

Illustrations are strongly encouraged.

References – up to 100 (exceptions are possible in special cases)

Author Contribution Statements:

- Benjamin A. Sikes - conceptualization, organize group, provide content on plant-microbial associations (1.2, 3.1), refine manuscript drafts and final manuscript (TO COME)
- Nicholas J.B Brereton - conceptualization, provide content (2.2 and 4.2), refine manuscript drafts and final manuscript (TO COME)
- Anna Simpson – conceptualization, provide content on ISS microbiome (3.2) ?
- Anna Lewkowicz – provide content on plant science history (1.1). ?

Contents

Title: Charting the path forward for plant science in space 2

1 A requirement for plant science in space 2

1.1 History of plant science experiments in space 2

1.2 CLDs, Gateway, returning to the moon, and the horizon 2

2 The immediate astrobotany frontiers 2

2.1 Media for sustainable plant growth off-Earth 2

2.1.1 Soil 2

2.1.2 Hydroponics 2

2.1.3 Regolith 2

2.2 Model organisms and crops in space 3

2.2.1 Arabidopsis, the green test tube in space 3

2.2.2 Food crops 3

2.2.3 Non-food crops 3

3 Plants require microbial interactions 3

3.1.1 Healthy functions 3

3.1.2 Microbial tracking on the ISS 3

3.1.3 Pathogens 3

4 Enabling a multiomics future in plant research 3

Figures 3

Tables 4

References 5

Annex 1 5

Annex 2 5

Title: Charting the path forward for plant-microbe systems in space

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1 A requirement for plant science in space

History of plant science experiments in space (Contributors: AL, NJB)

Plant seeds were sent to space for the first time in 1946 (Beischer and Fregly, 1962), but it was not until 1982 that *Arabidopsis thaliana* became the first plant cultivated in space aboard the Soviet Salyut 7 space station(Merkys et al., 1984). Plant research has steadily progressed aboard the International Space Station (ISS) since its establishment in 1998, with over 60 experiments conducted to date. These early milestones revealed the challenges of growing plants in space and advanced both experimental systems and our understanding of plant biology in space.

Seven Plant Growth Systems (PGSs) have been used aboard the ISS, with two currently operational. The first was the Advanced Astroculture (ADVASC) unit, flown 2001–2003, which performed three microgravity experiments using a single growth chamber regulating light, temperature, humidity, nutrients, and atmospheric conditions(Stankovic et al., 2002). In 2002, the Biomass Production System (BPS) and the Lada Greenhouse were installed, performing 17 plant experiments between 2002 and 2011(Brady et al., 2018; Zabel et al., 2016) (Lada Greenhouse remains on the ISS). Another PGS, the European Modular Cultivation System (EMCS), was installed on the ISS in 2006 and contained two rotors able to apply different levels of gravity (0.001–2.0g). Despite having a simple life support system, the EMCS was not suitable for growing plants for food production but performed ten experiments until its decommissioning in 2018 (Jost et al., 2015), which highlighted the need for modular, adaptable growth environments capable of supporting the fundamental research.

Two PGSs were launched to the ISS in 2009, including the eight Japanese Plant Experiment Units (PEUs), which conducted a seed-to-seed *Arabidopsis* experiment(Yano et al., 2013). The other PGS was the Advanced Biological Research System (ABRS), which conducted fundamental research on *Arabidopsis* (APEX01-TAGES), willow (APEX-Cambium), and white spruce (APEX-CSA2) before its return to Earth in 2015(Zabel et al., 2016). The Vegetable Production System (VEGGIE) is the latest and most advanced PGS(Massa et al., 2017a). Launched in 2014 and still operational, it consists of two single-chamber growth units, enabling experiments in food production due to its larger growth area compared to predecessors (0.17 m^2). As of August 2024, 30 experiments have been performed with VEGGIE, with more scheduled to further explore fundamental responses of plants to spaceflight but also advance the design of next-generation growth systems for food production in space.

The China National Space Administration has also contributed to plant science, first sending peony seeds on the Shenzhou 4 spacecraft in 2002. Nearly a decade later, in 2011, rice calli and *Arabidopsis thaliana* cell cultures were used to study the effect of microgravity aboard the Shenzhou 8 spacecraft, focusing on gene expression changes(Fengler et al., 2015; Jin et al., 2015). In 2022, on the Chinese Space Station (CSS), *Arabidopsis* was cultivated in the Wentian module laboratory over 120 days to observe a full life cycle(Jia et al., 2024), marking a significant step toward understanding reproductive success and long-term plant growth in space.

Model plants have been extensively used in space for fundamental studies, which are challenging to perform on crops(Borrill, 2020). These studies have provided the first insights into how plant biological processes respond to real space stressors and have laid the foundation for a transition from fundamental exploration to the development of operational systems for sustainable space missions through the cultivation of agricultural crops. With an increasing focus on food production, there is now an opportunity to integrate the lessons learned from these studies into the design of

modular growth systems capable of supporting diverse plant species across a range of space environments.

	Years in Use	Chambers	# of Experiments	Growth Area (cm^2)	Detailed data
ADVASC	2001 - 2003	1	3	520	Link
BPS	2002	4	2	4 x 260	Link
Lada	2002 - present	2	17	2 x 252	Link
EMCS	2006 - 2018	8	10	770	Link
PEU	2009	1 (8 installed)	1	8 x 270	Link
ABRS	2009 - 2015	2	3	2 x 268	Link
VEGGIE	2014 - present	2 (1 per unit)	30	2 x 1700	Link
APH	2017 - present	4	8	1700 shoot, 1850 root	Link
SIMBOX	2011		17		Link
MVP	2018 - present				Link
TG-2 (TongGong)	2016 - 2022				Zheng et al papers
GBCM	2022 – present	4			Link

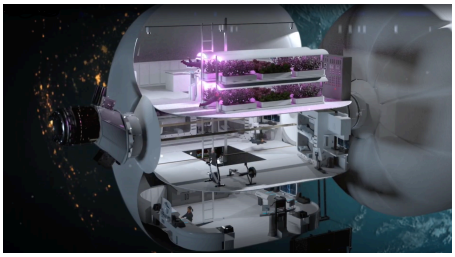
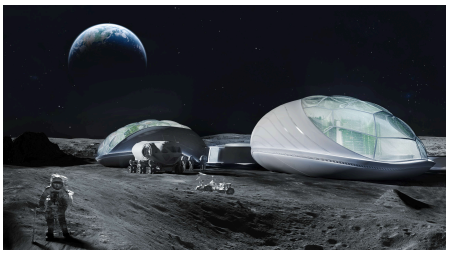
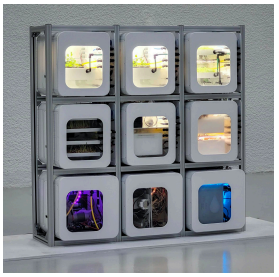
1.1 Commercial Low-Earth Orbit (CLDs) stations, Gateway, Lunar return, and the Horizon (Contributors: NLB, BAS)

The future of space exploration hinges on ensuring astronauts have a nutritious, resilient, and safe food source. This is crucial for maintaining astronaut health and metabolism during long-duration missions. NASA's Moon to Mars Objectives (2022) guide research within the ISS, upcoming commercial Low Earth Orbit (LEO) destinations, and the Lunar Gateway. Two key objectives are Human and Biological Sciences Goal 1 (HBS-1)- to understand the effects of exposure to the Lunar and Martian environment on biological systems and health, using humans, model organisms and plants and Applied Sciences Goal 5 (AS-1)- to develop crop plant systems capable of providing sustainable and nutritious food sources for lunar, deep Space transit, and Mars habitation. As on Earth, reliance on these crop systems means designing productive and resilient farming systems but with extensive added constraints.

As missions move beyond LEO, longer durations, harsher environments, and limited resupply opportunities will intensify the need for robust, self-sustaining agricultural systems. The substantial lessons learned on ISS and other space stations may also be less applicable. The ISS water processing facility (WPA), for example, has created a near closed loop on-board but integrating this with cropping system able to provide most food needs will be a challenge. Increasing the diversity of research into bioregenerative life support systems (BLSS) and space crop technologies in LEO, particularly in collaboration with commercial partners, will provide more tests, breakpoints, and

possible solutions. Specifically expanding research across a wide spectrum of crop types, inputs and control, mission scenarios, and hardware systems is essential (Wheeler, 2023). These diverse research efforts will provide more robust predictions for resilient BLSS beyond LEO to support astronaut health and the viability of human habitation on increasingly distant missions (ESA, 2021).

The European Space Agency's (ESA) Technology Strategy highlights the need for innovation in food production, especially within Environmental Control and Life Support Systems (ECLSS). This includes exploring new technologies for space crop production. There are several, agency-specific hardware designs for space crop production alongside commercial partners that include Interstellar Lab's Biopods, Sierra Space's Ohallo III, and Vertical Farming initiatives. Together, these and other similar efforts will shape the future of space agriculture. Though the funding future is unpredictable, the emerging landscape could lead to a rapid surge of new design possibilities, opening opportunities for breakthrough solutions in bioregenerative life support systems (BLiSS) to be used on Earth and long duration missions.

