Research Note • DOI: 10.2478/gsr-2024-0001 GSR • 12 • 2024 • 18–40

Gravitational and Space Research

Strategies, Research Priorities, and Challenges for the Exploration of Space Beyond Low Earth Orbit

R. Craig Everroad[1], Jamie S. Foster[2], Jonathan M. Galazka[1], Janet K. Jansson[3], Jessica A. Lee[1], Matthew P. Lera[1], Imara Y. Perera[4], Antonio J. Ricco[1,5], Nathaniel J. Szewczyk[6], Paul W. Todd[7], Ye Zhang[8], Lynn Harrison[9]*

[1]NASA Ames Research Center, Moffett Field, CA; [2]Department of Microbiology and Cell Science, Space Life Science Lab, University of Florida, Merritt Island, FL; [3]Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA; [4]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC; [5]Department of Electrical Engineering, Center for Integrated Systems, Stanford University, CA; [6]Ohio Musculoskeletal and Neurological Institute, Department of Biomedical Sciences, Ohio University, Athens, OH 7Magnaquant, New Albany, IN; [8]NASA John F. Kennedy Space Center, Merritt Island, FL; [9]Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport, LA.

Abstract

NASA's recent emphasis on human exploration of the Moon and, ultimately, Mars necessitates a transition from a focus of its research in the biological sciences from Low Earth Orbit (LEO) to platforms beyond LEO. Fundamental research questions need to be addressed to enable humans to thrive in deep space. Work beyond LEO necessitates a shift in technology and the utilization of organisms in autonomous experiments, especially in the near term. The Beyond LEO Instrumentation & Science Series Science Working Group (BLISS-SWG) was established to provide NASA's Space Biology Program input on its strategy for developing research priorities and tools for exploration beyond LEO. Here, we present an abridged version of the first annual report of the BLISS-SWG, which is publicly available on the NASA Technical Reports Server. Seven priority areas and pertinent research questions were identified for research beyond LEO in the coming 2-5 years. Appropriate experimental organisms and technology development needs for research addressing these questions are summarized. The BLISS-SWG aims for this review to serve as a resource for the space biology and science and engineering communities as they develop research to understand risks and mitigation strategies for deep-space stressors on human crew, plants, and their microbiomes.

Keywords

beyond low Earth orbit • gravity • space radiation

Preface

The Beyond LEO Instrumentation & Science Series Science Working Group (BLISS-SWG) was established in December 2020 to provide NASA's Space Biology Program with sustained input from a group of subject matter experts from the space biosciences community in its strategy for developing research priorities and tools for exploration beyond LEO. The purpose of the BLISS-SWG is to report upon scientific goals and technological developments that will be accessible within the upcoming years. The two specific aims were to define the technical capabilities that should be sought in order to enable biological research beyond LEO and to consider the potential scientific gains that can be made from utilizing different experimental organisms in future research beyond LEO. Seven priority areas were identified, and the research and the technology needs that can be performed in the next 2-5 years are discussed. The emphasis is on research that cannot be conducted within LEO (e.g., on the International Space Station) or through ground simulations. This overview is a summary of the conclusions and recommendations of the BLISS-SWG 2021 report, which can be found in full form at https://ntrs.nasa.gov/citations/20210023324. The full report was written by members of the scientific community with NASA as an intended audience. Likewise, this overview is a product of the scientific community and not of NASA. The views and opinions expressed herein do not necessarily state or reflect those of the US government.

Introduction

Human space exploration was never intended to stop within low Earth orbit (LEO). Although nearly all of NASA's biological research in space has taken place in LEO, on Shuttle and International Space Station (ISS) missions, NASA's recent shift in emphasis toward human exploration of the Moon and ultimately Mars necessitates a change in the focus of its research in the biological sciences [National Academies of Sciences,

†Corresponding author: Lynn Harrison
E-mail: lynn.clary@lsuhs.edu

Engineering & Medicine, 2018]. Specifically, from 2022 and beyond, the Division of Biological and Physical Sciences seeks to pivot toward a focus on Thriving In Deep Space (TIDES), furthering the fundamental research necessary for understanding risks and mitigation strategies for deep-space stressors on human crew, plants, and their microbiomes.

Living and working beyond LEO involves surviving a number of factors that generate stress on biological systems. Long-duration spaceflights will be required for the exploration of deep space; for instance, the transit to Mars using conventional rocket fuel and design will take at least 180 days one way [Sobel and Duncan 2020], and a mission is likely to be approximately two years long. This far exceeds the time most human crew currently spend in space. Further research is needed to understand the effects of space stresses such as microgravity on the human body for such long periods of time; and for shorter-lived organisms, such as plants and microorganisms, multigenerational exposure to such stresses may have evolutionary implications. Moreover, sustaining crew health for long time periods in space will require the further development of biological life support systems, *in situ* resource utilization, and space food and pharmaceutical production. The beyond LEO environment will also result in exposure to high energy ionizing radiation, altered gravity, and different chemical environments that exist on the Moon and Mars.

X-rays and gamma rays are common types of ionizing radiation used on Earth for medical treatment. The unit used to describe the amount of radiation absorbed by a material is the Gray (Gy), which is defined as 1 Joule of energy absorbed per kilogram of material [U.S.NRC]. However, different types of ionizing radiation induce different amounts of damage when biological samples absorb the same dose. To be able to compare the biological relevance of the different types of radiation, the absorbed dose is multiplied by a quality factor for the type of radiation, and this is expressed in the unit of the Sievert (Sv). X-rays are the standard, so 1 Gy of X-rays is equivalent to 1 Sv, while heavier ion particle radiation has a quality factor of 20, and 1 Gy would be equivalent to 20 Sv [U.S.NRC]. On an average six-month mission on the ISS, an astronaut receives a dose of ~100 mSv, while on Earth three medical chest x-rays would be equivalent to 1 mSv or 1 mGy [Sobel and Duncan 2020]. Ionizing radiation beyond LEO includes galactic cosmic radiation (GCR) and solar particle events (SPE). GCR consists of protons (~87%), helium (12%) and heavier ions (1%) [Norbury et al., 2019], while the main concern from SPE is proton exposure. SPE can also consist of heavier ions and helium. Exposure to GCR and SPE depends on sunspot activity during the 11-year solar cycle. When astronauts are on the surface of the Moon or Mars, back scattered (albedo) particles are generated from the GCR or SPE colliding with the surface of the planet. The most dangerous albedo particles for biological systems are neutrons. During periods just preceding and just following solar maxima, there will be coronal mass ejections resulting in energetic particle storms known to deliver up to 150 cGy, more than half of which may be attenuated by spacecraft structures. Currently, radiation and radiobiological research have been conducted within LEO, where there is protection from deep-space radiation due to the Earth's magnetic field. While the physics of deep-space radiation is well understood, few empirical measurements have been made of its effects on organismal growth, metabolism, and genetic stability. Ground simulation facilities have several limitations, one of which is that they commonly use unrealistic high dose rates. This is in part due to time and technology constraints at the available space radiation facilities, such as the NASA Space Radiation Laboratory. Radiation is considered a substantial health threat to human crew on long-duration missions and is likely to affect the biology of plants and microorganisms in unique ways. On a mission to Mars, astronauts could be exposed to 1000-1200 mSv of radiation [Sobel and Duncan 2020].

Living beyond LEO will also result in exposure to altered gravity environments. While microgravity is not unique to the BLEO environment, moving from Earth's gravity to microgravity during spaceflight, to partial gravity once a destination has been reached, is a feature specific to BLEO. On Mars and the Moon, the gravitational force is reduced to 1/3rd and 1/6th Earth's g, respectively. Some studies of partial gravity have been conducted on the ISS using centrifuges, but the scale of such studies is necessarily extremely limited. In addition, the combined effects of altered gravity and radiation experienced on the Moon and Mars can only be studied in those settings.

Many of the environments during missions beyond LEO will harbor specific chemistries that could affect biological systems. As has been demonstrated on the ISS, unique environmental conditions exist in closed habitable environments. An example is altered atmospheric conditions such as elevated carbon dioxide levels due to the metabolism of living organisms. Gateway will be the first lunar orbiting space station [Gateway] and astronauts will live and work on Gateway and use it as an outpost to transfer to a habitat on the Moon. However, Gateway and lunar habitats will only be inhabited for 30 days to three months a year [Canadian Space Agency: Lunar Gateway] and during the months when astronauts are not living in these BLEO habitats, it is expected that environmental conditions will be altered to reduce power usage and will be less hospitable to living organisms. Hence, the habitat interiors will likely have unique built-environment microbiomes. Lunar regolith is another element of the lunar environment that will likely infiltrate Gateway and lunar habitats. Lunar regolith is the loose material on the surface of the Moon which is 5-15 meters deep depending on the region [Noble 2013]. The Lunar maria are visibly darker areas on the Moon that originally were believed to be seas, and regolith is ~5 meters deep in these areas but can be ~10 meters deep in the highlands. Particle sizes less than 10-20 μm are defined as lunar dust, and lunar "soil", or regolith, is up to the sub-centimeter size range [Noble 2013]. The chemical composition and particle size varies depending on the sample collection sites [Apollo 16 preliminary examination team 1973; Li et al 2021; Ling et al 2015]. The Lunar maria consist mainly of basalt, and the average grain size is about 70 μm [McKay et al., 1991]. The Astromaterials Research and Exploration Science Division at NASA [ARES | Astromaterials Research and Exploration Science (nasa.gov)] curate the lunar and other cosmic samples and have information on lunar regolith simulants that mimic different regions of the Moon. While likely posing health risks to organisms [Lam et al., 2023], lunar regolith could also be a potential resource. This includes being incorporated into lunar habitat construction materials [Lee and van Riessen 2022] and in "soil" to grow plants [Paul et al., 2022].

To date, few biological experiments have been performed beyond LEO: Russia's Zond 5 sent a diverse group of organisms on a 6-day circumlunar mission, and the Biostack experiments I and II on Apollo 16 and 17 measured the effect of HZE particle radiation on immobilized organisms such as bacterial spores during lunar transit [Bücker and Horneck, 1975]. However, BLEO biology research is resuming. In 2019, China's Chang'e-4 lunar lander was the first mission to germinate plant seeds on the Moon, in an attempt to demonstrate a closed biological life support system. NASA's first BLEO biological experiments in decades recently launched on Artemis 1: BioSentinel sent an autonomous microfluidic culturing device on a SmallSat to measure the effect of deep-space radiation on two actively growing yeast strains (*Saccharomyces cerevisiae*) in heliocentric orbit [Ricco et al., 2020; Santa Maria et al., 2020; Padgen et al., 2023], and 4 biological experiments were on board Orion (BioExpt-1), and samples returned to Earth to determine the biological effects of exposure to space radiation and microgravity on plant seeds, algae (*Chlamydomonas reinhardtii*), and fungus (*Aspegillus nidulans* and *Saccharomyces cerevisiae*).

Feasibility challenges for biological work beyond LEO during the next five years

Beyond LEO flight opportunities typically require payloads to be ready months prior to launch, and the lead time could be up to 1 year between the time that a payload is prepared and the time that it reaches its destination. Experimental organisms must have the capacity to remain in stasis and viable for extended time periods and be able to be reactivated reliably. This limits the range of candidate organisms. Payloads will be exposed to extreme conditions, and experimental platforms must have the capacity to mitigate conditions that are not of scientific interest (e.g., the thermal environment), while simultaneously being exposed to and accurately measuring the parameters of interest, such as radiation. As an example, the thermal environment on the lunar surface changes drastically throughout the lunar "day" (28 earth days). Minimum night-time temperature is about 80 K at all latitudes and rises to maxima of 390 K at the equator, 330 K at 60° latitude, and 290 K at 75°. The poles are colder, 110-180 K in summer and 40 K in winter [Vasavada et al., 1999; Paige et al., 2010]. A spacecraft orbiting the Moon will be heated to about 100 K in the Moon's shadow and 300 K on its illuminated side. The near-future BLEO opportunities will also require autonomous experiments due to limited or no crew involvement, and it is unlikely that sample return will be possible, requiring data to be collected and transmitted to Earth.

Platforms and types of missions (Figure 1)

Within the next five years, research will be conducted on Commercial Lunar Payload Services (CLPS) landers, on crewed Artemis missions, and in free flyer spacecraft. CLPS and free-flyer payloads will be responsible for controlling their own environment and conducting their own experiments. The CLPS landers will provide power and data interface for returning data to Earth, while the free-flyer missions must generate their own power and have their own telemetry capability. Once free-flyers are placed in a stable orbit, they can support experiments months-to-years, whereas the CLPS landers currently have a maximum time of a lunar day as they cannot survive the lunar night. There will be limited capacity inside the crewed Orion capsule on future Artemis missions for science experiments. Since payloads will be inside the vehicle, the environment will be controlled by an Environmental Control and Life Support System (ECLSS). Crew time will be very limited, so experiments will need to be largely passive or autonomous, as for the CLPS and free-flyer payloads. Sample return may be available for some Artemis missions, but would be extremely limited in mass, and cold storage capacity is unlikely in early missions. Experiments for Artemis platforms would therefore benefit from using similar technologies and assays to those used for autonomous missions, though this platform does offer support for some experiments that require post-processing of samples on the ground, especially those using compact, passive hardware.