2 The immediate astrobotany frontiers

2.1 Media for sustainable plant growth off-Earth (Contributors BAS, NT + Who put original bullet points?)

In what should plants be grown to provide the robust and resilient BLSS necessary for experimentation and crop production in space? Extensive research over the last three decades on Earth and in space has refined several systems for plant growth in microgravity. These provide the foundation for current systems, including APH and VEGGIE. There remains a strong debate as to whether hydroponic or aeroponic systems can provide more simplicity and reliability than physical substrates, despite the long evolutionary relationship between plants and substrates. Recent innovations in plant growth systems though are providing an array of novel designs that need testing in the space environment. Longer duration missions are likely to challenge established and newer systems, particularly on their resilience, ability to provide robust food, and tight integration with other aspects of life support.

2.1.1 Plant substrates in space

Plant growth in space has included a diverse array of substrates. They provide a rooting surface, to which plants anchor, and a porous media through which nutrients and water can be provided and extracted in microgravity. Substrates have included rock wool, vermiculite/perlite, coconut coir, and other inert media. To explore differences in Arabidopsis growth, photosynthesis, and flowering between flight and ground controls, the Chromex experiments utilized a phenolic foam ("Oasis Foam") infused with nutrient solution (Musgrave et al., 1997). First flown in 1996, the Plant Generic Bioprocessing Apparatus (PGBA) was a Bioserve-designed plant growth chamber that could utilize a nutrient pack systems and agar (later soil) matrix for plant roots. This matrix allowed

for ~6cm of root growth in each cell over a porous plate membrane, with fans used to establish vertical flow for dehumidification and root aeration (Hoehn et al., 1997). Porous tube (PT) methods were adopted more broadly to control water and nutrients around roots (Dreschel and Sager, 1989). These tubes were combined with calcined clay substrates (“Arcillite”) in Astroculture (Morrow et al., 1994) and PESTO (Stutte et al., 2005) or aluminosilicate crystals (“Balkanine zeolite”) on Mir and shuttle flight experiments (Bingham et al., 2000; Jones and Or, 1999; Kostov et al., 2008). With pumps and fans, the substrate and tube combinations establish vertical flow of water and nutrient solutions, preventing hypoxic root conditions, while also providing a rooting substrate for growth.

On the ISS, the Advanced Plant Habitat (APH) and VEGGIE growth systems have built on these designs for robust and diverse plant growth experiments. The APH (Monje et al., 2020) uses Arcillite over porous tubes with negative pressure to provide root aeration and active flow of water/nutrient solutions. The VEGGIE system (Massa et al., 2017b) uses rooting “pillows”, which contain substrate, fertilizer, and germination wicks along with attached seeds. Much like the PT system, these rooting pillows interface with an underlying mat reservoir watering system to provide a capillary water column to growing plants (Massa et al., 2017d). Both APH and VEGGIE use pumps to circulate water and nutrients similar to hydroponic systems.

Hydroponic systems reduce the need for soil and soil-like substrates, delivering nutrients directly to plant roots. NASA has helped pioneer hydroponic development on Earth, including vertical farming and support for private company spinoffs, yet has not trialled hydroponics in space. On Earth, hydroponics can provide nutrients and water with root aeration because gravity makes oxygen gas rise. Microgravity therefore provides a direct challenge to this potentially more effective plant growth system (Hasenstein and Miklave, 2024). Plant Water Management (PWM) systems have been tested on the ISS to mechanically overcome this challenge, creating distinct “layers” in the solution flow through which plant roots might grow (Hatch et al., 2022; Wasserman et al., 2022). The use of hydrogels instead of Arcillite or a complete shift to aeroponic systems, that aerosolize water and nutrient solutions onto plant roots, may be important alternatives. Aeroponics demands fine-scale root moisture control and mechanisms to reduce excess moisture through vacuum or centrifugation. Over time, hydrogels can have similar root aeration issues to hydroponics but would more effectively allow for the incorporation of regolith-derived materials on Lunar and Martian missions (Hasenstein and Miklave, 2024). Both hydroponics and aeroponics systems may be limited to particular plant species and crops. Root vegetables like potatoes and carrots need the loose, aerated structure of soil for proper development (Johansen et al., 2015). Similarly, bulbous plants like onions rely on soil's pressure and resistance for bulb formation [ref]. These adaptations may create roadblocks for substrate-less cultivation methods to provide food for beyond LEO missions.

2.1.2 Earth Soil (agricultural or engineered)

Plants have a long evolutionary history with the complex soil substrates on Earth but that complexity has so far rarely been explored in space. Plant growth in soils has been limited in space, but is the next frontier - Shift of research to soils is needed to bring Earth’s biological complexity to space.

Potential concerns with using soils in a closed environment

- CO_2 release that can't necessarily be predicted - see what happened with Biosphere 2. May or may not cause problems, especially if soil plant growth chambers aren't isolated from main habitat. How this would be regulated or controlled - try and do most of the soil decomposition/formation in some kind of bioreactor?
- Obvious hotbed of microbial activity. Presumably the benefits of microbial diversity both for humans and plants outweigh the possible dangers of pathogens, but a reference or study here would be good.
- Bioaccumulation of undesirable elements over time - problems? Any papers we can find on this?
    - if using human feces - Biosolids in earth, problem has been accumulation of heavy metals in poo, but unlikely to be a problem if already monitoring for that in diet.

2.2 Model organisms and crops in space

2.2.1 Arabidopsis, the green test tube in space (Contributors NB)

Summary overview only with limiting to reviews or high impact papers only (ref)

CHAT-GPT version of bullet elements from before:

Arabidopsis thaliana has become a pivotal model organism for studying plant responses to spaceflight. Various experiments conducted using hardware systems like the Biological Research in Canisters (BRIC) and the Veggie system have provided key insights into how genetic variation affects plant adaptation to space conditions. Multiple Arabidopsis ecotypes have been flown in space, including Columbia (Col-0), Wassilewskija (Ws), Wassilewskija-2 (Ws-2), Landsberg erecta (Ler), and Cape Verde Islands (Cvi) (Choi et al., XXX). These studies show that different ecotypes and genotypes exhibit unique responses to spaceflight, with the Col-0 ecotype, for example, showing a more pronounced transcriptional response than Ws and Ler (Choi et al., XXX).

Environmental Factors and Experimental Design

The plant responses in space are also influenced by environmental factors, such as age, tissue type, and light conditions. The BRIC experiments have provided insights into how different tissue types at various developmental stages react to spaceflight conditions. Light is a key factor in determining plant responses, with studies comparing plants grown under ambient light versus darkness (Ferl et al., 2024). Spaceflight hardware systems like BRIC-LED, Veggie, EMCS, MVP, and the Advanced Biological Research System (ABRS) have enabled controlled photomorphogenesis under varying light conditions. However, it is important to consider that spaceflight hardware can degrade over time, leading to inconsistent light levels across missions. Thus, more precise measurements of light environments, particularly during germination, are needed to ensure accuracy in comparing results between studies.

Furthermore, the use of multi-omics approaches (e.g., RNA-seq, proteomics, and glycomics) in spaceflight research has provided valuable insights into plant biology. However, there is a gap in comprehensive ground control experiments that adequately support these spaceflight studies. Future research should aim to develop more robust ground-based controls that incorporate multiple omics technologies (Raul et al., XXX).

European Modular Cultivation System (EMCS) and TROPI Experiments

The European Modular Cultivation System (EMCS) has been instrumental in conducting plant growth experiments aboard the International Space Station (ISS). However, some observed

phenotypes remain unexplained. The TROPI and TROPI-2 experiments, which were conducted in the EMCS, focused on the interaction between phototropism and gravitropism in Arabidopsis seedlings, helping to clarify how plants perceive and respond to light and gravity in space.

Advanced Imaging Technologies in Space

Several advanced imaging systems aboard the ISS have enhanced our understanding of plant growth in space. These include:

- **ABRS:** A system for plant growth and observation.
- **SPECTRUM:** A spectral imaging tool for monitoring plant health.
- **Light Microscopy Module:** Enables high-resolution imaging of plant samples.

These systems have enabled time-lapse photography, morphometric analysis, and confocal fluorescence microscopy of plants in microgravity, providing insights into how plants develop and respond to space conditions.

Future Directions: Ground-Based Simulations

To deepen our understanding of plant responses to spaceflight, future research should focus on ground-based simulations that replicate spaceflight stressors. Potential areas for investigation include:

- Combining clinorotation with Galactic Cosmic Radiation (GCR) simulations.
- Replicating ISS atmospheric conditions, such as elevated CO2 levels.
- Integrating phototropism assays with microgravity simulations.

By simulating spaceflight conditions on Earth, researchers can refine experiments and further explore how plants adapt to the challenges of space environments.

2.2.2 Food crops (Contributors ?, NB)

The VEGGIE system has advanced space agriculture by testing of growth media within plant root pillows, such as commercial peat-based potting mixes, arcillite (calcined clay), and perlite-vermiculite blends, with peat-arcillite combinations generally supporting the best growth during spaceflight(Massa et al., 2011). These findings suggest the need to develop lightweight, water-efficient substrates tailored for space-based agriculture.

Crops cultivated in VEGGIE have included mizuna mustard, 'Wasabi' mustard, 'Waldmann's Green' lettuce, 'Outredgeous' red romaine lettuce, 'Tokyo Bekana' Chinese cabbage, 'Red Russian' kale, radishes, snow peas, and 'Extra Dwarf' pak choi. Early experiments, VEG-01, established proof-of-concept for growing 'Outredgeous' red romaine lettuce and zinnia flowers during spaceflight(Massa et al., 2017c). Subsequently, VEG-03 expanded the diversity of crops tested and introduced cut-and-come-again harvesting as a strategy to increase yields within the constraints of spacecraft systems. VEG-04 extended these investigations by optimising red-blue light ratios and energy-efficient methods for promoting plant growth(Massa et al., 2020). The space-grown lettuce aboard the ISS in 2015 represents an important milestone of the first consumption of space grown food *in situ* (figx) but also demonstrated the feasibility of incorporating psychologically and nutritionally important fresh food for astronauts during future long-duration lunar and Martian missions.

Partial gravity environments, such as those on the moon (0.16g) and Mars (0.38g), remain poorly characterised in terms of their effects on plant growth, development, and reproduction. Strategies for resource recycling, including water management, nutrient recovery, and radiation shielding, are still in early stages of development, and the role of plant-associated microbiota, including nitrogen-fixing and plant growth-promoting bacteria (PGPB) essential for crop growth on Earth, has yet to be explored in any detail under extraterrestrial conditions. Similarly, while the focus on food crops in space agriculture has advanced understanding of plant growth in small, highly controlled systems, the potential of non-food plants to complement food crops or contribute to a sustainable habitat remains almost entirely unexplored.

2.2.3 Non-food crops (Contributors SS, RB?)

Future-centric guidance, functions (waste, materials, energy, pharma, chemistry, phytoremediation and ISRU). Keep refs modern and tight – Sections 3 and 4 are more forward looking.

Contributors: SS (non-food), RB (ie. cotton)?,

Provisional draft deadline:

Core topic/take-home for this section is 1) we've done lots of plant experiments, 2) they often have growth issues and will in the future, leads to 3) microbes are and will be often important in success

3 Plants require microbial interactions

3.1 Healthy function at the plant-microbe interface (Contributors BAS)

Build from functions above – keep forward looking ie. "Path forward"

Plant growth and health are intricately linked to their interactions with microorganisms. The effects of a fungus, bacteria, or virus can vary based on microbial load, the host plant genotype or species, growth period, and environmental factors (Azarbad and Junker, 2024; Hoeksema et al., 2010; Zhao et al., 2023). Plant responses from inoculation with multiple microorganisms or whole microbiomes can be even more variable (Kaminsky et al., 2019; Liu et al., 2023). So how can we predict microbial effects for plants in space? First, extensive existing data from Earth-based studies should be brought to bear on the problem. These studies often quantify the relative importance of several factors to plant-microbiome outcomes (Fitzpatrick et al., 2020; Trivedi et al., 2020). Predicting these functional outcomes is essential to better understand responses of model plants like Arabidopsis, as well as for crops and other plants on which we will depend. Whenever possible, we should focus on data from systems that best offer insights into space-relevant stress. Extreme terrestrial environments, such as Antarctica, and closed systems (e.g., growth chambers) offer insights into how plant-microbe interactions may evolve under space conditions, informing future space plant science experiments.

Certain functions of plant-microbial systems are especially important in space. These include well known growth benefits, improved nutritional content, and water use efficiency (Compant et al., 2024). Each stress is distinctly impacted by the space environment. For example, plant water stress beyond Earth is influenced by the watering system, growth media, and the effects of microgravity (Hatch et al., 2022; Smirnova et al., 2023). Specific plant symbionts, like endophytic fungi, may enhance plant water relations and stomatal conductance (Augé, 2004; Augé et al., 2015) in space flight. Plant-associated microbes can aid plant hosts with nutrient capture (Mylona et al., 1995; Smith and Read, 2010) and enhance crop nutritional content (Consentino et al., 2022; Hart et al., 2015; Hart and Forsythe, 2012). These nutrients will be essential for astronaut health (Tang et al., 2021). Plant-microbe interactions that reduce radiation and oxidative stress may also

enhance plant resilience (Bacha et al., 2024; Gupta et al., 2022), during both exploration and settlements. Expanding the utility of plant-microbial systems to other planets will likely rely on increased resistance to drought (Ricks and Yannarell, 2023), salinity, heavy metals (Mishra et al., 2017), and temperature extremes (Rodriguez et al., 2008), all of which microbes have been shown to provide.

Which microorganisms might be most likely to provide enhanced resilience for mission critical plants? A good place to start is by more extensively testing the increasing diversity of recognized habitat-adapted plant symbionts (Bouremani et al., 2023; Rodriguez et al., 2012) in space and space-like conditions. These experiments are currently rare and represent low-hanging fruit for plant space biology research. For example, several studies have looked at legume growth responses to microgravity (Kamada et al., 2020; Levine and Piastuch, 2005; Lionheart et al., 2018; Nakajima et al., 2021). Far fewer explicitly quantify nitrogen-fixing bacteria, which is central to legume function and nutrition. Microgravity can impede nodulation or impact a plant's ability to form nodules after exposure (Stutte and Roberts, 2013). Similarly, the oldest and most common root fungal symbionts, arbuscular mycorrhizal (AM) fungi, regulate host growth, shaping plant productivity, nutrient content, and abiotic stress tolerance (Begum et al., 2019; Smith and Read, 2010). Ground-based studies have explored mycorrhizas in simulated gravity (Liu et al., 2018) and in lunar regolith simulants (Atkin and Oliveira Pedro dos Santos, 2024; Konings-Dudin et al., 2014), yet to date no space flight experiments have occurred. Expanded space flight testing for well characterized plant mutualists like these is essential and provides a key step to mechanistically understand how the space environment may shift microbial functions.

Functional tradeoffs for plants and microbes present an important ecological lens through which these outcomes should be considered. Many microbial associations exist on a continuum from mutualism to parasitism (Johnson et al., 1997). Microbes that aid nutrition under some conditions may compete or hinder growth under others. Resource availability often provides the context for mycorrhizal benefits, but with significant variation among hosts and systems (Hoeksema et al., 2010). Plant root system also plays a key role in mycorrhizal outcomes(Yang et al., 2015), providing a rubric to predict mycorrhizal effects on roots impacted by microgravity. Yet, parasitism in this symbiosis is rare (Purin and Rillig, 2008). In contrast, *Fusarium solani* causes root rot in several plant species, including legumes, potatoes, and cucurbits, and can even cause mycoses in humans. Under drought though, certain strains of *F. solani* can help reduce plant water stress (Kavroulakis et al., 2018). These switches are driven by environmental stress and include functional tradeoffs. Stressed plants are usually more susceptible to disease-causing pathogens, but on Earth, many of plant-microbial relationships are coevolved to ameliorate stress. Microgravity, radiation, and other space stresses may therefore increase plant-microbial inter-dependence and resilience, but microorganisms that can act as pathogens may be increasingly likely to do so. The recent example of *Fusarium oxysporum* infecting Zinnias on the ISS (Schuerger et al., 2021) provided a key example. Plant microbiomes help suppress *F. oxysporum* and other pathogens (Carrión et al., 2019; Sikes et al., 2009) so may be useful tools. Without active plant-microbial experiments, however, plant space biology will learn about microbial functions only in reaction to contamination and opportunistic infections like Fusarium. These may increase in frequency and represent a pivotal problem for longer duration missions that rely on plants.

How do microbes on missions colonize plants? The largest source of microbes that can colonize plants is likely to be the spacecraft environment and astronauts themselves. Extensive efforts to

surface sterilize seeds, growth systems, and nutrient solutions in ISS experiments still produce plants with diverse microbiomes. Several VEGGIE experiments, for example, show lettuce, tomato, and mixed green plants with similar or even greater abundance and diversity of bacteria and fungi (Hummerick et al., 2021; Khodadad et al., 2023, 2020; Spern et al., 2025). Some may be present as endophytic taxa within the seeds, but many are common airborne taxa or those present in water lines that are colonizing after germination. The VEGGIE pillow systems for substrates are prepared sterile but develop a distinct microbial community that has the greatest overlap with crop roots grown during the experiment (Hummerick et al., 2021). Microbiome connections between plants and substrate are common on Earth, with distinct niches in bulk soil, on the surface of, and within plant roots (Edwards et al., 2015; Peiffer et al., 2013; Schlaeppi et al., 2014). Shifts across these niches show response to, and impact on, host abiotic stress (Wang et al., 2024) and disease (Zhang et al., 2017). The habitat microbiome therefore may be critical to shape plant microbiome structure and function. Over the last decade, we have built an extensive understanding of this environment on-board the ISS in ways that can inform the colonize and subsequent effects of habitat microbes colonizing plants.