Questions of Importance for Beyond LEO Investigations

Seven priority areas were identified by the BLISS-SWG that require investigation to advance humans thriving in deep

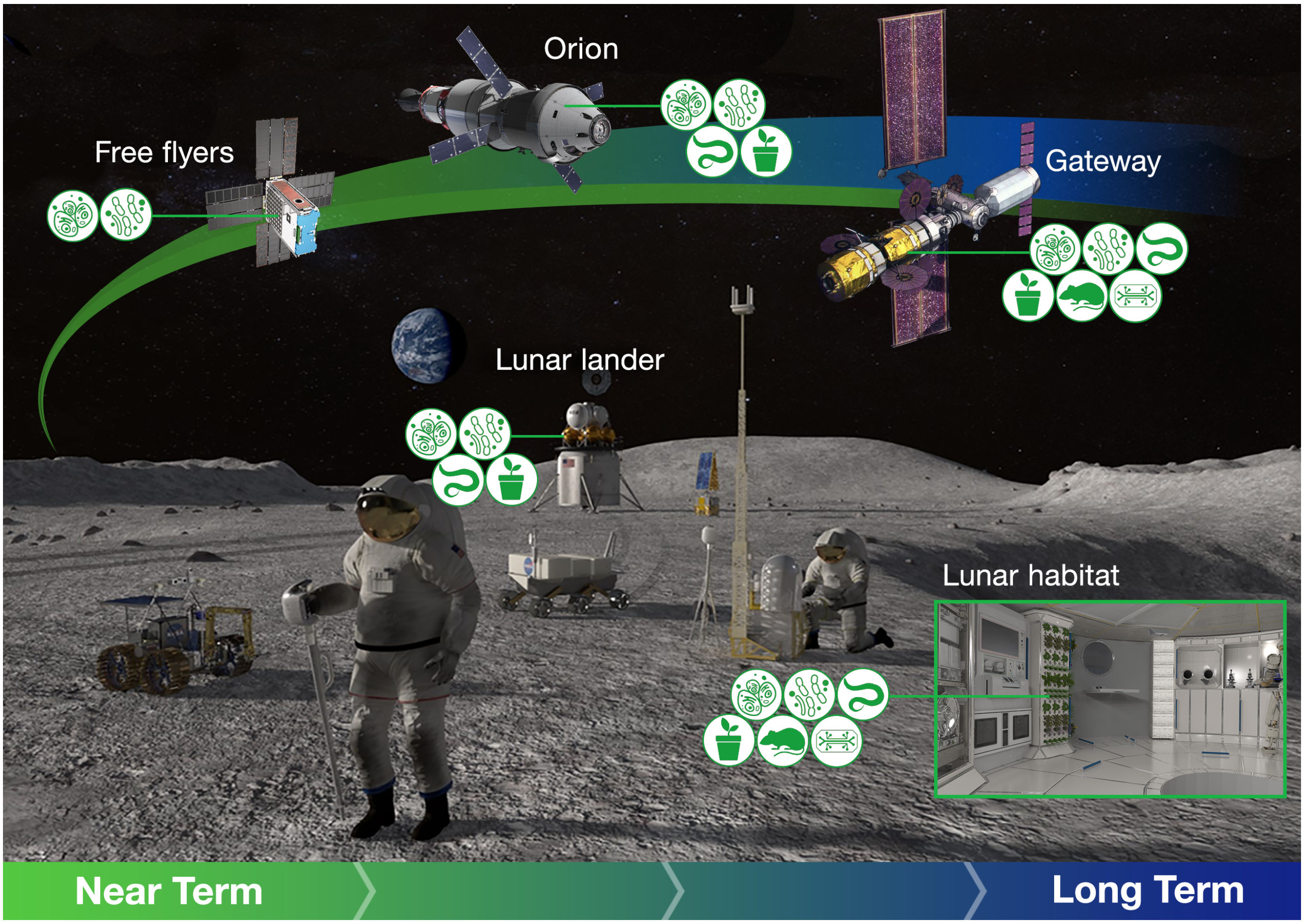

Figure 1. Summary of future platforms and the likely increasingly complex organisms each can support.

space: Cellular functions, microorganisms and microbial communities, physiology of multi-cellular animals, plant development and physiology, host-microbe interactions, evolutionary processes, and biotechnological processes. Key questions that need to be addressed for each of these subjects are provided in this section (Figure 2).

How does the beyond LEO environment impact cellular functions?

There are fundamental common processes within cells that are critical to the functioning of the cell, and these are found in prokaryotes and eukaryotes, and both single-cell and multicellular organisms. Examples relevant to beyond LEO include DNA structure, transcription, metabolism, and oxidative stress responses. Alterations in these critical functions or pathways can result in changes in phenotype or the ability of the cell to survive. This section considers questions that need to be addressed to understand how essential cell processes could be altered by conditions beyond LEO and, in particular, by the environment on the Moon. Understanding how these fundamental cell processes change will be essential to proposing and testing countermeasures that will allow humans to thrive in deep space.

How does exposure to beyond LEO alter DNA structure?

DNA is prone to damage, and ionizing radiation can directly damage the DNA by ionizing the DNA molecule or by generating reactive oxygen species (ROS) that oxidize the bases and deoxyribose backbone. The severity of damage induction can be modulated by the DNA structure. DNA in all organisms is protected by proteins that bind and wrap the DNA. Proteins bound to DNA can change DNA compaction and influence transcription. Alterations in the levels of these proteins could change the compaction of the DNA and the sensitivity to radiation-induced damage.

How does exposure to beyond LEO alter the epigenome

The DNA and eukaryotic histones can be modified: DNA can be methylated, and histone modifications include acetylation, phosphorylation, methylation, and ubiquitinylation. These

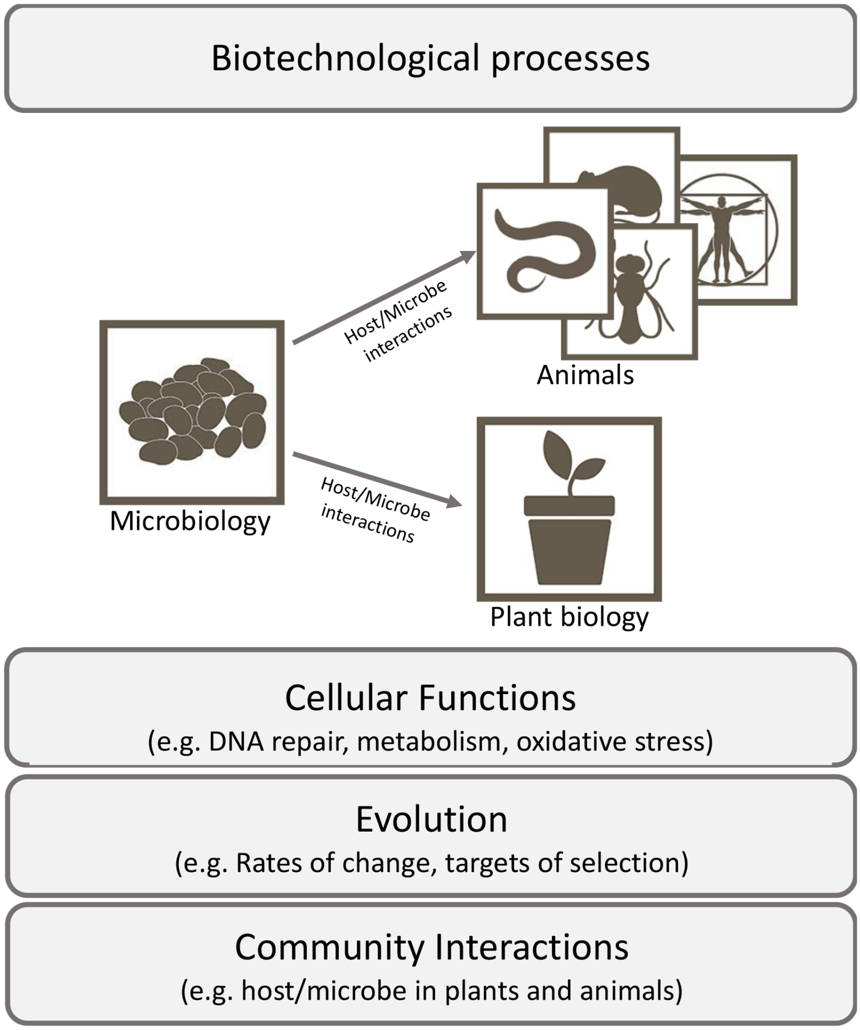

Figure 2. Summary of Questions of Importance. Evolution and cellular functions are foundational as they impact everything above. Biotechnology is applied and relies on everything.

modifications form the epigenome. DNA in eukaryotes and prokaryotes can be methylated and exposure to radiation changes DNA methylation patterns [Shi et al., 2014; Miousse et al., 2017], although dramatic alterations were not detected during LEO spaceflight in the Twins Study [Garrett-Bakelman et al., 2019]. Alterations to DNA methylation and chromatin modifications are biologically relevant as they alter gene expression and DNA repair. Chromatin remodeling and chromatin modifying proteins are important for the initiation, accuracy, and completion of DNA repair [Hunt et al., 2013; Kim, 2019; Mackenroth and Alani, 2021]. Changes to the epigenome due to living conditions beyond LEO will be important for cell survival and possibly evolution as the epigenome is heritable and has been linked to human disease [Ramzan et al., 2021].

How does exposure to beyond LEO alter DNA damage, DNA repair, and DNA mutations?

Deep space radiation can induce complex clustered DNA damage that is more lethal [Moore et al., 2014] and mutagenic [Malyarchuk et al., 2004; Sage and Harrison, 2011] than individual damages introduced by ROS generated from metabolism on Earth. The steady state level of DNA damage in a cell is determined by the induction of the damage and the removal of the damage by DNA repair. The repair of oxidative base damage and double strand break repair are relevant to removal of complex radiation damage and hence to survival after radiation exposure. Misrepaired and unrepaired DNA damage can result in DNA mutations if cells survive. Extensive studies have examined chromosome aberrations and micronuclei formation in mammalian cells

[Furukawa et al., 2020] and mutations in genes such as the adenine phosphoribosyl transferase (APRT) in mice [Turker et al., 2017] using ground-based simulated space radiation. These studies demonstrated an increase in mutations and genetic instability at astronaut-relevant doses of particle radiation. Studies are needed to determine whether space radiation and partial gravity are synergistic with respect to increasing mutation frequency and inducing cell death. The synergy between reduced gravity and radiation may initiate at a threshold gravitational and/or radiation level, which may be cell type dependent. DNA damage, signaling, DNA repair, and DNA mutations need to be examined to determine possible long-lasting effects of living beyond LEO on humans and other organisms, as beyond LEO living conditions may induce inheritable mutations, increase virulence, or increase drug resistance of organisms.

What changes occur to the transcriptome of cells due to the BLEO environment?

Alterations to the transcriptome have the potential to change every characteristic of the cell, as changes in transcript levels can alter the proteome. Transcription is influenced by DNA structure, by the epigenome, and by the presence of DNA damage. DNA microarrays and more recently RNA-Seq are able to probe changes in gene expression in prokaryotes and eukaryotes in single cells, mammalian cells in culture, cells in animals and plants, and host-pathogen or host-symbiotic partners. In prokaryotes, a common factor identified as responding to simulated microgravity and to LEO stress is Hfq [Wilson et al., 2007; Crabbé et al., 2010; Crabbé et al., 2011; Castro et al., 2011; Soni et al., 2014; Duscher et al., 2018]. Hfq is an RNA chaperone that binds small regulatory RNAs (sRNA) and promotes the binding of sRNAs to target RNAs. This changes the half-life and the translation of the target RNA, and Hfq is therefore classed as a global gene regulator [Vogel and Luisi, 2011]. Other pathways altered by simulated microgravity or spaceflight include stress responses, chemotaxis, motility, and metabolic pathways [Li et al., 2014; Orsini et al., 2017; Aunins et al., 2018; Acres et al., 2021]. Pathways found to change in mammalian cells also include, but are not limited to, oxidative stress, DNA repair, metabolism, circadian regulated genes, and NFKB [Liu and Wang, 2008; Ranieri et al, 2015; Zhang et al., 2017; Paul et al., 2021]. Few published studies have examined partial gravity, which has required the use of centrifuges on the ISS or the use of microgravity simulation devices, such as a random positioning machine on Earth. Two transcriptome studies used centrifuges on the ISS to examine cell growth and cell proliferation in *Arabidopsis thaliana* seedlings at different gravity levels. These studies did identify gene expression changes that were different at microgravity, partial gravity (lunar or Mars), and normal gravity [Herranz et al., 2019; Villacampa et al., 2021]. This demonstrates the importance of examining the transcriptome of different types of cells exposed to different gravitational forces. Other beyond LEO conditions, such as higher CO_2 level, human/animal isolation during spaceflight, and lunar dust on the lunar surface, may induce synergistic changes and add to the combined effect of radiation and partial/microgravity on biological systems. Venturing further to the Moon and beyond to reveal transcriptome modifications will be essential to understanding the stress pathways activated under conditions of living beyond low Earth orbit.

How does beyond LEO influence metabolism?

Transcriptomic studies of prokaryotes and eukaryotes subjected to LEO spaceflight or simulated microgravity have revealed changes in transcript levels of genes involved in metabolic pathways. These pathways include oxidative phosphorylation [Versari et al., 2013; Suzuki et al., 2020], lipid metabolism [Pecaut et al., 2017; Suzuki et al., 2020], carbohydrate metabolism [Suzuki et al., 2020; Crabbé et al., 2013; Uda et al., 2021], and anaerobic metabolism [Crabbé et al., 2011]. Few studies have measured metabolites or the activity of specific metabolic pathways. Suzuki et al. (2020) did find that mouse plasma levels of glycerol, glycine, and succinate were altered in a similar way by LEO spaceflight and aging in humans on Earth [Suzuki et al., 2020]. This suggests that countermeasures may be required to prevent metabolic human aging on long missions. Ground-based radiation studies have identified metabolite disturbances for nucleotides, amino acids, and metabolic markers of inflammation in the intestines of irradiated mice [Cheema et al., 2014] and differences were detected between γ-ray and ^{56}Fe irradiated mice. GCR and SPE could therefore induce specific changes to metabolism. Metabolism directly affects the ability of the cell to generate energy and produce cell components required for growth and cell maintenance. Metabolism is also important for generating NADPH, which is needed to maintain the epigenome and to maintain antioxidants such as thioredoxin and reduced glutathione. Specific types of metabolism such as microbial carbon fixation [Rubin-Blum et al., 2019; Mandal et al., 2021] and plant photosynthesis will be useful to humans for developing technology and growing food and so will be essential to life beyond LEO.

Does exposure to beyond LEO increase oxidative stress in cells?