3.2 Microbial tracking on the ISS and Pathogens

The seeds of plants grown aboard the ISS have exclusively been surface-sterilized prior to launch . Any microbes (i.e. bacteria, archaea, fungi, and protists) and viruses colonizing or infecting ISS live plant tissues that did not originate inside seeds must perforce come from the native microbiome of the ISS itself – a microbial ecosystem composed of the microbes and viruses shed by astronauts, as well as any microbes hitching a ride on supplies, building materials, and experiments brought on board. The ISS microbiome is primarily composed of human-associated species ((Checinska Sielaff et al., 2019; Salido et al., 2025), including many strains that act as secondary pathogens to humans (Singh et al., 2018; Tierney et al., 2022). These microbes have inadvertently affected plant growth and the presence or absence of plant diseases aboard the ISS, as well as potential food safety, but whether these effects have been positive, negative, or neutral for plant growth and human health remains unknown.

The microbial communities of the surfaces, air, and water supplies aboard the ISS are both mundane and profoundly fascinating, as multiple studies and reviews have discussed over the past decade (Be et al., 2017; Checinska et al., 2015; Checinska Sielaff et al., 2019; Mora et al., 2019, 2016; Salido et al., 2025; Singh et al., 2018; Urbaniak et al., 2022; Venkateswaran et al., 2014). The total species pool of the ISS is subject to both dispersal limitation (as a sealed habitat in space) and concomitant historical contingency (due to the transient nature of astronaut stays) (Costello et al., 2012). The ISS is a ultra-low-biomass environment, similar to hospitals and cleanrooms in that it is desiccated, nutrient-poor, and undergoes constant cleaning and air filtration; unlike cleanrooms and hospitals, microbial input from natural soil and water systems is virtually non-existent. Areas where studies of the microbiome or of individual isolates have been conducted, including metagenomes and isolates from both hard and cloth surfaces where there is significant skin contact or usage (Checinska Sielaff et al., 2019; Mora et al., 2019; Salido et al., 2025; Urbaniak et al., 2022), amplicon sequencing and isolates from flex lines of the Urine Processor Assembly (UPA) (Nguyen et al., 2023), isolates from the Water Process Assembly (WPA) (Castro et al., 2024). Initial characterization of the microbiome of ISS surfaces found it to be most similar to that of build environments such as office buildings (Checinska Sielaff et al., 2019). A dataset collected over a larger number of surfaces from all modules further showed that the ISS microbiome is significantly depleted in microbes native to Earth soil, water, and air; it is most

similar to Earth built environments with lower exchange with Earth's biosphere, such as COVID-19 quarantine facilities, unoccupied hospitals and closed-habitat analog study sites (Salido et al., 2025). Dust collected aboard the ISS is significantly lower in diversity and biomass, has a lower fungi to bacteria ratio, and is much more heavily dominated by *Aspergillus* spp. compared to dust collected in U.S. homes on Earth (Nastasi et al., 2024).

Microbial growth and dispersal in microgravity vs on Earth

Conditions for microbial dispersal and colonization differ for microgravity in multiple respects. In liquid under microgravity , non-motile microbes rely almost on mass diffusion for signalling, nutrient intake, and removal of waste products (Rosenzweig, Ahmed et al. 2014), forming nutrient depletion zones and potentially inhibiting metabolic processes. Microbes experience 'bubbles' of waste product and dissolved CO_2 accumulation, which have the non-intuitive effects of decreasing lag phase growth, increasing log phase growth, and increasing total cell number in growth experiments (Li, Diaz et al. 2023). In liquid growth experiments in microgravity where gases are not fully removed, gases in enclosed containers assume a toroidal shape (Nicholson, Fajardo-Cavazos et al. 2021), rather than the separation of liquid below gas under gravity. This greatly increases the surface area of the liquid-gas interface, including much greater access to O_2. Biofilm formation complicates this area of research and is a controversial subject for the study of microbial growth in microgravity; some studies have observed increased biofilm formation with microgravity (Diaz, Dixit et al. 2023) while others have seen no particular increase or change in biofilm morphology with microgravity (Flores, Luo et al. 2024). Biofilms inside built environments are a concern for human health, due to the increased difficulty of cleaning surfaces where biofilms are growing, the potential for biofouling of plumbing (Diaz, Dixit et al. 2023, Nguyen, Sharp et al. 2023), and the increased likelihood of bacteria gaining antimicrobial resistance while in biofilm form. All of these factors are likely limited to either liquid systems or to pockets (however small) of liquid that may form in overlooked/hidden areas of the ISS. Meanwhile, microbial transfer from one surface to another via air is likely limited but possible – the ISS undergoes strong filtering ventilation to remove particulates and create air movement to mimic density-difference-based airflow (which is non-existent in microgravity). This creates an atmosphere in which the baseline number of air particulates is zero, with single spikes indicating potential moments of dust disturbance/dust and hair dispersal such as vacuuming or crew movement (Marit Meyer, personal comm.).

Health concerns from the ISS microbiome

The focus of most studies of the ISS microbiome has been on its potential negative effects on human health, specifically microbes that might cause disease. Secondary pathogens and genes for antimicrobial resistance (AMR) and virulence have certainly been detected via both shotgun metagenomic sequencing and isolation of live strains (Mora et al., 2019; Singh et al., 2018), and some secondary pathogens have persisted aboard the ISS for many years, as evidenced by the proliferation of the same clonal strains of known or newly discovered species over multiple samplings in the past decade (Tierney et al., 2022; Urbaniak et al., 2019) (Hill et al., in submission). Based on amplicon and shotgun metagenomic data, there is no evidence that the microbiome of the ISS is more enriched in genes for AMR or virulence than any other indoor environment on Earth (Mora et al., 2019; Singh et al., 2018), as opposed to the enrichment in AMR and virulence genes found in hospital environments (Gupta et al., 2019; Klassert et al., 2021) and livestock enclosures and wastewater. Phenotypic studies of AMR and virulence in bacteria and fungi isolated from the ISS have shown that in some cases ISS strains are far more virulent and/or resistant to a much wider array of antibiotics than closely related Earth strains (Tierney et al., 2022; Urbaniak et al., 2019) despite no differences from Earth strains in genes encoding for AMR and virulence - an effect that is likely therefore due to differences in transcriptional regulation acquired

due to the stress of the ISS environment. This has only observed in some secondary pathogens, with other non-pathogenic strains showing no difference in antimicrobial resistance or virulence compared to Earth strains (Mora et al., 2019).

Potential interplay between ISS surfaces, astronauts, and plants

Microbes present within surface-sterilized seeds and the general microbiome of the ISS are (in theory) be the only pools available to colonize plant tissue. The vertical transmission of the plant holobiont from seeds is therefore dependent on species and seed batch/source, while horizontal transmission from the environment is dependent on the core microbiome of the ISS as well as the individual microbiomes of astronauts.

In one respect, plants grown in a closed environment such as the ISS or hydroponic facilities/vertical farms on Earth are much less likely to host microbes pathogenic to humans, because they are not exposed to or fertilized with, animal waste or compost containing animal/human waste.

Anti-microbial resistance/virulence and plants

Plant stress and the switch from commensal to pathogenic microbes

Microbes introduced as plant-beneficial may affect a space-station microbiome (or may end up attacking the plant under stress conditions)

-

- humans are inured to most microbes living on plants and mostly it's not a concern – except in space, humans may be much weaker and cancerous, microbes may become more virulent.

Forest bathing?

- Is the microbiome of a space station diverse and robust enough to provide plants with sufficient beneficial microbes for robust plant growth and stress tolerance? How would this change in the long-term if resupply was reduced or non-existent for years?

Notes from microbial food safety in space production systems white paper

Pre-packaged food types include thermostabilized (retort process)
dishes, irradiated meat items, dried and freeze-dried foods, extended shelf-life bread products, dry beverage mixes, and natural-form foods, supplemented by a few fresh foods as preference items and, only recently, some space-grown crops. Food safety in processed foods relies on safe production methods: the Hazard Analysis and Critical Control Point (HACCP) system and Good Manufacturing Practices (GMPs). Foods that are not commercially sterile must meet standards for microbial tolerances (2x104 CFU/g total aerobic count; 103 CFU/g yeasts and molds; 102 CFU/g coliform or coagulase-positive *Staphylococci*; no *Salmonella*) [6].

The great unknown: Specific concerns for plant viruses in space

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Plant viruses in space

While surface sterilization techniques are rigorously employed to ensure the health of these crops [ref], a hidden threat persists – seed-borne viruses [ref]. These pathogens, residing within the seed itself, can bypass surface sterilization and pose significant challenges to successful plant cultivation on the ISS.

Seed transmission occurs when viruses infect a parent plant and subsequently infiltrate the developing seeds. This can happen through various pathways, including the vascular system or direct infection of the embryo [ref]. These viruses can remain dormant within the seed, evading detection and surviving harsh conditions [ref]. Once the seed is planted and germinates in the controlled environment of the ISS, the virus reactivates, potentially compromising the plant's health and jeopardizing the entire crop.

The implications of seed-borne viral infections are particularly concerning in the confined and resource-limited environment of the ISS. An outbreak could lead to significant crop losses, impacting food availability for astronauts. Furthermore, the closed system of the ISS and exceptional conditions, such as high radiation doses, microgravity, and compact spaces, increases the risk of rapid spread, with limited options for containment and treatment [ref]. Radiation is a known mutagen. It can damage DNA and RNA, the genetic material of viruses, leading to changes in their sequence [ref]. These changes can be random, potentially leading to new traits in the virus. Some of these traits might make the virus more infectious, more resistant to treatments, or even change its ability to jump between species [ref]. While the direct effects of microgravity on virus mutation are less clear, studies suggest it can alter the behavior of cells and potentially influence how viruses replicate within them [ref] and their transmission [ref]. Additionally, lunar or martian regolith contains various minerals and compounds that could interact with viruses. Some of these substances might be mutagenic themselves, or they could cause stress to the host [ref], potentially increasing the viral mutation rate. Viruses already mutate rapidly in normal environments [ref]. The space environment could accelerate this process, potentially leading to faster evolution of viruses. This poses a serious challenge to the sustainability of space agriculture and the success of long-duration missions.

Several factors contribute to the difficulty in eliminating seed-borne viruses. Firstly, these viruses are often deeply embedded within the seed, beyond the reach of surface sterilization techniques. While methods like chlorine gas and UV radiation can effectively eliminate surface contaminants, they may not penetrate the seed coat to neutralize internal pathogens [ref]. Secondly, the diversity of seed-borne plant viruses, including bean common mosaic virus [ref], cucumber mosaic virus [ref], lettuce mosaic virus [ref], tomato mosaic virus [ref], among others, and their complex interactions with host plants make it challenging to develop universal detection and elimination methods.

Addressing this challenge requires a multi-pronged approach. Firstly, rigorous testing and screening procedures are essential to identify and eliminate infected seeds before they reach the ISS. This could involve advanced molecular techniques like PCR or ELISA to detect viral presence with high sensitivity [ref]. Secondly, developing virus-resistant plant varieties through genetic engineering or traditional breeding methods could provide a long-term solution. Finally, implementing strict phytosanitary measures on the ISS, such as isolating infected plants and using sterile tools, can help contain outbreaks and prevent further spread.

In conclusion, while surface sterilization is a critical step in ensuring plant health on the ISS, it cannot fully eliminate the risk of seed-borne viruses. These hidden pathogens pose a significant challenge to space agriculture and require a comprehensive approach involving rigorous testing, resistant varieties, and strict phytosanitary measures. Since most plant viruses have an RNA genome [ref], this would include optimization of sample collection and storage for further investigation of potential infections. This would entail longitudinal collection of samples across tissues and location in the ISS, aliquoting samples before RNA depletion and/or RNA extraction, genomic sequencing for identification of the pathogen and assessment of viral diversity, and phylodynamic modeling to reconstruct the viral evolutionary rate and transmission dynamics in space [ref]. If for any reason this is not possible, preservation of tissues or samples would allow exploration of these and other questions in the future. Ultimately, these guidelines would facilitate overcoming this challenge is crucial for ensuring food security and the success of future space exploration endeavors.

Contributor: NST – BAS edited a bit: I like this writing but we haven't introduced plant microbes yet. As such, it's not appropriate in it's current form. If we follow the pattern so far, we want a short paragraph about the innovations of hydroponics/aeroponics for plant growth in space.

Hydroponics has clear advantages for controlled environment agriculture but has limitations when it comes to certain food crops. Soil aeration and drainage also creates niches that sustain a more diverse microbial community on which plants rely, aspects that are hard to replicate hydroponically. The structural complexity of soil increases fungal dominance and supports a complex food web that that better retains nutrients, suppresses plant diseases, and enhances overall plant health (Saleem et al., 2019). In contrast the hydroponic environment is relatively homogenous, increasing bacterial dominance (Thomas et al., 2024). Plant-microbial coevolution to the soil environment underlies many recent advances in sustainable agriculture (Lyu et al., 2021). These adaptations represent roadblocks to the singular use of hydroponic or aeroponic systems for growth. Soil-less systems do offer better direct nutrient and moisture control for plant growth environments, particularly in microgravity. New research to enhance the hydroponic microbiome may improve the generalizability and utility of these systems (Stegelmeier et al., 2022)

Integrating plant-microbial associations into space-plant biology must be done at a foundational level. Apart from targeted experiments, all plant space experiments should have standard protocols to preserve material in the integrated biobank for Space Life Sciences (ibSLS) for future microbiome analysis. Experiments described above should parallel efforts to measure pathways for microbes to disperse to plants. Some of this data already exists. Common microbial taxa on the International Space Station are consistently surveyed (see below). How have these been altered by the presence of plants in the Advanced Plant Habitat (JAXA module) or VEGGIE systems (Columbus module)? How well do plant microbiomes resemble local surfaces compared to an ISS-wide microbiome? These surveys can provide hypotheses that can be experimentally tested by the growing array of commercial players in spaceflight. Plant microbiomes may also be effective tools that can aid other mission challenges. For example, does water from cycled through plant microbiomes differ in biofilm formation, which challenge both ISS (Vaishampayan and Grohmann, 2019; Wong et al., 2010) and extended missions (Zea et al., 2020). On Earth, strategies to control plant pathogens increasingly is a process to establish and maintain a healthy microbiome, not just eliminate pathogen sources. With plants and their associated partners as a foundation, this concept can be extended to human habitation in space. Plant-microbial interactions will be

influenced by the humans that care for them, ranging from design of greenhouses to microbial exchanges. Removing humans from plant cultivation might reduce some disease risk. However, efforts to fully automate plant production overlook the essential role they play in human mental health (Lee et al., 2015; Oluwafemi et al., 2021), human-plant coevolution (Murphy, 2007), and the critical microbial exchanges between humans and plants (Krishna et al., 2019). Plant space biology experiments that help optimize plant-microbial functions and resilience are the foundation for our continued adventure.

4 Enabling a multiomics future in plant- microbial research

Contributors: N.J.B.B. (transcriptomics), E.G (metagenomics), E.S (metabolomics)

Plant omics data from spaceflight cultivation experiments have predominantly focused on host transcriptomics (Barker et al., 2023) or, to a lesser degree, metagenomics (whole metagenome or amplicon sequencing) (Khodadad et al., 2020). The integration of these data, alongside an expansion of assays to include metabolomics and proteomics (Olanrewaju et al., 2023; Zeng et al., 2022), is paving the way for a multiomics-driven future of plant science in space. These integrated datasets, which encompass both host-focused and community-wide perspectives, capture signals from both plants and their associated microbial communities, and understanding the contributions of these signals represents a challenge for astrobotany as well as for contemporary agriculture on Earth.

4.1 Transitioning from transcriptomics to Metatranscriptomics

Transcriptomics has been the most regularly used omics approach in plant space science to-date, with 36 datasets in NASA's GeneLab (Berrios et al., 2021). These datasets, generated using RNA sequencing, capture changes in gene expression, providing insights into the biological processes occurring within plants under spaceflight conditions.

Best practices in data analyses are crucial. After sequencing, genome-guided methods, such as HISAT2 (Kim et al., 2019) or STAR (Dobin et al., 2013), can be used for mapping of RNASeq reads to known transcripts when reliable references are available. However, discrepancies between the reference genomes can lead to mapping errors. *De novo* assembly provides an alternative, reconstructing transcripts directly from sequencing reads using tools like Trinity [31], SOAPdenovo-Trans [32], IDBA-Tran [33], and rnaSPAdes [34]. This avoids reference bias, preserving unknown biology and capturing alternative splicing events and isoforms, a key challenges in polyploid plant species. Assembly also removes reliance on reference genomes for differential expression statistics, which can compromise analysis[35], while still benefiting from detailed *a priori* knowledge in reference genomes during later competitive annotation of transcripts. This retains the diversity of transcriptomic data, minimising arbitrary loss of sequences that do not map, or mistakenly map[36], to provide a multiomic-ready framework for transcriptomic analysis in plant systems.

Traditional plant transcriptomics workflows poly(A) enrich RNA libraries, a standard practice in plant science since its development in the 1970s (Darnell et al., 1971). Poly(A) enrichment selectively targets eukaryotic mRNA by enriching transcripts with polyadenylated tails. While effective for partially enriching eukaryotic mRNA, the technique introduces biases towards longer poly(A) tail length[37], and selects against bacterial RNA, as polyadenylation in bacteria functions as a transient degradation signal rather than a stabilising mechanism (Mohanty and Kushner, 2016; Sarkar, 1997). As a result, polyadenylated bacterial RNA constitutes a smaller fraction of the total

bacterial RNA population at any given time, typically ranging from 15–25% under specific conditions[38,39]. This bias makes PolyA enrichment unsuitable for future integrated multiomics studies and metatranscriptomics, decreasing bacterial RNA and adding undue noise to eukaryotic signals from plants and other organisms commonly detected in plant RNASeq data (particularly rhizosphere), such as fungi and helminths [40,41].