Cells have protective mechanisms to combat the day-to-day ROS generated through everyday metabolism, but an imbalance in ROS production or ROS removal results in oxidative stress. Microorganisms exposed to LEO spaceflight [Blachowicz et al., 2019] or simulated microgravity [Crabbé et al., 2010] do elicit oxidative stress responses and have altered sensitivity to exogenous oxidative stress. Ground-based analogs for microgravity and radiation, and LEO spaceflight studies have detected oxidative stress in mammalian cells by staining for lipid peroxidation [Limoli et al, 2007; Overby et al., 2019; Mao et al., 2020], analyzing the transcriptome [Versari et al., 2013], and measuring enzymes and antioxidants [Lawler et al., 2003]. Transcriptome studies have also implicated oxidative stress in plant responses to space flight [Choi et al., 2019]. Whereas the mitochondria are the predominant site for ROS production in animal cells, chloroplasts produce more ROS in plants than the mitochondria [Speijer et al., 2020]. The main type of ROS produced by these organelles is also different: chloroplasts produce hydrogen peroxide, while mitochondria produce superoxide ions that are enzymatically converted to hydrogen peroxide by superoxide dismutase. The hydrogen peroxide and hydroxyl radicals that can form from the hydrogen peroxide can be detoxified by antioxidants and enzymes to limit oxidative damage to lipid, protein, and DNA. A decrease in antioxidant capacity, altered mitochondrial gene expression, and an increase in DNA damage was detected in the LEO Twins Study [da Silveira et al., 2020]. Exposure to low dose particle radiation also results in a persistent oxidative stress [Tseng et al., 2014] that can last weeks to months. ^{56}Fe ion irradiation of mice resulted in increased ROS in the cerebral cortex for up to 12 months [Suman et al., 2013], and increased mitochondrial ROS production was detected in mouse intestinal epithelial cells one year after irradiation of mice with ^{56}Fe ions [Datta et al., 2012]. Increasing the antioxidant capacity in the mitochondria by overexpressing catalase did protect from oxidative stress generated from 0.5 Gy proton radiation [Liao et al., 2013], which supports the idea that the radiation-induced increased ROS and oxidative stress originates in the mitochondria in eukaryotes. Oxidative stress is implicated in multiple pathophysiological human conditions including neurodegeneration, osteoporosis, cardiovascular disease, diabetes, and cancer. Exposure to deep space radiation and partial or microgravity on long missions beyond LEO on the Moon or Mars could result in persistent oxidative stress and increase the risk of astronauts developing early-onset degenerative diseases.

How does the Beyond LEO environment impact microorganisms and microbial communities?

Microorganisms can serve as models to elucidate fundamental biological effects of Beyond LEO conditions on higher organisms, including humans, or they can be studied to determine the effects on the microorganism itself or the microbial community it is part of. Single microbes and/or communities of microbes can provide essential services that are needed for life support during long-duration missions. These services include water recycling, waste management, vitamin production [Horneck et al., 2010], human probiotics, and plant growth promotion. Coordinated metabolisms of microbial communities are responsible for key services, including nutrient cycling and plant growth promotion. It is currently not known how the space environment beyond LEO will influence microbial dynamics, their interspecies/interkingdom interactions, and the overall ecology of the microbial community. To answer this question, it is important to take advantage of model microbial systems that have sufficient simplicity to allow experimental control in beyond LEO conditions [Jessup et al., 2004].

What are the impacts of deep-space radiation and partial gravity on microbial biology?

It is currently not known how partial gravity, deep-space radiation, and the combination of these factors will impact different types of microorganisms. For studies of individual microbial species, investigations of interest include studies of beyond LEO conditions on genetics, growth, reproduction, and physiology. Prior studies have examined the impact of microgravity on microbial cultures [section 4.1 above, Manti et al., 2006], and a number of microbes show altered growth, aggregation, and resistance to antibiotics in liquid culture under simulated microgravity or LEO spaceflight [Castro et al., 2011; Abshire et al 2016; Rosenzweig et al., 2010; Ricco et al 2011; Clary et al., 2022]. Therefore, additional investigations of interest include studies of growth dynamics, susceptibility or resistance to antibiotics, biofilm formation, and synthesis of secondary metabolites under beyond LEO conditions.

How do conditions on the lunar surface impact fundamental microbial properties?

Specific to the lunar surface, studies of interest include determination of the effect of albedo particles, lunar dust, and the lunar chemical environment on microbial biology.

What is the potential for microbial pathogens to emerge beyond LEO?

An important area for beyond LEO research concerns understanding the threat of microorganisms as pathogens. Some microorganisms that are typically non-pathogenic in Earth environments may pose a threat when conditions change beyond LEO. Opportunistic pathogens may be able to survive and colonize new niches in the beyond LEO environment that could pose a threat to human, animal, or plant health. For example, *Salmonella typhiurium* was significantly more virulent when grown in space [Wilson et al., 2007]. This topic was also highlighted as a future space microbiology NASA Research Announcement (NRA) in the Space Biology Science Plan, which is a document produced by NASA and poses the following question: “Under the reduced microbial-diversity conditions of space habitats, do opportunistic pathogens have a greater survival capacity, and do they have a greater propensity to infect as compared with ground controls?” [Space Biology Science Plan, 2016].

How are communities of microorganisms (synthetic communities, 'Syncoms', or characterized assemblies) impacted by beyond LEO conditions?
It is important to understand how beyond LEO conditions impact interactions between members of microbial communities and their coordinated functions. On Earth, microbial communities have evolved to coordinate metabolic and other interactions between species. Interactions vary between beneficial mutual interactions, including commensalism and symbiosis, to negative interactions, including competition and predation [Jansson and Hofmockel, 2020; Großkopf and Soyer, 2014]. Direct examples of microbe-microbe interactions include biomass turnover, production of extracellular polysaccharides, and competitive exclusion. Molecular interactions include syntrophic interactions that can be either directional or commensal, quorum sensing, production of antibiotics, and metabolic division of labor. Questions to address for microbial communities beyond LEO include: Do microbial communities persist over time? Are they stable? Does biodiversity remain stable, or change? Do commensal, cooperative, or competitive interactions differ in beyond LEO conditions compared to those on the ground? Questions relevant to microbial systems biology can also intersect with human microbiome and plant microbiome ecosystems.

How does Life Beyond LEO impact the Physiology of Multi-Cellular Animals?
Since cells make up organs, which in turn make up multi-cellular animals, life beyond LEO is expected to impact physiology. Thus, it is important to consider studying Beyond LEO impact on multi-cellular animals at the level of cells as discussed above and in terms of host-microbe interactions and evolution. The impact of LEO on human and animal physiology has been well studied and is regularly reviewed by the National Academy of Sciences via decadal surveys, the most recent being published in 2011 with the most recent decadal survey for the Biological and Physical Sciences was published in 2023.

How does Beyond LEO impact physiologic systems?
Since this topic was recently extensively reviewed by the NASA Life Below Low Earth Orbit Science Working Group, readers are directed to this review [67] which highlights the Immune, Muscle and Skeletal, Cardiovascular, and Central Nervous Systems to be of particular interest for animal physiology research beyond LEO [NASA Science Working Group Life Beyond Low Earth Orbit Report, 2018].

How does Life Beyond LEO impact Plant development and Physiology?
Plants are a vital and valuable component of bioregenerative life support systems (BLSS) for long-duration space missions. Plants provide several crucial functions from production of food to helping with air purification and recycling of water [Kordyum and Hasenstein, 2021] as well as psychological benefits [Odeh and Guy, 2017]. However, there are challenges to growing plants in LEO and beyond [De Pascale et al., 2021]. These include providing the essential requirements for optimal plant growth such as lighting, water, and nutrients. Additionally, strategies are needed to mitigate the detrimental effects of radiation and microgravity that are particular hazards of the Beyond LEO environment. In order to maximize the potential of plants for BLSS, several key science questions will need to be addressed concerning seed germination, plant quality and growth in space.

What are the effects of different g levels on germination, growth, tropisms, secondary metabolite production, and food quality?
The Moon, Mars, and spacecraft with artificial gravity represent intermediate g levels between that of Earth and that of orbital flight. At about 1/6th g, the lunar surface would be an ideal venue for exploring this question. Intermediate g levels have been simulated in the laboratory, finding, for example, lunar gravity impacting root growth parameters in a similar manner to microgravity and Mars gravity impacting root growth in a similar manner to Earth gravity [Manzano et al., 2018]. The European Modular Cultivation System (EMCS) on the ISS was also used to explore the effects of a five-day exposure to microgravity or partial g (0.53-0.88 g) levels on gene expression of *Arabidopsis* seedlings [Sheppard et al., 2021]. A subset of genes was identified where expression changes were correlated with changes in g, and these genes were related to transcription regulation, defense, heat shock, and the cell wall.

How can root zone water, nutrient, and O_2 provision be optimized for plant quality and growth in space?
The optimization of water, nutrients, and O_2 to the root zone is critical for plant health and the behavior of water and nutrient solutions under partial gravity conditions needs to be understood. While numerous plant species have been grown on orbit, some with astounding success, root matrix selection, and design require continued exploration, and the relative merits of porous media, hydroponic seal, and aeroponic mist (which is of rising interest) are still under discussion. NASA is implementing a Passive Orbital Nutrient Delivery System (PONDS) prototype into a flight-qualified Enhanced Passive Water Delivery System (EPWDS) for the eventual purpose of most effectively delivering aqueous nutrient solutions to the roots of plants intended for food. A lunar settlement might use regolith as porous root-zone media to minimize equipment, upmass, and energy. Seed germination tests with lunar regolith simulant and deionized water [Wamelink et al., 2014], as well as root zone aeration by oxygen producing polymers

[MacDonald et al., 2020], have yielded encouraging results and need to be explored further. More recently, *A. thaliana* was grown in lunar regolith, but plants developed slowly and exhibited signs of stress [Paul et al., 2022].

How do plant-microbe interactions affect plant quality and growth in space (beneficial as well as pathogens)?
Beneficial microbes can promote plant growth, increase resistance to pathogens, and reduce the need for fertilizer input [Gopalakrishnan et al., 2015; Backer et al., 2018]. Therefore, microbes would be valuable additions to increase plant productivity in space. In nature, the plant microbiome is varied and diverse and more ground-based studies are needed to develop minimal synthetic consortia to supplement non-soil-based growth media in space. Beneficial microbial strains will need to be carefully vetted to ensure safety and efficacy. Studies are also needed to understand the response of plants in space to opportunistic pathogens. Some bacterial pathogens were found to be more virulent in space, which could increase the risk of plant disease. Zinnia plants growing in Veggie hardware on the ISS were more susceptible to *Fusarium* infection when their roots were under hypoxia and excess water [Schuerger et al., 2021]. Currently, plant seeds are sanitized to minimize crew health risks. However, this could lead to a higher susceptibility to opportunistic pathogens from the unique microbiome of a transit vehicle. Understanding the impact of long-duration culture and fractional gravity on interactions between the microbe, the host, and the environment will be important for humans traveling and living beyond LEO.

What are the effects of different radiation levels on plant quality and growth in space?
Numerous published findings have shown that the effect of ionizing radiation on plants depends upon species, cultivar, development stage, tissue architecture, and genome organization, as well as radiation features, e.g. quality, dose, and duration of exposure [De Micco et al., 2011; Arena et al., 2014; Caplin et al., 2018]. In deep space, GCR is present as an extremely low dose background radiation, which may have less impact on short term plant growth experiments. For example, the maximum accumulative GCR dose is in the milli Gray (mGy) range for a 10-day exposure, and earth-based studies have demonstrated that 290 mGy of simulated GCR did not reduce the germination rate of *Arabidopsis* seeds and did not significantly alter the length of the roots of the seedlings from the germinated seeds [Zhang et al., 2022]. Thus, for imbibed seeds, protons released from a large SPE pose a more significant impact than GCR. However, dry seeds in long-term storage during deep space missions will be exposed to a much higher accumulative GCR dose, which will affect seed viability over a long-duration mission. Long-duration exposure of seeds to the space environment have been carried out using MISSE, EXPOSE-E and R, and LDEF platforms. In general, these studies have shown that seed viability and germination are negatively impacted, although the severity of the response varied between experiments and plant species tested [Novikova et al., 2015; Sugimoto et al., 2016; Tepfer and Leach, 2017]. Following the EXPOSE-E mission, *Arabidopsis* seed survival was 23%; however, germination dropped to 3% with no survival, following the EXPOSE-R mission where total UV and cosmic radiation doses were >1.4 times higher. In a recent experiment (CRESS 1U CubeSat), *Arabidopsis* seeds (under 1 atm) were exposed to the stratosphere (36-40 km) environment above Antarctica in a 30 day long-duration high altitude balloon mission. In a parallel experiment, seeds were exposed to 40 cGy GCR simulation at NSRL. GCR- and stratosphere-exposed seeds showed significantly reduced germination rates of 76.4% and 82.5%, respectively compared to 98% for the controls. Significantly elevated somatic mutation rates (and developmental aberrations) were also revealed in these GCR- or stratosphere-exposed seeds, with the GCR exposure generating a significantly higher mutation rate than that of Antarctica. These mutations also resulted in the death or delayed growth of certain plant organs. Heritable mutations were found in the second generation of the GCR-irradiated seeds [Califar et al., 2018]. Heritable epigenetic changes have also been detected in rice seeds following space flight [Ou et al., 2009]. It is clear that more studies need to be conducted, on a variety of space crops, to determine the impact of deep space radiation on critical developmental stages in the plant life cycle.

What conditions are necessary for successful lunar agriculture?
Maximizing the lunar environment for crop growth would involve a minimally pressurized containment, maximum use of natural ambient light, and lunar regolith as root matrix [Ellery, 2021]. Potential challenges faced by plants in a pressurized enclosure on the Moon include sunlight intensity (1.37 vs. 1.0 kW/cm^2 on Earth), spectrum (UV below 250 nm) and cycle (14 d vs. 12 h on/off), temperature (+120°C) and its fluctuations (to -170°C), and regolith composition (basalt, pyroxene, olivine).

How does atmospheric composition and pressure affect plant quality and growth in space?
Maintaining atmospheric pressure during long-duration missions imposes costs associated with mass and energy requirements. Defining the limits of pressure and composition that are needed for optimal plant growth is therefore of great interest [Paul and Feri, 2006]. Much of our current understanding of plant adaptations to low atmospheric pressure comes from experiments conducted at high altitude locations as well as in hypobaric chambers. These studies have revealed that low atmospheric pressure results in hypoxia as well as increased water loss by transpiration.

Transcriptional studies have shown that the effects of hypobaria can be partially mitigated by sufficient O_2 and water availability [Paul et al., 2004; Zhou et al., 2017]. However, hypobaria also constitutes a unique stress, and more studies are needed to enable plants to adapt and thrive under these unfamiliar environmental conditions.

What plants and novel organisms should be used and or developed for food production and BLSS in space?
The ideal plants for food production would be high yielding (high harvest index) with minimum hardware requirements, small upmass, and energy provision. A fully consumable plant with less waste would be valuable (i.e., 10-day aeroponic beet). Microgreens are good candidates [Kyriacou et al., 2017] as well as tuberous crops with high edible biomass such as potatoes [Wheeler et al., 2019; Paradiso et al., 2020]. Additionally, cyanobacteria or unicellular algae could be used to recycle oxygen from CO_2 as well as provide food at the end of their growth cycle; however, palatability issues will need to be solved by further research for the feasibility of crew consumption. Research will also be needed to generate crop cultivars with improved traits either by breeding/selection or genetic engineering. Traits of interest include the ability to withstand stress, enhanced plant performance under unfavorable conditions, resistance to pathogens/pests, and improved nutritional content.

What are the effects of different magnetic field levels on plant quality and growth in space?
Although some claims have been made concerning the effects of modified magnetic environments on plant processes, there has been no evidence thus far that removal of plants from the Earth's 3×10^{-5} Tesla field will have a catastrophic effect on plant performance. More work may be needed to fully understand the consequences of altered magnetic fields on long term plant propagation.

Multi-stressor effects
It is clear that plants on Earth are exposed to multiple stressors simultaneously, which may have antagonistic or synergistic interactions. Recent work has shown that plant responses to multiple stress combinations are unique and cannot be extrapolated from the response to a single stress treatment [Suzuki et al., 2014; Zandalinas et al., 2018]. Similarly, plants in spaceflight are exposed to a combination of unfavorable conditions, such as radiation, altered gravity, non-optimal growth conditions (including water stress, high CO_2 and VOC levels, and altered air pressure). To date, combined effects have not been studied in crop plants and other candidate biology for deep space BLSS. Ground-based simulation studies are able to provide some insight; however, to obtain high fidelity, data, seeds, and plants still need to be tested in the true deep space environment to prove the knowledge base and validate mitigation concepts developed from ground-based studies.

What are the comparative effects of ambient vs. built-environment (LED) illumination on photosynthesis and tropisms?
While spectrally ideal combinations of LEDs have been identified, it would still be valuable to investigate a means of using the ambient continuous daylight of interplanetary space to potentially save energy and spacecraft complexity.

How does the Beyond LEO environment impact host/microbe positive and negative interactions?
Gravity represents one of the few constant evolutionary drivers of life on Earth [Morey-Holton, 2003]. How multicellular organisms respond to gradients in gravity or how these gradients shape the evolution of life is not fully understood [Volkmann and Baluška, 2006]. Compounding our understanding of the mechanisms underlying the effects of changing gravity conditions on eukaryotic health is the lack of understanding of the impact of changes in gravity on host-associated microbiomes [Turroni et al., 2020]. A microbiome is typically defined as the sum of the microbes, genomes, and community interactions that interact with the body [Whipps, 1988; Lederberg and McCray, 2001; Eisen, 2015]. The term has been quickly adopted to represent the connectivity and interactions between complex host-microbe associations [Eisen, 2015]. Initial surveys indicate that for every host gene there are hundreds of microbial genes, thereby providing the host with millions of genes of additional metabolic functional potential [Dethlefsen et al, 2007]. Because of the intricate interplay between the host and its associated microbiome, it is imperative to also understand how radiation impacts the host microbiome and if the microbiome can be harnessed to counteract some of the negative impacts.
Together, these efforts to understand the diversity and stability of host-microbe interactions under changing gravity and radiation conditions will provide important insight into the resiliency of the host microbiome to withstand the stress of spaceflight. Regular disturbances and perturbations may result in a loss of biodiversity or extirpation (i.e., the extinction of a species in a localized area within the host) that may potentially drive the community towards dysbiosis and disease of the host. Therefore, it is critical to provide a comprehensive assessment not only of the complement of microbiota associating with plant and animal hosts in the space environment, but how the interactions between a host and its associated microbiome are initiated, persist, and are maintained over long-duration spaceflight. Through the examination of these processes, it is likely that signatures of host-microbe co-evolution within the spaceflight environment

will emerge and may be used to help mitigate and attenuate any negative impacts on host health.

How does the host microbiome change over long-duration space travel?

Long-duration space travel microbiome research needs to be a critical area of study. There has been a rapid rise in the number of microbiome studies conducted under spaceflight or modeled microgravity conditions, especially regarding astronaut health [Garrett-Bakelman et al., 2019; Jiang et al., 2019; Voorhies et al., 2019; Liu et al., 2020]. However, most of these studies have either focused on short-term changes in hosts or have included very small sample sizes. As the number of space stations increase and are inhabited, it will be important to monitor the microbiome of the crew as well as the station to understand the changes and exchanges that occur between the human host and the habitat. It will be important to know if host microbiomes are stable over time, to what extent there is exchange between habitats and hosts, whether probiotic supplements are helpful to hosts if key taxa are extirpated, and whether the stability of the space station habitat microbiome can mitigate the spread of pathogens for plant and animal hosts.

How are beneficial interactions with microbes established in the space environment?

Understanding whether the space environment negatively impacts the formation of host microbe interactions will be essential for long-duration space flight and ecosystem maintenance. For example, as the growth of food crops is likely to diversify beyond lettuce and chili peppers, the initiation and establishment of the rhizosphere and host microbiome will be necessary under spaceflight or lunar gravity conditions. Evidence using partial gravity simulations of plants have found distinctive thresholds of cell growth and proliferation [Manzano et al., 2018], but the impact on the associated microbes has yet to be fully explored. Likewise, animal physiology under a changing gravity continuum also shows changes [Hariom et al., 2021]; however, only a few studies have examined the initiation of animal-microbe interactions in modeled microgravity conditions [Foster et al., 2014; Casaburi et al., 2017]. Key areas of study should evaluate whether there are gravity thresholds for successful colonization of host tissues, assess whether there are changes in colonization phenotypes across the gravity continuum, and determine whether host-microbe interactions change due to the space environment.

How are functional activities of beneficial interactions with microbes maintained throughout the life of the host organism in the space environment?

It is not known whether long-term spaceflight conditions will negatively impact the persistence and normal healthy functions of the host-microbe interactions. There is very little data on the metabolic activity and exchange that occurs between a host and its microbiome in the space environment over long periods of time (e.g., > six months). Key areas of study should evaluate the signaling pathways used by microbes and their host to communicate under the stress of the space environment and assess whether microbes regulate and control host processes in different ways under a gravity continuum and/or changes in radiation.

How does the Beyond LEO environment impact the evolutionary process?

Exploration scenarios in the Beyond LEO context will expose Earth life to new mutagenic sources and selection pressures. For the crew and associated biology (e.g., seeds and plants for fresh food), it is likely that physiological acclimation will dominate over evolutionary processes. However, the co-occurring microbial bio-load and microbiome, be it viruses, fungi and other small eukaryotes, or bacteria, will be exposed to spaceflight stressors on evolutionarily relevant timescales. With their large population sizes and short generation times and the inability of flight programs to completely control the microbiota of spacecraft and crew, understanding how these microbes adapt evolutionarily to life beyond LEO is critical. The evolution of bacteria, fungi, plant-microbe interactions, and population-level genetics in the context of *in-situ* resource utilization (ISRU), food production, and human health in spaceflight are long-term targets for fundamental research.

Microbes will play key roles in the development of biologically based closed-loop regenerative life support, food production, and ISRU and will have extensive interactions with human and plant hosts. Further, microbes will pose challenges through contamination, as nuisance factors such as biofilms, and through enhanced pathogenicity and antibiotic resistance [Padgen et al., 2020; Clary et al., 2022]. Previous spaceflight experiments with microbes have documented striking physiological and phenotypic changes including differences in growth rates, enhanced antibiotic resistance, and virulence [Juergensmeyer et al., 1999; Nickerson et al., 2000; Nickerson et al., 2004; Klaus et al., 2006; Nicholson et al., 2011; Ott et al., 2004; Ott et al., 2020]. New bacterial species have been identified on the ISS [Bijlani et al., 2021], though it is unclear if they evolved there, and there is evidence of colonization of crew microbiomes by ISS microbes [Lee et al., 2021; Morrison et al., 2021]. Potentially virulent bacteria exist onboard ISS, with some evidence of persistence and even an increase in virulence factors [Singh et al., 2018].

Although many studies have detailed physiological adaptation to the space environment [Leys et al., 2004], studies that examine underlying genetic changes that might also occur via evolutionary change or adaptation are lacking. Long-term evolutionary studies are a logistical and technical challenge in the context of spaceflight, where experimental requirements specify automation with minimal to no human intervention, and dictate limitations on experimental duration, power, mass, storage, and sample return.

Evolution is complex and includes multiple aspects, including epigenetics, e.g., methylation, as well as neutral and population-level processes, and the co-evolution of microbes with the built environment, and with plant and human hosts. In order to advance understanding of how life evolves in the space exploration environment, fundamental science questions will need to be addressed concerning microbial evolution and adaptation, microbe-host interactions, and risks and countermeasures in space.

How does long-duration spaceflight and exploration affect rates of evolutionary change?

Experimental evolution studies with bacteria on Earth have revealed general rates and processes for mutation, adaptation, and bacterial evolution in laboratory settings [Elena et al., 2003; Wielgoss et al., 2011]. Adaptation to a new, benign environment, as indicated by clear increases in growth rate, can take up to 1,000 generations to be clearly observable, with examples of even faster adaptation occurring under selective conditions, and it has been previously noted that increasing growth rate is a hallmark of adaptation to selective conditions [Nicholson et al., 2011; Lenski and Travisano, 1994; Barrick et al., 2009; Maughan and Nicholson, 2011]. Comparable evolution studies in spaceflight are lacking. In space, particularly with ISS-based microbial studies, a wealth of information on the diversity and distribution of microbial taxa has been reported, including the collection of microbial isolates, sequences, and genomes [Singh et al., 2018]. However, there is little to no ability to know the provenance of an individual sequence, genome, or isolate; is it representative of a lineage that has persisted and evolved for decades onboard the ISS, or is it representative of a microbe newly arrived with the latest crew transfer or resupply mission? Controlled multi-generational evolution studies are needed to explore the mechanistic nature of the evolutionary process. In particular, understanding the evolutionary responses to variable gravity and radiation will be foundational in understanding how life is impacted across generations at the molecular genetic level.

What are the targets of genetic, molecular, and biochemical processes that are selected upon in the Beyond LEO space environment?

More specific to a general understanding of changes in rates of mutation and the evolutionary process in spaceflight is the question of what genes, pathways, and processes are specifically affected. Does the space environment cause epigenetic changes, and which genes are susceptible or affected, and how does this impact biological function in space and after return to Earth gravity? Studies that target specific phenotypic traits in an evolutionary context (e.g., antibiotic resistance and virulence, motility, membrane transport, and cell adhesion) will be of particular interest. Further, population-level selection will occur on microbial communities in the beyond LEO environment, including microbe-microbe and microbe-host interactions. Studies that can elucidate how these microbial communities adapt to spaceflight will be important. Adaptation of microarray technology to flight, or targeted gene-expression studies will be invaluable, although linking the data expected to be collected to evolution (versus acclimation) may be a challenge without the possibility of sample return.

How does the Beyond LEO environment impact biotechnological processes?

Biotechnological processes have unique features that make them appealing in the deep space environment (low temperature, low pressure, regenerable, expandable, programmable) and are the only means of manufacturing certain products (e.g., protein products such as enzymes and biologics). They can also make a far wider range of products or chemicals (e.g., drugs) available on a space mission to address contingencies than could be manifested as cargo. Because of this, NASA and other space agencies are developing new biological technologies to fill defined technology gaps [Bryan, 2020] and enable new mission architectures. For example, CUBES (Center for the Utilization of Biological Engineering in Space) is a 5-year $15 M multi-institute effort to develop concepts and technologies to support a biotechnology ecosystem on Mars [McNulty et al., 2021], and various perspectives on the utility of biotechnology for space are available [McNulty et al., 2021; Karouia et al., 2017; Menezes et al., 2015; Sleator and Smith, 2019; Snyder et al., 2019; Shiwei et al., 2020]. Examples of biotechnological processes under development to advance space travel include the MELiSSA (Micro-Ecological Life Support System Alternative) project, 3D printing, and in-space manufacturing of organs for medical use.

The MELiSSA (Micro-Ecological Life Support System Alternative) project aims to develop a closed-loop system for air, water, and waste management in space habitats. MELiSSA relies upon four subsystems: an anaerobic liquifying compartment that converts heterogeneous wastes to ammonium ion, H_2, CO_2, volatile fatty acids, and minerals; a photoheterotrophic compartment that removes the remaining volatile fatty acids; a nitrifying compartment that converts ammonium ion to nitrates; and a photoautotrophic compartment responsible for regenerating oxygen. MELiSSA has operated a pilot process on Earth to improve integration between these systems [Godia et al., 2004], and a set of spaceflight experiments has assessed the performance and stability of individual components [Ilgrande et al., 2019].