Metatranscriptomic workflows aiming to capture cross-kingdom interactions essential for understanding plant-microbe dynamics are being developed, with tools such as HUMAnN2[42] widely used for profiling functional pathways in well-characterised microbial systems. In contrast, assembly-based workflows[43,44] could be better suited for plant-associated systems with less well-characterised microbiomes. Despite these advances, many workflows may not adequately reflect the complexity of diverse datasets and future advancements for plant systems should move beyond "push-the-button" approaches, emphasising flexibility to retain unknown diversity, scalability to integrate multiomics datasets, and adaptability to specific ecological and experimental conditions, such as those encountered in spaceflight environments.

To align transcriptomics with the goals of multiomics to support reproducibility, cross-study and cross-technology comparability, future studies should focus on:

i. Adopt inclusive RNA capture strategies over Poly(A) enrichment,

ii. Use *de novo* co-assembly to maintain data complexity and minimise reference bias,

iii. Ensure complete reporting of read use and fate.

Assembly strategies are particularly important for metatranscriptomics in space systems, where the contribution of plant-associated microbiota to ecosystem function and resilience still represents a substantial knowledge gap.

4.2 Metagenomics for an inventory of plant-microbe functions

As plant metatranscriptomics reveals functional interactions with microbial communities, metagenomics is becoming invaluable to provide the genomic context necessary to interpret functional data, offering the genetic blueprint of functional potential.

Metagenomics can use targeted amplicon sequencing or shotgun whole metagenome sequencing (WMS). Amplicon sequencing, such as 16S rRNA or ITS sequencing, focuses on marker genes for taxonomic profiling, while WMS aims to assemble the genomes of all taxa within a community. Amplicon sequencing has notable limitations, including bias of “universal” primers, reliance on imperfect and incomplete reference databases, and often lacking the resolution to differentiate closely related species or strains. Nonetheless, amplicon sequencing remains an essential tool for high-throughput comparative studies, particularly in spaceflight experiments or when characterising change in complex rhizosphere communities. Moreover, when used with advanced methods [45-48], amplicon sequencing can often reach species-level identification in a taxa specific manner, even accurately reflecting gene copies and generating biologically relevant information of highly complex environments.

De novo WMS assembly constructs contigs directly from sequencing reads without prior knowledge of the genome using algorithms like Ray Meta [49], MetaSPAdes [50], or MEGAHIT [51], employing De Bruijn graph-based methods to identify overlaps between reads. In cases where multiple samples are available, co-assembly is more commonly being used to improve the recovery of low-abundance genomes[52], combining reads from multiple samples. is particularly

useful for studies investigating microbial dynamics across between multiple samples and conditions. Despite a demanding computational resource, co-assembly is particularly useful for comparative statistical analysis across samples through improved assembly quality and enhanced the resolution of rare genomes, or bacterial “dark matter”[53].

By employing these assembly strategies, researchers can generate high-quality contigs that form the foundation for subsequent analyses, including binning, taxonomic profiling, functional annotation, and abundance estimation. Taxonomic classification with tools like Kraken2 [54] uses k-mer matching for rapid classification to reference databases, while marker gene-based approaches like MetaPhlAn4 [55] profile microbial communities by targeting clade-specific gene collections. Taxonomic and functional profiling can use *de novo* assembly of reads into contigs. Similar to transcriptomics, this can be advantageous for environments with novel or poorly characterized microbes. Tools like Prokka [56] and MEGAN [57] can be used for taxonomic and functional profiling in assembled metagenomes, but accurate metagenomic annotation is still a challenge in plant-associated microbiomes, especially from soil environments, where uncharacterised microbial genomes lead to misclassifications. Metagenome-assembled genomes (MAGs) offer potential for reconstructing genomes directly from complex microbial communities, to resolve annotation challenges. Widely used tools such as MetaBAT2 [58], CONCOCT [59], or MaxBin2 [60] group contigs based on these sequence features; however, their accuracy depends heavily on assembly quality and relatedness of taxa. MAGs offer important insights into microbial community composition and functional potential, but should be interpreted cautiously in poorly characterized environments while methods evolve to improve database coverage, computational algorithms, and sequencing technologies. Downstream analysis in metagenomics includes differential abundance analysis. Statistical approaches for differential abundance analysis, such as those implemented in tools like DESeq2 [61], can account for variability in sequencing depth and is particularly useful for detecting microbial changes to environmental shifts, host health, or experimental treatments.

To align metagenomics with the goals of multiomics, future studies should:

i. focus on assembly-based whole metagenome sequencing (WMS) which retains data complexity and minimises filtering and reference bias,

ii. Incorporate biological replication through *de novo* co-assembly,

iii. Distribution reads accurately across samples, and report complete read use and fate

The use of biological replication within data processing and robust statistical analyses can help detection of important community shifts, particularly those in rare taxa that will help drive plant-microbe discoveries on earth and in space.

Similar to transcriptomics assembly-based metagenomics workflows, can preserve data integrity within complex samples more effectively than read-mapping methods (ref). Discarding unmapped reads or contigs outside gold standard MAGs removes important information. Complete reporting of read use, assembly metrics, and sequence fate is critical for transparency and cross-study comparisons (ref). In comparative analysis of complex metagenomics samples, co-assembly-based workflows use biological replication to include shared sequence information in the assembly process (ref). This leveraging of replication has the potential to improve detection of differential abundance of rare taxa (as it does in gene expression studies (ref)). Detailed reporting of assembly metrics, including read retention, filtering and thresholding, assembly completeness, and annotation parameters, is necessary to ensure reproducibility and facilitate integration with other omics datasets.

4.3 Untargeted metabolomics for multiomics integration

Untargeted metabolomics can also provide the inclusive and unbiased datasets required for a multiomic future of plant science, but require standardisation of protocols for reproducibility and reliability(Kodra et al., 2022), which are less widely adopted than those in the transcriptomics or metagenomics fields. This standardisation is ongoing (ref) to enable high-resolution mass spectrometry (HR-MS) and tandem mass spectrometry (MS/MS) for more accurately and confidently identify metabolites, integrate retention time and ion mobility data along with MS data to improve the annotation and reduce ambiguities. Similarly, improvement and integration of bespoke and diverse public spectral databases will be critical to maximising annotation coverage and confidence in order to meet the current extraordinary shortfall of metabolite identification (ref).

The further adoption of standardised reporting practices can help improve literacy in these protocols, and is necessary for metabolomics to effectively contribute to multiomics studies. The required standardisation of data reporting for metabolomics originates from the Metabolomics Standards Initiative (MSI)(Fiehn et al., 2007a) which outlines a distinct reporting framework for plant metabolomics(Fiehn et al., 2007b), as well as cell cultures and microbiology(Morrison et al., 2007a), environmental studies(Morrison et al., 2007b), and human and animal studies(Griffin et al., 2007). These build upon general guidelines(Goodacre et al., 2007; Sumner et al., 2007) for: experimental design, sample preparation, data generation (chromatography, mass spectrometry, RMN), data processing and metabolite identification (including how to report unknown metabolites) as well as minimum information for public metabolomic repositories (Spicer et al., 2017).

Looking forward, multiomics-ready untargeted metabolomics requires improved use of computational tools and algorithms for better data processing, annotation, structural prediction, statistical analysis and networking (equivalent to those used in contemporary transcriptomics and metagenomics). While data preprocessing steps are largely accepted components of untargeted metabolite workflows, including feature detection, chromatogram building, deconvolution, isotope grouping, cross-sample alignment, quality filtering, gap filling, correlation grouping and minimal occurrence filtering, the retention of currently unknown or lower-confidence signals that can play significant roles in biological systems are logical but less wildly accepted practices (Sas et al., 2024). Data transformation and differential abundance approaches analogous to those commonly used (such as Eigen MS (Karpievitch et al., 2014)) are being applied in discovery-rich non-model organisms (Sas et al., 2024) but their application in model systems should help create the foundation required for multiomics study.

To align metabolomics with the goals of multiomics, future studies should focus on:

i. Untargeted metabolomics
ii. Biological replication and accurate alignment across samples
iii. Advanced statistical differential abundance analysis

4.4 Multiomics Analysis: Bridging Data for Complex Biological Systems

Individual omics approaches capture distinct molecular snapshots but lack the depth to resolve dynamic plant-microbe interactions across multiple biological layers. A core advantage of multiomics is its ability to validate and refine hypotheses derived from single-omics data, and reveal functional relationships that would be overlooked in isolation. Combining metagenomics with

metatranscriptomics and metabolomics allows researchers to determine not only the genetic potential of microbial communities but also their active contributions to plant responses.