In-space additive manufacturing could enable new mission architectures and 3D printing is under continual development. Currently, Made In Space operates a 3D filament printer on the ISS capable of utilizing various input substrates (presently ABS, HDPE, and PEI-PC polymers are authorized). Future deep space 3D printing operations could be constrained by the need for continual resupply of substrate from Earth. To relieve these constraints, various approaches for generating these substrates from locally sourced materials are being investigated. Amongst these are microbially generated polyhydroxyalkanoates (PHAs). PHAs serve as a source of stored carbon for multiple microbial species, and in some conditions, PHAs can make up >50% of cellular dry mass. Over 150 different varieties of PHAs have been discovered, all with different material characteristics [Surendran et al., 2020]. Using a variety of input materials, microbially generated PHAs could be procured in-space at sufficient scale to improve missions' architectures [Menezes et al., 2015].

Microbes can be used for processes that can support sustainable human exploration of space. For example, bacteria and fungi can be used to extract and recover valuable metals from minerals [Kaksonen et al., 2020]. In fact, this is commonly done on Earth and 15% and 5% of copper and gold, respectively, currently on the market come from biomining processes. Additionally, microorganisms can be used to extract rare earth elements from ores (e.g., asteroid regolith), as well as electronic waste (printed circuit boards). The effectiveness of biomining processes has already been tested on ISS under a European project, including reduction of vanadium [Cockell et al. 2021]. Another biotechnological process that may be implemented beyond LEO using bacteria is the bioremediation of habitat air (CO_2 removal, O_2 generation) and water (removal of human- and machine-produced toxic compounds) [Ojuederie & Babalola, 2017]. Microbes can also aid in soil formation efforts to enable crops to grow on regolith (unconsolidated and heterogeneous rock deposits, such as on the lunar surface and Mars). An additional application is bioconcrete production (microbiologically induced calcite precipitation (MICP)) [Mujah et al., 2016].

In-space repair/manufacture/assembly of (certain) human organs would improve in-space medical capabilities. Moreover, the microgravity environment of space may result in improved organ characteristics, which could lead to a terrestrial market for in-space manufactured organs. As such, in-space organ printing is being pursued. Recently, a scaffold-free and nozzle-free magnetic levitation-based process has successfully generated tissue spheroids (chondrospheres) on the ISS [Parfenov et al., 2020].

Beyond these examples, biotechnology promises to be flexible enough to provide multiple services including generation of edible nutrients, pharmaceuticals, materials, catalysts, and fuels. As bioengineering and synthetic biology tools continue to improve, biotechnology will become more desirable and competitive to traditional approaches for obtaining key materials (i.e., resupply or strictly physicochemical systems) and resources during space missions.

How does the lunar gravity environment affect biotechnological processes?

Reduced gravity could directly or indirectly impact cellular and biochemical processes. These changes would influence biotechnological processes by altering the ambient baseline conditions under which a cellular factory would operate and may impair or improve biological processes. For example, production of valuable secondary metabolites were alternately increased or decreased in distinct strains of *Aspergillus nidulans* grown on the ISS [Romsdahl et al., 2019]. Beyond this, there are additional concerns with reduced gravity that only become relevant in the context of a biotechnological process. For example, foaming within terrestrial bioreactors is a major concern that must be managed, and it is reasonable to expect that the severity of this problem and the effectiveness of different mitigation strategies may be altered in the lunar gravity environment. The same is true for all aspects of gas or fluid management in a biotechnological process, particularly those related to mass transport. Thus, there would be great value in experiments designed to test and validate these aspects of a biotechnological process.

How does the Lunar radiation environment affect biotechnological processes?

The lunar environment - particularly the lunar radiation environment - could lead to increased mutation rates and an altered biologically selective landscape. This could be of particular concern for biotechnological processes that need to be reliably operated within specified parameters. Even on Earth, continuously operated systems face issues with culture stability, as the metabolic burden associated with production can select for cells with reduced productivity [Kopp et al., 2019]. This is because high production output necessitates diversion of carbon and protein synthesis capacity away from core processes necessary for cell growth and replication and towards the synthesis of pathway enzymes and/or products. Thus, cells with reduced productivity will usually grow faster. Developing methods to measure and respond to cellular burden is a major goal of synthetic biology [Han and Zhang, 2020]. Approaches include the development of "anti-mutator" strains of *E. coli* [Deatherage et al., 2018], pathway synthesis on orthogonal ribosomes [Darlington et al., 2018], feedback control circuits [Ceroni et al., 2018; Liu and Zhang, 2018], metabolic switching through two-stage fermentation [Yang et al., 2018; Gao et al., 2019], population quality control with sensor-selector [Rugbjerg et al., 2018; Guo et al., 2019], or growth-coupled production approaches [Wang et al., 2019].

How can biotechnological processes best utilize lunar resources?

Any biotech process at scale will need to acquire resources (carbon, oxygen, nitrogen, water) on site to avoid costly delivery from Earth. As the Moon effectively lacks an atmosphere, all resources must be sourced from the lunar regolith. The lunar surface can be subdivided into the ancient lunar highlands and the younger lunar mare ('seas'). The lunar highlands are rich in calcium, aluminum, silicon, and oxygen in the form of anorthite ($CaAl_2Si_2O_8$) [Crawford, 2015], but poor in magnesium and iron. The lunar maria are relatively rich in magnesium, iron and titanium in the form of anorthite ($CaAl_2Si_2O_8$), orthopyroxene ((Mg,Fe)SiO_3), clinopyroxene (Ca(Fe,Mg)Si_2O_6), olivine ((Mg,Fe)$_2SiO_4$), and ilmenite ($FeTiO_3$), but poorer in calcium and aluminum. At the surface, these minerals exist as a layer of loose regolith several meters thick with an average grain size of 60-70 μm. The lunar surface is constantly bombarded by the solar wind, which consists primarily of hydrogen and helium nuclei (by number) with heavier elements making up less than 0.1%. These solar wind particles accumulate in the regolith with volatile carbon present at a concentration of ~125 ppm (μg/g). In 2009, the Lunar Crater Observation and Sensing Satellite (LCROSS) impacted Cabeus crater on the Moon's south pole and revealed the presence of CO_2, light hydrocarbons (CH_4, C_2H_4) [Colaprete et al., 2010], and CO [Gladstone et al., 2010]. Overall, the Moon is highly depleted of water, but recent discoveries show its presence within Permanently Shadowed Regions (PSR) at the poles where water delivered from comets or formed through reactions with the solar wind has been trapped. In addition, there is evidence for hydrated minerals outside of the PSR at high latitudes, likely formed through reaction with the solar wind. Oxygen is present within the various sources of water but also within anhydrous oxide and silicate minerals, making up >40% of lunar regolith by mass.

Extraction of these mineral and water resources for use in biological processes would take place in the context of a larger In Situ Resources Utilization (ISRU) system focused on the extraction and generation of critical life- and mission-support resources (e.g., oxygen, propellant). Biotech processes would comprise one component of the larger ISRU ecosystem that would rely on lunar regolith [Sanders and Duke, 2005]. For example, over 20 processes have been identified to extract oxygen from lunar regolith, with two having demonstrated their effectiveness at human-relevant scales. They have been developed to a low-medium fidelity level and have demonstrated overall performance in critical areas. In NASA, technology/hardware development has specific milestones to define the Technology Readiness Level (TRL) [NASA Technology Readiness definition], and these two processes are at least TRL 4-5, with TRL 9 defined as successfully operated in a flight mission. The two types are a Hydrogen Reduction process where iron oxide is reduced to iron and water with hydrogen at 900°C and a Carbothermal Reduction process where silicates are reduced at 1600°C to generate CO and H_2, which are then converted to CH_4 and water. The water is then electrolyzed to O_2 and H_2 [Sanders and Duke, 2005]. As the larger lunar ISRU framework is further developed, it would be valuable to test the integration of biotechnological processes with this infrastructure on the Moon. This could include experiments that are directly attached to future ISRU validation hardware, or stand-alone missions that have dedicated mechanisms for the sampling and processing of lunar regolith.

Feasible Research Beyond LEO in the Next Five Years

Experiments in the next five years will need to be autonomous with no sample return and require the test cell/organism to be able to survive long pre-launch and transit times. Beyond LEO experiments will likely use the Orion capsule, lunar lander, and free-flyer platforms. Table 1 summarizes the organisms/cell systems that will allow some of the questions posed above to be addressed. In the near future, single cell organisms, simple multi-cellular organisms, or seeds that can survive dormant until experiments are initiated can be used to interrogate questions for cellular function, microorganisms and microbial communities, plant physiology, and biotechnological processes. Experiments using fish or mice will not be possible, but hardware to study nematodes will be available. Technology advancements are needed to allow autonomous culturing of microbes, mammalian cells, and organ-on-a chip to move the experiments to multi-generational experiments to study evolution and multi-cellular experiments to study microbe-host interaction. Differentiated mammalian cells and organs-on-a-chip need only medium changing to keep the cultures alive, and advancements in hardware to maintain appropriate environmental conditions should be available in the next five years. To move beyond the study of seeds, autonomous seed germination, plant maintenance, and monitoring of small plants will be needed to address questions of plant development and physiology.

Autonomous processing of samples to perform autonomous RNA-Seq and metabolomics will likely be unavailable in the near future. Recent advances in microfluidics for PCR amplification and small single molecule sequencing platforms, such as nanopore (MinION) sequencing, should allow studies into evolution and microbial genome stability, especially with single organism cultures. With the use of fluorescence and light

Table 1. Organisms and Sample Types Proposed for Space Biology Research Beyond LEO in the Next 5 years.

		Research Themes / Sections						
Organism / Sample Type	**Considerations & Rationale**	**SECTION A. Cellular Functions**	**SECTION B. Fundamental Microbiology and Ecology**	**SECTION C. Multicellular Physiology**	**SECTION D. Plant Development and Physiology**	**SECTION E. Host-Microbe Interactions**	**SECTION F. Evolution**	**SECTION G. Biotechnological Processes**
Cell Cultures	Mammalian and plant experiments are possible without experiment and hardware requirements necessary for vertebrates or large plants	Mammalian cell culture *Specifically, cell types related to the Human Research Program risk gaps*	Not applicable	Organ on a chip	Plant cell culture	Organ on a chip (JJ)	Open	Mammalian, human and plant cell cultures Organ on a chip
Model Bacteria and Archaea	Extensive published understanding of organism characteristics, often with flight heritage and established experimental systems	Single-celled bacteria and archaea	Representatives of functional guilds of interest: photosynthetic, anaerobic, nitrogen cycle, carbon cycle, etc. Stress tolerant microbes: radiation tolerant, spore formers, psychrophiles, etc.	Not applicable	Pathogenic and plant growth promoting bacteria Cyanobacteria	Open *more applicable to co-cultures and complex communities*	*Bacillus* *Deinococcus* *Escherechia* *Pseudomonas* *Salmonella* Cyanobacteria	Single-celled bacteria and archaea
Model Eukarya	Extensive published understanding of organism characteristics, often with flight heritage and established experimental systems	Single-celled yeasts *Arabidopsis*	Representatives of fungi and protists that carry out specific functions and/or are stress tolerant.	Yeasts Small animal eukaryote *e.g. worms, flies, fish* *Mus*	Green algae including *Chlorella* Moss species *Arabidopsis* Crop species as seeds and mature plants *e.g. lettuce, tomato, peppers, maize*	*Arabidopsis* Crop species *e.g. lettuce, mizuna, peppers* *Hydra* Rotifers *Chlorella*	Green algae *i.e. Chlorella* Yeasts and filamentous fungi Small animal eukaryotes *e.g. Nematodes and Tardigrades* Small plants *e.g. Brassica cultivars, Arabidopsis*	Single-celled yeasts Filamentous fungi
Organisms Useful for Targeted Functions or Questions	Studies of specific species, biological behaviors or processes of interest in spaceflight and BLEO; can include non-model organisms	Engineered organisms *e.g. with promoter-reporter constructs, fluorescent protein vector*	Nitrogen-cycle bacteria Oxygenic and anoxygenic photosynthetic bacteria Sulfur metabolism Halotrophy and radiation resistance Chemo- and autotrophic metabolisms	Open	Pathogenic and plant growth promoting bacteria Plants suited for efficient food production (tubers, beets, microgreens)	Probiotics for plants and humans	Nitrogen-cycle bacteria Oxygenic and anoxygenic photosynthetic bacteria Chemo- and autotrophic metabolisms	Chemolithoautotrophs Thermophiles
Co-Cultures	The effects of the BLEO environment on interaction effects between organisms in a defined and controlled manner	Open	Metabolic interactions of microbes and coordinated functions Symbiosis, commensalism and syntrophy Competition and predation	Open	Pathogenic and plant growth promoting bacteria	*Hydra* and algae Model host-microbe symbiotic systems *e.g. hydra and algae* Organ-on-a-chip (human cells co-cultured with specific microbes)	Plant-associated and plant growth promoting bacteria Symbiosis, commensalism and syntrophy Competition and predation	Syntrophy
Complex Communities	The responses of complex, natural communities to the BLEO and extreme built environment that cannot be reliably predicted from reductionist approaches.	Combined phenotypes	Synthetic model communities Naturally-evolved communities *e.g. soils, microbial mats* Cell cultures of gut, skin, plant with associated microbes. Built microbiome (potential living space BLEO)	Not applicable	Naturally-evolved communities *e.g. soils* Gut-, skin-, plant- and built-microbiome	Gut-, skin-, plant- and built-microbiome Termites	Synthetic model communities Gut-, skin-, plant- and built-microbiome	Biofilms

microscopy, studies of targeted gene expression, organelle function, oxidative stress, and microbial interaction using fluorescent-tagged cells are possible. Sensors to measure fluorescence and light of different wavelengths opens the possibility of monitoring fluorescent activity-based probes for specific enzyme activity, bioluminescence to monitor growth, and viability and growth using colorimetric metabolic activity dyes. The ability to capture images and videos of seed germination or plant growth should also be available in the next five years and will advance our understanding of plant development beyond LEO.

Concluding Remarks

This summary of the 2021 report of the BLISS committee provides recommendations for research beyond LEO from space biologists who work with a variety of organisms and study different aspects of space biology. Here, the focus has been on the important questions identified by the committee. The full report is available at the NASA Technical Reports Server (https://ntrs.nasa.gov/citations/20210023324) and provides greater detail about the beyond LEO environment, potential experiments, and technology needed to perform experiments.

Moving space biology research beyond LEO will advance the understanding of how organisms from bacteria to humans can adapt to and survive microgravity, partial gravity, and the radiation environment. These experiments cannot be fully simulated on Earth, especially work utilizing the lunar environment to cultivate plants for food and to develop materials to sustain human habitation. The basic knowledge obtained by performing experiments beyond LEO will be essential for eventual remote human survival on other planets.

Acknowledgments

We would like to thank Sonja Caldwell (Graphic Designer, KBR-NASA Ames Research Center) for the graphics and Stephanie Perreau Rainey (Project Scientist, KBR-NASA Ames Research Center) for assistance with editing and reference organization.

References

Abshire CF, Prasai K, Soto I, Shi R, Concha M, Baddoo M, Flemington EK, Ennis DG, Scott RS, Harrison L (2016) Exposure of *Mycobacterium marinum* to low-shear modeled microgravity: effect on growth, the transcriptome and survival under stress. *NPJ Microgravity* **2**: 16038. https://doi.org/10.1038/npjmgrav.2016.38116

Acres JM, Youngapelian MJ, Nadeau J (2021) The influence of spaceflight and simulated microgravity on bacterial motility and chemotaxis. *NPJ Microgravity* **7**: 7. https://doi.org/10.1038/s41526-021-00135-x

Apollo 16 preliminary examination team (1973) The apollo 16 lunar samples: petrographic and chemical description. *Science* **179**: 23-24. doi: 10.1126/science.179.4068.23

Arena C, De Micco V, Macaeva E, Quintens R (2014) Space radiation effects on plant and mammalian cells. *Acta Astronautica* **104**: 419. https://doi.org/10.1016/j.actaastro.2014.05.005

ARES – Astromaterials Research and Exploration Science. https://ares.jsc.nasa.gov/ Visited on November 12th 2023.

Aunins TR, Erickson KE, Prasad N, Levy SE, Jones A, Shrestha S, Mastracchio R, Stodieck L, Klaus D, Zea L, Chatterjee A (2018) Spaceflight modifies *Escherichia coli* gene expression in response to antibiotic exposure and reveals role of oxidative stress response. *Frontiers in Microbiology* **9**: 310. https://doi.org/10.3389/fmicb.2018.00310

Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science* **9**: 1473. https://doi.org/10.3389/fpls.2018.01473

Barrick JE, Yu DS, Yoon SH, Jeong H, Oh TK, Schneider D, Lenski RE, Kim JF (2009) Genome evolution and adaptation in a long-term experiment with *Escherichia coli*. *Nature* **461**(7268): 1243-1247. https://doi.org/10.1038/nature08480

Bijlani S, Singh NK, Eedara VVR, Podile AR, Mason CE, Wang CCC, Venkateswaran K (2021) *Methylobacterium ajmalii* sp. nov., Isolated From the International Space Station. *Frontiers in Microbiology* **12**: 639396. https://doi.org/10.3389/fmicb.2021.639396

Blachowicz A, Chiang AJ, Romsdahl J, Kalkum M, Wang CCC, Venkateswaran K (2019) Proteomic characterization of *Aspergillus fumigatus* isolated from air and surfaces of the International Space Station. *Fungal Genetic Biology* **124**: 39-46. https://doi.org/10.1016/j.fgb.2019.01.001

Bryan W (2020) Technology Taxonomy. In: NASA [internet cited 3 Feb 2023]. Available: http://www.nasa.gov/offices/oct/taxonomy/index.html

Bücker H, Horneck G (1975) The biological effectiveness of HZE-particles of cosmic radiation studied in the Apollo 16 and 17 Biostack experiments. *Acta Astronautica* **2**: 247-264. https://doi.org/10.1016/0094-5765(75)90095-8

Califar B, Tucker R, Cromie J, Sng N, Schmitz RA, Callaham JA, Barbazuk B, Paul A-L, Fer RJ (2018) Approaches for surveying cosmic radiation damage in large populations of seeds - antarctic balloons and particle beams. *Gravitational and Space Research* **6**: 54-73. https://doi.org/doi:10.2478/gsr-2018-0010

Canadian Space Agency: Lunar Gateway https://www.asc-csa.gc.ca/eng/atronomy/moon-exploration/lunar-gateway.asp visited November 12th 2023.

Caplin N, Willey N (2018) Ionizing radiation, higher plants, and radioprotection: from acute high doses to chronic low doses. *Frontiers in Plant Science* **9**: 847. https://doi.org/10.3389/fpls.2018.00847

Casaburi G, Goncharenko-Foster I, Duscher AA, Foster JS (2017) Transcriptomic changes in an animal-bacterial symbiosis under modeled microgravity conditions. *Science Reports* **7**: 46318. https://doi.org/10.1038/srep46318

Castro SL, Nelman-Gonzalez M, Nickerson CA, Ott CM (2011) Induction of attachment-independent biofilm formation and repression of Hfq expression by low-fluid-shear culture of Staphylococcus aureus. *Applied Environmental Microbiology* **77**: 6368-6378. https://doi.org/10.1128/AEM.00175-11

Ceroni F, Boo A, Furini S, Gorochowski TE, Borkowski O, Ladak YN, Awan AR, Gilbert C, Stan GB, Ellis T (2018) Burden-driven feedback control of gene expression. *Natural Methods* **15**(5): 387-393. https://doi.org/10.1038/nmeth.4635

Cheema AK, Suman S, Kaur P, Singh R, Fornace AJ Jr, Datta K (2014) Long-term differential changes in mouse intestinal metabolomics after gamma and heavy ion radiation exposure. *PLoS One* **9**: e87079. https://doi.org/10.1371/journal.pone.0087079

Choi WG, Barker RJ, Kim SH, Swanson SJ, Gilroy S (2019) Variation in the transcriptome of different ecotypes of *Arabidopsis thaliana* reveals signatures of oxidative stress in plant responses to spaceflight. *American Journal Botany* **106**: 123-136. doi: 10.1002/ajb2.1223

Clary JL, France CS, Lind K, Shi R, Alexander JS, Richards JT, Scott RS, Wang J, Lu X-H, Harrison L (2022) Development of an inexpensive 3D clinostat and comparison with other microgravity simulators using *Mycobacterium marinum*. *Frontiers in Space Technologies* **3**: 1032610. https://doi.org/10.3389/frspt.2022.1032610

Cockell CS, Santomartino R, Finster K, Waajen AC, Nicholson N, Loudon CM, et al. (2021) Microbially-enhanced vanadium mining and bioremediation under micro- and Mars gravity on the International Space Station. *Frontiers in Microbiology* **12**: 641387. https://doi.org/10.3389/fmicb.2021.641387

Colaprete A, Schultz P, Heldmann J, Wooden D, Shirley M, Ennico K, Hermalyn B, Marshall W, Ricco A, Elphic RC, Goldstein D, Summy D, Bart GD, Asphaug E, Korycansky D, Landis D, Sollitt L (2010) Detection of water in the LCROSS ejecta plume. *Science* **330**: 463-468. https://doi.org/10.1126/science.1186986

Crabbé A, Nielsen-Preiss SM, Woolley CM, Barrila J, Buchanan K, McCracken J, Inglis DO, Searles SC, Nelman-Gonzalez MA, Ott CM, Wilson JW, Pierson DL, Stefanyshyn-Piper HM, Hyman LE, Nickerson CA (2013) Spaceflight enhances cell aggregation and random budding in *Candida albicans*. *PLoS One* **8**: e80677. https://doi.org/10.1371/journal.pone.0080677

Crabbé A, Pycke B, Van Houdt R, Monsieurs P, Nickerson C, Leys N, Cornelis P (2010) Response of *Pseudomonas aeruginosa* PAO1 to low shear modelled microgravity involves AlgU regulation. *Environmental Microbiology* **12**: 1545-1564. https://doi.org/10.1111/j.1462-2920.2010.02184.x

Crabbé A, Schurr MJ, Monsieurs P, Morici L, Schurr J, Wilson JW, Ott CM, Tsaprailis G, Pierson DL, Stefanyshyn-Piper H, Nickerson CA (2011) Transcriptional and proteomic responses of *Pseudomonas aeruginosa* PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. *Applied Environmental Microbiology* **77**:1221-30. doi: 10.1128/AEM.01582-10

Crawford IA (2015) Lunar resources: A review. *Progress in Physical Geography: Earth and Environment* **39**: 137-167. https://doi.org/10.1177/0309133314567585

Datta K, Suman S, Kallakury BV, Fornace AJ, Jr (2012) Exposure to heavy ion radiation induces persistent oxidative stress in mouse intestine. *PLoS One* **7**: e42224. https://doi.org/10.1371/journal.pone.0042224

Darlington APS, Kim J, Jimenez JI, Bates DG (2018) Dynamic allocation of orthogonal ribosomes facilitates uncoupling of co-expressed genes. *Natural Communications* **9**: 695. https://doi.org/10.1038/s41467-018-02898-6

Deatherage DE, Leon D, Rodriguez AE, Omar SK, Barrick JE (2018) Directed evolution of *Escherichia coli* with lower-than-natural plasmid mutation rates. *Nucleic Acids Res* **46**: 9236-9250. https://doi.org/10.1093/nar/gky751

De Micco V, Arena C, Pignalosa D, Durante M (2011) Effects of sparsely and densely ionizing radiation on plants. *Radiation and Environmental Biophysics* **50**: 1-19. https://doi.org/10.1007/s00411-010-0343-8

De Pascale S, Arena C, Aronne G, De Micco V, Pannico A, Paradiso R, Rouphael Y (2021) Biology and crop production in space environments: Challenges and opportunities. *Life Science Space Research* **29**: 30-37. https://doi.org/10.1016/j.lssr.2021.02.005

Dethlefsen L, McFall-Ngai M, Relman DA (2007) An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* **449**: 811-818. https://doi.org/10.1038/nature06245

Duscher AA, Conesa A, Bishop M, Vroom MM, Zubizarreta SD, Foster JS (2018) Transcriptional profiling of the mutualistic bacterium *Vibrio fischeri* and an hfq mutant under modeled microgravity. *NPJ Microgravity* **4**: 25. https://doi.org/10.1038/s41526-018-0060-1

Eisen J (2015) What does the term microbiome mean? And where did it come from? A bit of a surprise. *The Winnower* **2**: e142971.16196. doi: 10.15200/winn.142971.16196

Elena SF, Lenski RE (2003) Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nature Reviews Genetics* **4**: 457-469. https://doi.org/10.1038/nrg1088

Ellery A (2021) Supplementing closed ecological life support systems with in-situ resources on the Moon. *Life* **11**: 770. https://doi.org/10.3390/life11080770

Foster JS, Wheeler RM, Pamphile R (2014) Host-microbe interactions in microgravity: assessment and implications. *Life (Basel)* **4(2)**: 250-266. https://doi.org/10.3390/life4020250

Furukawa S, Nagamatsu A, Nenoi M, Fujimori A, Kakinuma S, Katsube T, Wang B, Tsuruoka C, Shirai T, Nakamura AJ, Sakaue-Sawano A, Miyawaki A, Harada H, Kobayashi M, Kobayashi J, Kunieda T, Funayama T, Suzuki M, Miyamoto T, Hidema J, Yoshida Y, Takahashi A (2020) Space radiation biology for "living in space". *BioMed Research International* **2020**: 4703286. https://doi.org/10.1155/2020/4703286

Gao C, Hou J, Xu P, Guo L, Chen X, Hu G, Ye C, Edwards H, Chen J, Chen W, Liu L (2019) Programmable biomolecular switches for rewiring flux in *Escherichia coli*. *Nature Communications*, 10: 3751. https://doi.org/10.1038/s41467-019-11793-7

Garrett-Bakelman FE, Darshi M, Green SJ, Gur RC, Lin L, Macias BR et al. (2019) The NASA twins study: a multidimensional analysis of a year-long human spaceflight. *Science* **364**: 6436. https://doi.org/10.1126/science.aau8650

Gateway https://www.nasa.gov/mission/gateway/ visited November 12th 2023.

Gladstone GR, Hurley DM, Retherford KD, Feldman PD, Pryor WR, Chaufray JY, et al. (2010) LRO-LAMP observations of the LCROSS impact plume. *Science* **330**: 472-476. https://doi.org/10.1126/science.1186474

Godia F, Albiol J, Perez J, Creus N, Cabello F, Montras A, Masot A, Lasseur, C (2004) The MELISSA pilot plant facility as an integration test-bed for advanced life support systems. *Advanced Space Research* **34**: 1483-1493. https://doi.org/10.1016/j.asr.2003.08.038

Gopalakrishnan S, Srinivas V, Prakash B, Sathya A, Vijayabharathi R (2015) Plant growth-promoting traits of *Pseudomonas geniculata* isolated from chickpea nodules. *3 Biotech* **5**: 653-661. https://doi.org/10.1007/s13205-014-0263-4.