In space, multiomics must bridge technologies to help understanding of how plant-microbe systems adapt to microgravity, radiation, and other new space-specific stressors to enable sustainable plant growth on Gateway, the moon and Mars. Importantly, multiomics workflows must retain complexity of datasets rather than filtering out low-confidence signals, ensuring that biologically relevant but poorly characterised processes are not lost. Statistical methods, including regression models and machine learning, are increasingly applied to predict interactions and refine functional annotations, enhancing the interpretability of high-dimensional datasets. Future research must benefit from open science initiatives for cross-study comparisons to address challenges in data integration. By prioritising data retention and robust experimental designs, multiomics will continue to shape the future of space agriculture, enabling plant growth in extreme conditions while providing fundamental insights into plant health and microbial interactions.

5 Plant-microbial experiments beyond LEO (what we need to know)

Bucket for Priorities for Future Plant-Microbial Research (Concise Sentence what you think!)

- All plant experiments should preserve data, tissues, etc that can be explored for microbes and interactions
-

5.1 Observing and understanding microbe-plant interactions in space

5.2 Bridging space and earth microbe-plant knowledge

Linking to vast ground data on plant-microbial associations (might BEHOOOVE the space biology community to start assembling good quality metagenomics data from Earth plants of the same species typically sent to the ISS - the range of healthy or unhealthy microbiomes for a single species may be much vaster than from a single experiment. We need better non-space controls!)

5.3 Altering and harnessing microbe-plant interactions

5.4 Integrating in situ resources as the environment for plant-microbial environments

5.4.1 Lunar, Martian and asteroidal regolith

Keep brief and focused on shaping plant-microbial outcomes

What about asking BISRU-C people? Laura Fackrell, Rafael Loureiro, Andy Palmer?

Their recent paper here: https://www.nature.com/articles/s44264-024-00013-5

Lunar (keep it immediate) – current research, pressing need ASAP to enable Artemis goals.

Slag from ISRU - is it useful in any way for plant growth?

Permanently shadowed regions - LCROSS experiment. Is there actually mercury there in quantity? What about PAHs? Tons of sulphur compounds. Since there's no data beyond that, probably best to keep this short. RIP Viper 😕

Mars - perchlorate bioremediation - tons of experiments going on there.

- Plant Trek - specialised microbial community
- Arizona person/Drew - single strains, wastewater

- Plant-based bioremediation - isn’t someone here doing that

- Figures

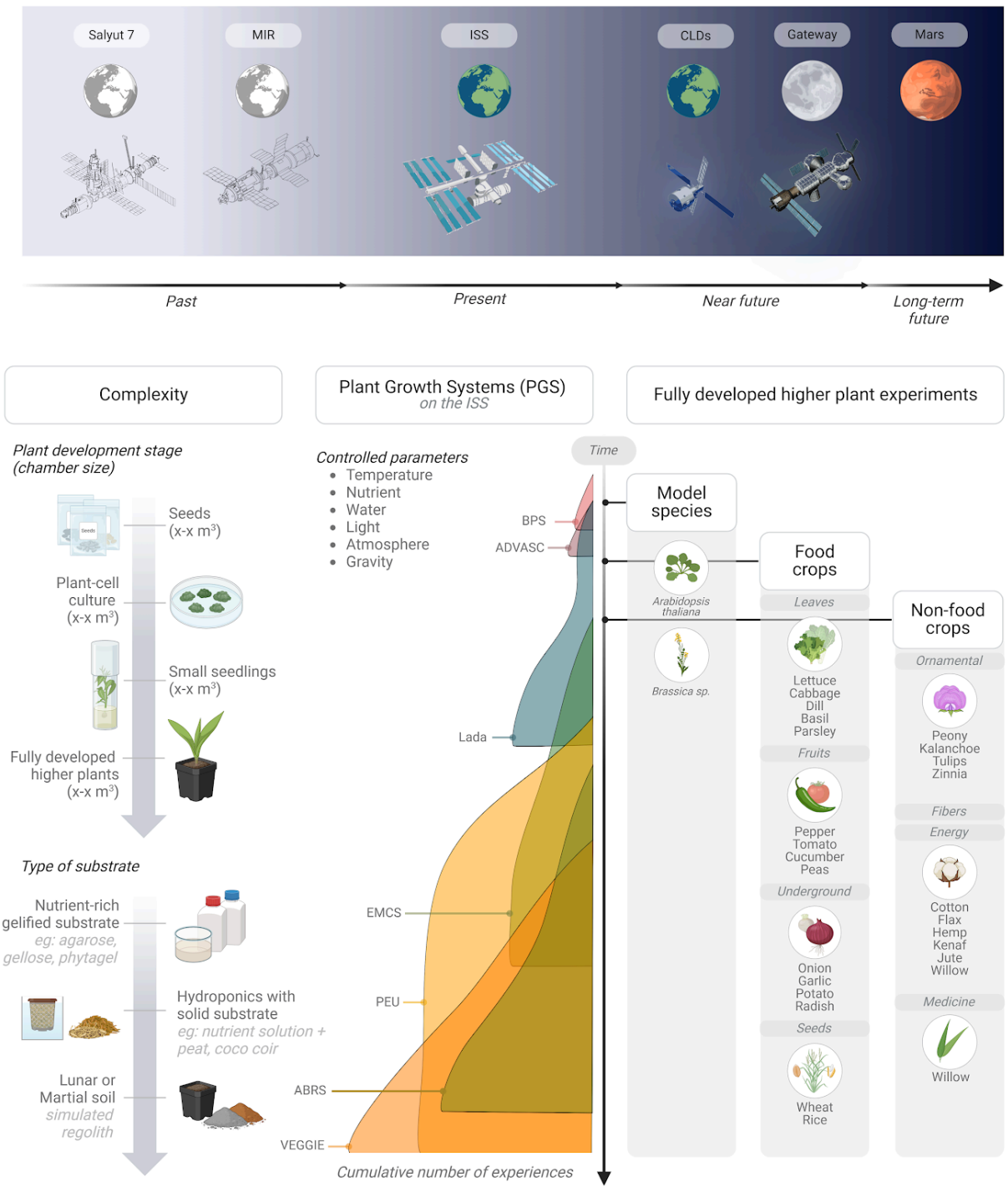


Figure 1 Progression of plant growth systems across spaceflight missions

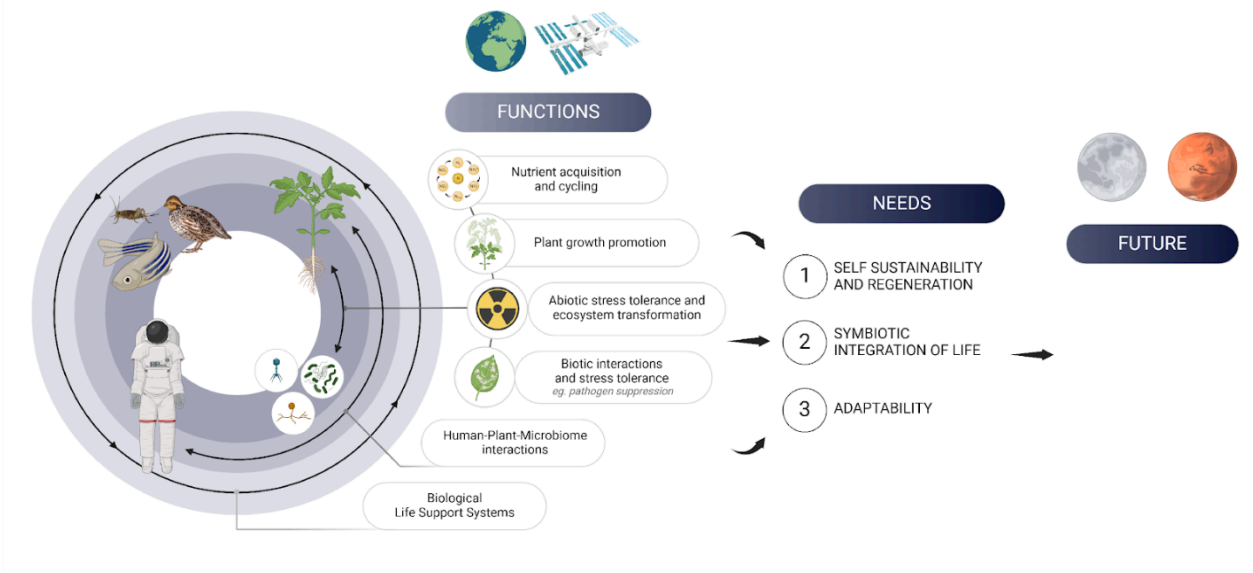


Figure 2 Designing plant systems to meet the needs of space habitation

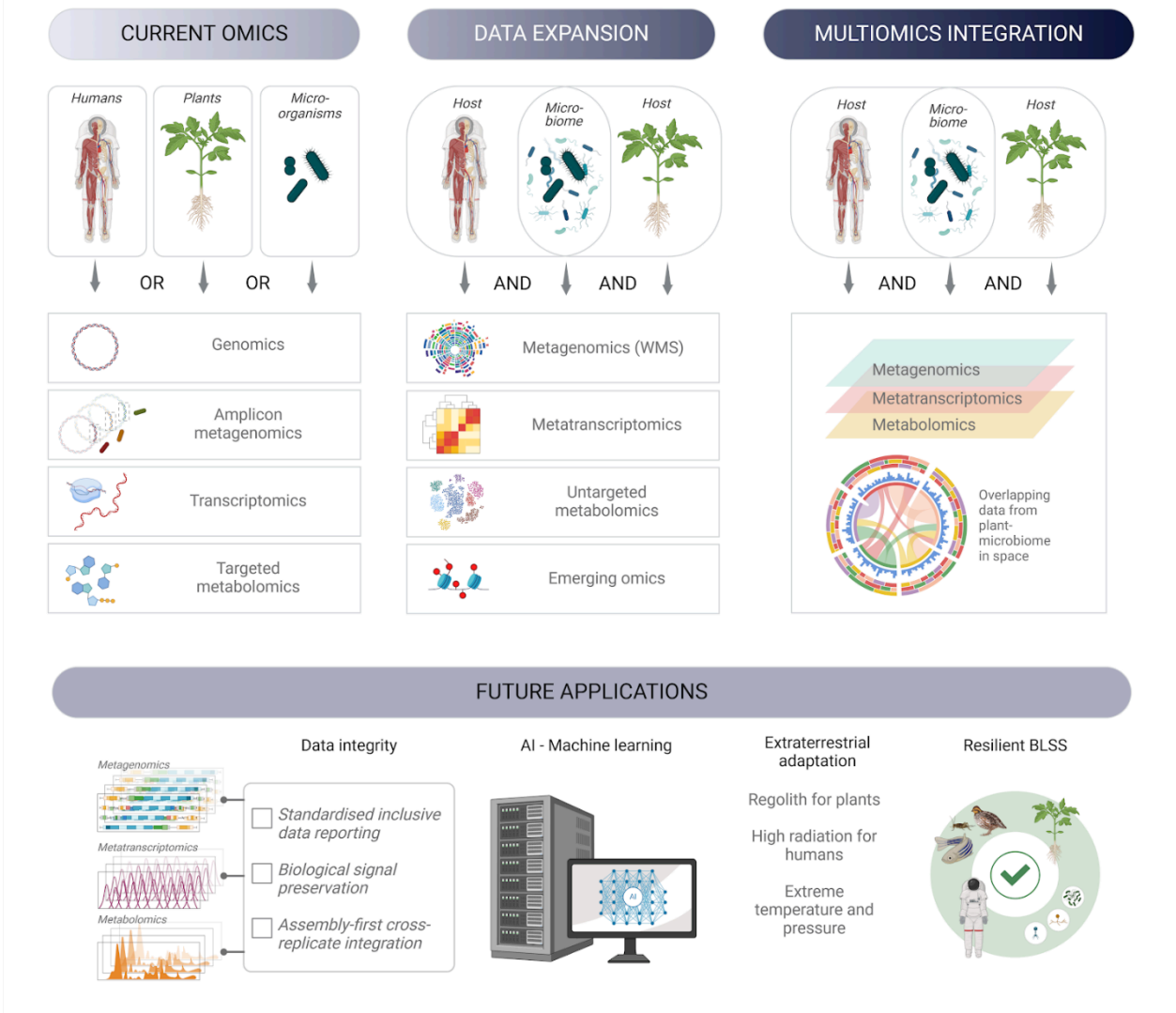


Figure 3 Preparing plant–microbiome research for a multiomics future

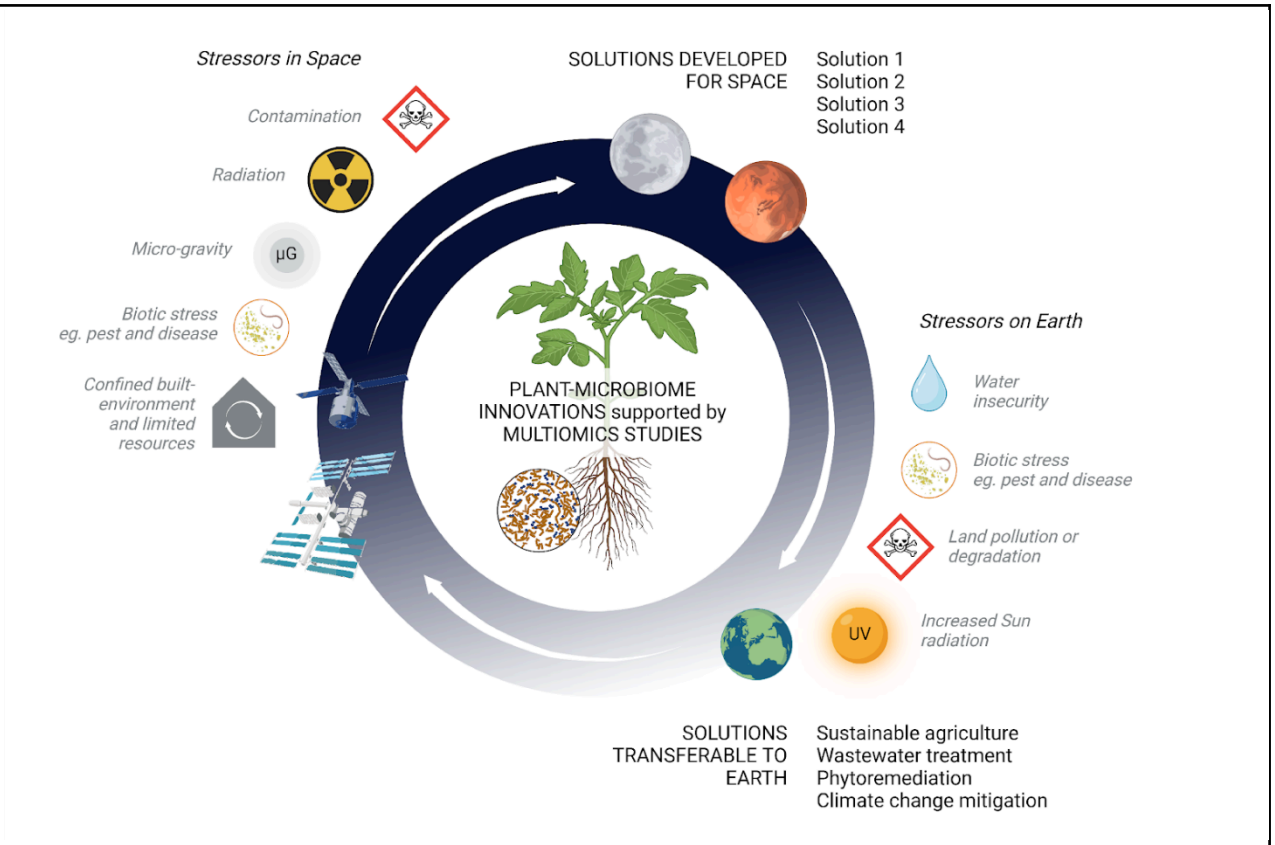


Figure 4 Harnessing plant–microbe functions to sustain humans on the Moon and Mars

- Tables

Common multiomics-ready steps for plant science in space			
	Transcriptomics	Metagenomics	Metabolomics
Tissues	Minimum separate above and belowground tissue	Minimum separate above and belowground tissue	Minimum separate above and belowground tissue
Replication	>2n biological replicates (not pseudo-replication)	>2n biological replicates (not pseudo-replication)	>2n biological replicates (not pseudo-replication)
Library preparation	No polyA enrichment	Amplicon, WMS	
Data generation	Platform, long, short, new	Platform, long, short, new	Chromatography, Mass spec, NMR, HR-MS, MS/MS, +ve/-ve
Read and spectra filtering	QC, pre-assembly host	QC, pre-assembly host	Signal/noise threshold
Assembly/alignment	(Co-)assembly criteria	(Co-)assembly criteria	Cross sample Alignment
Competitive annotation	Databases, spiked genomes and sequences, similarity criteria	Databases, spiked genomes and sequences, similarity criteria	Databases, similarity criteria, Sumner confidence levels, structural prediction
Treatment	Normalisation, (back-)mapping criteria	Normalisation, (back-)mapping criteria	Normalisation, alignment criteria
Thresholding	Minimum occurrence, sparsity, zero control,	Minimum occurrence, sparsity, zero control	Minimum occurrence, sparsity, zero control
Differential expression & abundance analysis	DESeq2	DESeq2, ANCOM, LEfSe, ALDEx2	ANOVA/t-tests, PLS-DA, Wilcoxon Rank-Sum Test, DESeq2
Summary reporting	Data representation (total read use and filters), summary statistics	Data representation (total read use and filters), summary statistics	Data representation (complete feature reporting and filters), summary statistics
Ontology and networking	Reduced complexity and pathway enrichment (KEGG, GO, Brite), network analysis	Gene inventory/functional inference from pathway enrichment (KEGG, GO, Brite)	functional inference from pathway enrichment, network analysis
Data management	Unfiltered storage, Fair, metadata	Unfiltered storage, Fair, metadata	Unfiltered storage, Fair, metadata

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- **Annex 1**
  - **Curated table of plant science in space papers**
  - **Raw table of plant science in space papers**

- **Annex 2 – Notes, suggestions and figure drafts**