Großkopf T and Soyer OS (2014) Synthetic microbial communities. *Current Opinions on Microbiology* **18**: 72-77. https://doi.org/10.1016/j.mib.2014.02.002

Guo X, Li Z, Wang X, Wang J, Chala J, Lu Y, Zhang H (2019) De novo phenol bioproduction from glucose using biosensor-assisted microbial coculture engineering. *Biotechnology and Bioengineering.* **116**: 3349-3359. doi: 10.1002/bit.27168

Han Y and Zhang F (2020) Control strategies to manage trade-offs during microbial production. *Current Opinions on Biotechnology* **66**: 158-164. https://doi.org/10.1016/j.copbio.2020.07.004

Hariom SK, Ravi A, Mohan GR, Pochiraju HD, Chattopadhyay S, Nelson EJR (2021) Animal physiology across the gravity continuum. *Acta Astronautica* **178**: 522- 535. https://doi.org/https://doi.org/10.1016/j.actaastro.2020.09.044

Herranz R, Vandenbrink JP, Villacampa A, Manzano A, Poehlman WL, Feltus FA, Kiss JZ, Medina FJ (2019) RNAseq analysis of the response of *Arabidopsis thaliana* to fractional gravity under blue-light stimulation during spaceflight. *Frontiers in Plant Science* **10**: 1529. https://doi.org/10.3389/fpls.2019.01529

Horneck G, Klaus DM, Mancinelli RL (2010) Space microbiology. *Microbiology and Molecular Biology Reviews* **74**: 121-156. https://doi.org/10.1128/mmbr.00016-09

Hunt CR, Ramnarain D, Horikoshi N, Iyengar P, Pandita RK, Shay JW, Pandita TK (2013) Histone modifications and DNA double-strand break repair after exposure to ionizing radiations. *Radiation Research* **179**: 383-392. https://doi.org/10.1667/RR3308.2

Ilgrande C, Mastroleo F, Christiaen MER, Lindeboom REF, Prat D, Van Hoey O et al. (2019). Reactivation of microbial strains and synthetic communities after a spaceflight to the International Space Station: corroborating the feasibility of essential conversions in the MELiSSA loop. *Astrobiology* **19**: 1167-1176. https://doi.org/10.1089/ast.2018.1973

Jansson JK and Hofmockel KS (2020) Soil microbiomes and climate change. *Nature Reviews Microbiology* **18**: 35-46. https://doi.org/10.1038/s41579-019-0265-7

Jessup CM, Kassen R, Forde SE, Kerr B, Buckling A, Rainey PB, Bohannan BJM (2004) Big questions, small worlds: microbial model systems in ecology. *Trends in Ecology & Evolution* **19**: 189-197. https://doi.org/10.1016/j.tree.2004.01.008

Jiang D, Armour CR, Hu C, Mei M, Tian C, Sharpton TJ, Jiang Y (2019) Microbiome multi-omics network analysis: statistical considerations, limitations, and opportunities. *Frontiers in Genetics* **10**: 995. https://doi.org/10.3389/fgene.2019.00995

Juergensmeyer MA, Juergensmeyer EA, Guikema JA (1999) Long-term exposure to spaceflight conditions affects bacterial response to antibiotics. *Microgravity Science Technology* **12**: 41-47. https://www.ncbi.nlm.nih.gov/pubmed/11543359

Kaksonen AH, Deng X, Bohu T, Zea L, Khaleque HN, Gumulya Y, Boxall NJ, Morris C, Cheng KY (2020) Prospective directions for biohydrometallurgy. *Hydrometallurgy* **195**: 105376. https://doi.org/https://doi.org/10.1016/j.hydromet.2020.105376

Karouia F, Peyvan K, Pohorille A (2017) Toward biotechnology in space: high-throughput instruments for in situ biological research beyond Earth. *Biotechnology Advances* **35**: 905-932. https://doi.org/10.1016/j.biotechadv.2017.04.003

Kim JH (2019) Chromatin remodeling and epigenetic regulation in plant DNA damage repair. *International Journal of Molecular Science* **20**: 4093. https://doi.org/10.3390/ijms20174093

Klaus DM and Howard HN (2006) Antibiotic efficacy and microbial virulence during space flight. *Trends in Biotechnology* **24**: 131-136. https://doi.org/10.1016/j.tibtech.2006.01.008

Kopp J, Slouka C, Spadiut O, Herwig C (2019) The rocky road from fed-batch to continuous processing with *E. coli*. *Frontiers in Bioengineering and Biotechnology* **7**: 328. https://doi.org/10.3389/fbioe.2019.00328

Kordyum E and Hasenstein KH (2021) Plant biology for space exploration - building on the past, preparing for the future. *Life Science Space Research (Amsterdam)* **29**: 1-7. https://doi.org/10.1016/j.lssr.2021.01.003

Kyriacou MC, De Pascale S, Kyratzis A, Rouphael Y (2017) Microgreens as a component of space life support systems: a cornucopia of functional food. *Frontiers in Plant Science* **8**: 1587. https://doi.org/10.3389/fpls.2017.01587

Lam C-W, Castranova V, Driscoll K, Warheit D, Ryder V, Zhang Y et al. (2023) A review of pulmonary neutrophilia and insights into the key role of neutrophils in particle-induced pathogenesis in the lung from animal studies of lunar dusts and other poorly soluble dust particles. *Critical Review of Toxicology* Oct 18: 1-39 doi: 10.1080/10408444.2023.2258925

Lawler JM, Song W, Demaree SR (2003) Hindlimb unloading increases oxidative stress and disrupts antioxidant capacity in skeletal muscle. *Free Radical Biological Medicine* **35**: 9-16. https://doi.org/10.1016/s0891-5849(03)00186-2

Lederberg J and McCray A (2001) `Ome sweet `omics--a genealogical treasury of words. *Scientist* **15**: 8-8.

Lee MD, O'Rourke A, Lorenzi H, Bebout BM, Dupont CL, Everroad RC (2021) Reference-guided metagenomics reveals genome-level evidence of potential microbial transmission from the ISS environment to an astronaut's microbiome. *iScience* **24**: 102114. https://doi.org/10.1016/j.isci.2021.102114

Lee S and van Riessen A (2022) A review on geopolymer technology for lunar base construction. *Materials (Basel)* **15**: 4516. doi: 10.3390/ma15134516

Lenski RE and Travisano M (1994) Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. *Proceedings of the National Academy of Science USA* **91**: 6808-6814. https://doi.org/10.1073/pnas.91.15.6808

Leys NM, Hendrickx L, De Boever P, Baatout S, Mergeay M (2004) Space flight effects on bacterial physiology. *Journal of Biologically Regulated Homeostasis Agents* **18**: 193-199. https://www.ncbi.nlm.nih.gov/pubmed/15471227

Li C, Hu H, Yang M-F, Pei Z-Y, Zhou Q, Ren X et al. (2021) Characteristics of the lunar samples returned by the Chang'E-5 mission. *National Science Review* **9**: nwab188. https://doi.org/10.1093/nsr/nwab188

Li J, Liu F, Wang Q, Ge P, Woo PC, Yan J, Zhao Y, Gao GF, Liu CH, Liu C (2014) Genomic and transcriptomic analysis of NDM-1 *Klebsiella pneumoniae* in spaceflight reveal mechanisms underlying environmental adaptability. *Science Report* **4**: 6216. https://doi.org/10.1038/srep06216

Liao AC, Craver BM, Tseng BP, Tran KK, Parihar VK, Acharya MM, Limoli CL (2013) Mitochondrial-targeted human catalase affords neuroprotection from proton irradiation. *Radiation Research* **180**: 1-6. doi: 10.1667/RR3339.1

Limoli CL, Giedzinski E, Baure J, Rola R, Fike JR (2007) Redox changes induced in hippocampal precursor cells by heavy ion irradiation. *Radiation Environmental Biophysics* **46**: 167-172. https://doi.org/10.1007/s00411-006-0077-9

Ling Z, Jolliff BL, Wang A, Li C, Liu J, Zhang J, et al. (2015) Correlated compositional and mineralogical investigations at the Chang'e-3 landing site. *Nature Communications* **6**: 8880. doi: 10.1038/ncomms9880

Liu D and Zhang F (2018) Metabolic feedback circuits provide rapid control of metabolite dynamics. *ACS Synthetic Biology* **7**: 347-356. https://doi.org/10.1021/acssynbio.7b00342

Liu Y, Wang E (2008) Transcriptional analysis of normal human fibroblast responses to microgravity stress. *Genomics Proteomics Bioinformatics* **6**: 29-41. https://doi.org/10.1016/S1672-0229(08)60018-2

Liu Z, Luo G, Du R, Sun W, Li J, Lan H, Chen P, Yuan X, Cao D, Li Y, Liu C, Liang S, Jin X, Yang R, Bi Y, Han Y, Cao P, Zhao W, Ling S, Li Y (2020) Effects of spaceflight on the composition and function of the human gut microbiota. *Gut Microbes* **11**: 807-819. https://doi.org/10.1080/19490976.2019.1710091

MacDonald JG, Rodriguez K, Quirk S (2020) An oxygen delivery polymer enhances seed germination in a Martian-like environment. *Astrobiology* **20**: 846-863. http://doi.org/10.1089/ast.2019.2056

Mackenroth B and Alani E (2021) Collaborations between chromatin and nuclear architecture to optimize DNA repair fidelity. *DNA Repair* **97**: 103018. https://doi.org/10.1016/j.dnarep.2020.103018

Malyarchuk S, Brame KL, Youngblood R, Shi R, Harrison L (2004) Two clustered 8-oxo-7,8-dihydroguanine (8-oxodG) lesions increase the point mutation frequency of 8-oxodG, but do not result in double strand breaks or deletions in *Escherichia coli*. *Nucleic Acids Research* **32**: 5721-5731. https://doi.org/10.1093/nar/gkh911

Mandal A, Dutta A, Das R, Mukherjee J (2021) Role of intertidal microbial communities in carbon dioxide sequestration and pollutant removal: a review. *Marine Pollution Bulletin* **170**: 112626. https://doi.org/10.1016/j.marpolbul.2021.112626

Manti L (2006) Does reduced gravity alter cellular response to ionizing radiation? *Radiation Environmental Biophysics* **45**: 1-8. https://doi.org/10.1007/s00411-006-0037-4

Manzano A, Herranz R, den Toom LA, Te Slaa S, Borst G, Visser M, Medina FJ, van Loon J (2018) Novel, Moon and Mars, partial gravity simulation paradigms and their effects on the balance between cell growth and cell proliferation during early plant development. *NPJ Microgravity* **4**: 9. https://doi.org/10.1038/s41526-018-0041-4

Mao XW, Nishiyama NC, Byrum SD, Stanbouly S, Jones T, Holley J, Sridharan V, Boerma M, Tackett AJ, Willey JS, Pecaut MJ, & Delp MD (2020) Spaceflight induces oxidative damage to blood-brain barrier integrity in a mouse model. *FASEB Journal* **34**: 15516-15530. https://doi.org/10.1096/fj.202001754R

Maughan H, Nicholson WL (2011) Increased fitness and alteration of metabolic pathways during *Bacillus subtilis* evolution in the laboratory. *Applied Environmental Microbiology* **77**: 4105-4118. https://doi.org/10.1128/AEM.00374-11

McKay DS, Heiken G, Basu A, Blanford G, Simon S, Reedy R, French BM, Papike J (1991) The lunar regolith. In: Lunar Sourcebook: A User's Guide to the Moon. Eds. GH Heiken, DT Vaniman, BM French. Chapter 7: 285-356. Cambridge University Press.

McNulty MJ, Xiong YM, Yates K, Karuppanan K, Hilzinger JM, Berliner AJ, Delzio J, Arkin AP, Lane NE, Nandi S, McDonald KA (2021) Molecular pharming to support human life on the Moon, Mars, and beyond. *Critical Review of Biotechnology* **41**: 849-864. https://doi.org/10.1080/07388551.2021.1888070

Menezes AA, Montague MG, Cumbers J, Hogan JA, Arkin AP (2015) Grand challenges in space synthetic biology. *Journal of the Royal Society of the Interface* **12**: 20150803. https://doi.org/10.1098/rsif.2015.0803

Miousse IR, Kutanzi KR, Koturbash I (2017) Effects of ionizing radiation on DNA methylation: from experimental biology to clinical applications. *International Journal of Radiation Biology* **93**: 457-469. https://doi.org/10.1080/09553002.2017.1287454

Moore S, Stanley FK, Goodarzi AA (2014) The repair of environmentally relevant DNA double strand breaks caused by high linear energy transfer irradiation--no simple task. *DNA Repair* **17**: 64-73. https://doi.org/10.1016/j.dnarep.2014.01.014

Morey-Holton ER (2003) The impact of gravity on life. In: Evolution on Planet Earth. Eds LJ Rothschild and AM Lister. Chapter 9: 143-159. London Academic Press. doi:10.1016/B978-012598655-7/50036-7

Morrison MD, Thissen JB, Karouia F, Mehta S, Urbaniak C, Venkateswaran K, Smith DJ, Jaing C (2021) Investigation of spaceflight induced changes to astronaut microbiomes. *Frontiers in Microbiology* **12**: 659179. https://doi.org/10.3389/fmicb.2021.659179

Mujah D, Shahin MH, Cheng L (2016) State-of-the-art review of biocementation by microbially induced calcite precipitation (MICP) for soil stabilization. *Geomicrobiology Journal* **34**: 524-537, https://doi.org/10.1080/01490451.2016.1225866

NASA Technology Readiness Definition Microsoft Word - TRL Definitions.doc (nasa.gov) visited 11/8/2023.

NASA Science Working Group Life Beyond Low Earth Orbit (2018) Report of a science working group to the nasa human exploration and operations mission directorate and space life and physical sciences division. Available: https://nspires.nasaprs.com/external/viewrepositorydocument/cmdocumentid=625219/solicitationId=%7B87B32BFC-87BB-9A8E-51FA-B884B658D0A5%7D/viewSolicitationDocument=1/LBLEO%20Report%2001082018.pdf

National Academies of Sciences, Engineering & Medicine. (2018) A midterm assessment of implementation of the decadal survey on life and physical sciences research at NASA. The National Academies Press. https://doi.org/doi:10.17226/24966

Nicholson WL, Ricco AJ, Agasid E, Beasley C, Diaz-Aguado M, Ehrenfreund P, Friedericks C, Ghassemieh S, Henschke M, Hines JW, Kitts C, Luzzi E, Ly D, Mai N, Mancinelli R, McIntyre M, Minelli G, Neumann M, Parra M, Piccini M, Rasay RM, Ricks R, Santos O, Schooley A, Squires D, Timucin L, Yost B, Young A (2011) The O/OREOS mission: first science data from the Space Environment Survivability of Living Organisms (SESLO) payload. *Astrobiology* **11**: 951-958. https://doi.org/10.1089/ast.2011.0714

Nickerson CA, Ott CA, Mister SJ, Morrow BJ, Burns-Keliher L, Pierson DL (2000) Microgravity as a novel environmental signal affecting *Salmonella enterica* serovar Typhimurium virulence. *Infection and Immunity* **68**:3147-3152. doi: 10.1128/IAI.68.6.3147-3152.2000

Nickerson CA, Ott CM, Wilson JW, Ramamurthy R, Pierson DL (2004) Microbial responses to microgravity and other low-shear environments. *Microbiological Molecular Biology Review* **68**: 345-361. https://doi.org/10.1128/MMBR.68.2.345-361.2004

Noble S (2013) The lunar regolith. NASA Technical Reports Server Document ID 20090026015 M09-0381_Final Paper.pdf (nasa.gov)

Norbury JW, Slaba TC, Aghara S, Badavi FF, Blattnig SR, Clowdsley MS, Heilbronn LH, Lee K, Maung KM, Mertens CJ, Miller J, Norman RB, Sandridge CA, Singleterry R, Sobolevsky N, Spangler JL, Townsend LW, Werneth CM, Whitman K, Wilson JW, Xu SX, Zeitlin C (2019) Advances in space radiation physics and transport at NASA. *Life Science Space Research* **22**: 98-124. https://doi.org/10.1016/j.lssr.2019.07.003

Novikova N, Deshevaya E, Levinskikh M, Polikarpov N, Poddubko S, Gusev O, Sychev V (2015) Study of the effects of the outer space environment on dormant forms of microorganisms, fungi and plants in the “Expose-R” experiment. *International Journal of Astrobiology* **14**: 137-142. https://doi.org/doi:10.1017/S1473550414000731

Odeh R and Guy C (2017) Gardening for therapeutic people-plant interactions during long-duration space missions. *Open Agriculture* **2**: 1 - 13.

Ojuederie OB and Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. *International Journal of Environmental Research and Public Health* **14**: 1504. https://doi.org/10.3390/ijerph14121504

Ott CM, Bruce RJ, Pierson DL (2004) Microbial characterization of free floating condensate aboard the Mir space station. *Microbiology and Ecology* **47**: 133-136. https://doi.org/10.1007/s00248-003-1038-3

Ott CM, Crabbé A, Wilson JW, Barrila J, Castro-Wallace SL, Nickerson CA (2020) Microbial stress: spaceflight-induced alterations in microbial virulence and infectious disease risks for the crew. In: Stress Challenges and Immunity in Space. Eds. Choukèr A. Springer, Cham. https://doi.org/10.1007/978-3- 030-16996-1_18

Orsini SS, Lewis AM, Rice KC (2017) Investigation of simulated microgravity effects on *Streptococcus mutans* physiology and global gene expression. *NPJ Microgravity* **3**: 4. https://doi.org/10.1038/s41526-016-0006-4

Ou X, Long L, Zhang Y, Xue Y, Liu J, Lin X, Liu B (2009) Spaceflight induces both transient and heritable alterations in DNA methylation and gene expression in rice (Oryza sativa L.). *Mutation Research* **662**: 44-53. https://doi.org/10.1016/j.mrfmmm.2008.12.004

Overbey EG, da Silveira WA, Stanbouly S, Nishiyama NC, Roque-Torres GD, Pecaut MJ, Zawieja DC, Wang C, Willey JS, Delp MD, Hardiman G, Mao XW (2019) Spaceflight influences gene expression, photoreceptor integrity, and oxidative stress-related damage in the murine retina. *Science Report* **9**: 13304. https://doi.org/10.1038/s41598-019-49453-x

Padgen MR, Liddell LC, Bhardwaj SR, Gentry D, Marina D, Parra M, Boone T, Tan M, Ellingson L, Rademacher A, Benton J, Schooley A, Mousavi A, Friedericks C, Hanel RP, Ricco AJ, Bhattacharya S, Maria SRS (2023) BioSentinel: a biofluidic nanosatellite monitoring microbial growth and activity in deep space. *Astrobiology* **23**: 6, 637-647. https://doi.org/10.1089/ast.2020.2305

Padgen MR, Lera MP, Parra MP, Ricco AJ, Chin M, Chinn TN, Cohen A, Friedericks CR, Henschke MB, Snyder TV, Spremo SM, Wang JH, Matin AC (2020). EcAMSat spaceflight measurements of the role of sigma(s) in antibiotic resistance of stationary phase *Escherichia coli* in microgravity. *Life Science Space Research* **24**: 18-24. https://doi.org/10.1016/j.lssr.2019.10.007

Paige DA, Foote MC, Greenhagen BT, Schofield JT, Calcutt S, Vasavada AR, Preston DJ, Taylor FW, Allen CC, Snook KJ, Jakosky BM, Murray BC, Soderblom LA, Jau B, Loring S, Bulharowski J, Bowles NE, Thomas IR, Sullivan MT, Avis C, de Jong EM, Hartford W, McCleese DJ (2010) The lunar reconnaissance orbiter diviner lunar radiometer experiment. *Space Science Reviews* **150**: 125-160. https://doi.org/10.1007/s11214-009-9529-2

Paradiso R, Ceriello A, Pannico A, Sorrentino S, Palladino M, Giordano M, Fortezza R, De Pascale S (2020) Design of a module for cultivation of tuberous plants in microgravity: the esa project “Precursor of Food Production Unit” (PFPU). *Frontiers in Plant Science* **11**: 417. https://doi.org/10.3389/fpls.2020.00417

Parfenov VA, Khesuani YD, Petrov SV, Karalkin PA, Koudan EV, Nezhurina EK, et al. (2020) Magnetic levitational bioassembly of 3D tissue construct in space. *Scientific Advances* **6**: eaba4174. https://doi.org/10.1126/sciadv.aba4174

Paul AL, Elardo SM, Ferl RJ (2022) Plants grown in Apollo lunar regolith present stress-associated transcriptomes that inform prospects for lunar exploration. *Communications in Biology* **5**: 382. doi: 10.1038/s42003-022-03334-8

Paul AL and Ferl RJ (2006) The biology of low atmosphere pressure-implications for exploration mission design and advanced life support. *Gravitational and Space Biology* **19**: 3-18.

Paul AL, Schuerger AC, Popp MP, Richards JT, Manak MS, Ferl RJ (2004) Hypobaric biology: *Arabidopsis* gene expression at low atmospheric pressure. *Plant Physiology* **134**: 215-223. https://doi.org/10.1104/pp.103.032607

Paul AM, Overbey EG, da Silveira WA, Szewczyk N, Nishiyama NC, Pecaut MJ, Anand S, Galazka JM, Mao XW (2021) Immunological and hematological outcomes following protracted low dose/low dose rate ionizing radiation and simulated microgravity. *Science Report* **11**: 11452. https://doi.org/10.1038/s41598-021-90439-5

Pecaut MJ, Mao XW, Bellinger DL, Jonscher KR, Stodieck LS, Ferguson VL, Bateman TA, Mohney RP, Gridley DS (2017) Is spaceflight-induced immune dysfunction linked to systemic changes in metabolism? *PLoS One* **12**: e0174174. https://doi.org/10.1371/journal.pone.0174174

Ramzan F, Vickers MH, Mithen RF (2021) Epigenetics, microRNA and metabolic syndrome: a comprehensive review. *International Journal of Molecular Science* **22**: 5047. https://doi.org/10.3390/ijms22095047

Ranieri D, Cucina A, Bizzarri M, Alimandi M, Torrisi MR (2015) Microgravity influences circadian clock oscillation in human keratinocytes. *FEBS Open Bio* **5**: 717-723. https://doi.org/10.1016/j.fob.2015.08.012

Ricco AJ, Maria SRS, Hanel RP, Bhattacharya S (2020) BioSentinel: a 6U nanosatellite for deep-space biological science. *IEEE Aerospace and Electronic Systems Magazine* **35**: 6-18. https://doi.org/10.1109/MAES.2019.2953760

Ricco AJ, Parra M, Niesel D, Piccini M, Ly D, McGinnis M, Kudlicki A, Hines J, Timucin L, Beasley C, Ricks R, McIntyre M, Friedericks C, Henschke M, Leung R, Diaz-Aguado M, Kitts C, Mas I, Rasay M, Agasid E, Luzzi E, Ronzano K, Squires D, Yost B (2011) PharmaSat: drug dose response in microgravity from a free-flying integrated biofluidic/optical culture-and-analysis satellite. *SPIE Microfluidics, BioMEMS, and Medical Microsystems IX Proceedings* Vol. **7929**: 79290T. https://doi.org/10.1117/12.881082

Romsdahl J, Blachowicz A, Chiang AJ, Chiang Y-M, Masonjones S, Yaegashi J, Countryman S, Karouia F, Kalkum M, Stajich JE, Venkateswaran K, Wang CCC (2019) International Space Station conditions alter genomics, proteomics, and metabolomics in *Aspergillus nidulans*. *Applied Microbiology and Biotechnology* **103**:1363-1377. doi: 10.1007/s00253-018-9525-0

Rosenzweig JA, Abogunde O, Thomas K, Lawal A, Nguyen Y-U, Sodipe A, Jejelowo O (2010) Spaceflight and modeled microgravity effects on microbial growth and virulence. *Applied Microbiology and Biotechnology* **85**: 885-891. https://doi.org/10.1007/s00253-009-2237-8

Rugbjerg P, Sarup-Lytzen K, Nagy M, Sommer MOA (2018) Synthetic addiction extends the productive life time of engineered *Escherichia coli* populations. *Proceedings of the National Academy of Science USA* **115**: 2347-2352. https://doi.org/10.1073/pnas.1718622115

Rubin-Blum M, Dubilier N, Kleiner M (2019) Genetic evidence for two carbon fixation pathways (the calvin-benson-bassham cycle and the reverse tricarboxylic acid cycle) in symbiotic and free-living bacteria. *mSphere* **4**: e00394-18. https://doi.org/10.1128/mSphere.00394-18

Sage E, Harrison L (2011) Clustered DNA lesion repair in eukaryotes: relevance to mutagenesis and cell survival. *Mutation Research* **711**: 123-133. https://doi.org/10.1016/j.mrfmmm.2010.12.010

Sanders GB and Duke M (2005) In-Situ Resource Utilization (ISRU) capability roadmap progress review. NASA Technical Report Server NTRS 20050205045. Available: https://ntrs.nasa.gov/citations/20050205045

Santa Maria SR, Marina DB, Massaro Tieze S, Liddell LC, Bhattacharya S (2020) BioSentinel: long-term *Saccharomyces cerevisiae* preservation for a deep space biosensor mission. *Astrobiology* **20**: 1-14. https://doi.org/10.1089/ast.2019.2073

Schuerger AC, Amaradasa BS, Dufault NS, Hummerick ME, Richards JT, Khodadad CL, Smith TM, Massa GD (2021) *Fusarium oxysporum* as an opportunistic fungal pathogen on Zinnia hybrida plants grown on board the International Space Station. *Astrobiology* **21**: 1029-1048. https://doi.org/10.1089/ast.2020.2399

Sheppard J, Land ES, Toennisson TA, Doherty CJ, Perera IY (2021) Uncovering transcriptional responses to fractional gravity in *Arabidopsis* roots. *Life* **11**: 1010. https://doi.org/10.3390/life11101010

Shi J, Lu W, Sun Y (2014) Comparison of space flight and heavy ion radiation induced genomic/epigenomic mutations in rice (*Oryza sativa*). *Life Science Space Research* **1**: 74-79. https://doi.org/10.1016/j.lssr.2014.02.007

Shiwei N, Dritsas S, Fernandez JG (2020) Martian biolith: A bioinspired regolith composite for closed-loop extraterrestrial manufacturing. *PLoS One* **15**: e0238606. doi: 10.1371/journal.pone.0238606

da Silveira WA, Fazelinia H, Rosenthal SB, Laiakis EC, Kim MS, Meydan C, Kidane Y, Rathi KS, Smith SM, Stear B, Ying Y, Zhang Y, Foox J, Zanello S, Crucian B, Wang D, Nugent A, Costa HA, Zwart SR, Schrepfer S, Elworth RAL, Sapoval N, Treangen T, MacKay M, Gokhale NS, Horner SM, Singh LN, Wallace DC, Willey JS, Schisler JC, Meller R, McDonald JT, Fisch KM, Hardiman G, Taylor D, Mason CE, Costes SV, Beheshti A (2020) Comprehensive multi-omics analysis reveals mitochondrial stress as a central biological hub for spaceflight impact. *Cell* **183**: 1185-1201 e1120. https://doi.org/10.1016/j.cell.2020.11.002

Singh NK, Wood JM, Karouia F, Venkateswaran K (2018) Succession and persistence of microbial communities and antimicrobial resistance genes associated with International Space Station environmental surfaces. *Microbiome* **6**: 204. https://doi.org/10.1186/s40168-018-0585-2

Sleator RD, Smith N (2019) Terraforming: synthetic biology's final frontier. *Archives of Microbiology* **201**: 855-862. https://doi.org/10.1007/s00203-019-01651-x

Snyder JE, Walsh D, Carr PA, Rothschild LJ (2019) A makerspace for life support systems in space. *Trends in Biotechnology* **37**: 1164-1174. https://doi.org/10.1016/j.tibtech.2019.05.003

Sobel A, Duncan R (2020) Aerospace environmental health: considerations and countermeasures to sustain crew health through vastly reduced transit time to/from Mars. *Frontiers in Public Health* **8**: 327. doi: 10.3389/fpubh.2020.00327

Soni A, O'Sullivan L, Quick LN, Ott CM, Nickerson CA, Wilson JW (2014) Conservation of the low-shear modeled microgravity response in enterobacteriaceae and analysis of the trp genes in this response. *Open Microbiology Journal* **8**: 51-58. https://doi.org/10.2174/1874285801408010051

Space Biology Science Plan. Space Biological Sciences Plan 2016-2025. NASA (2016). Available: https://www.nasa.gov/sites/default/files/atoms/files/16-03-23_sb_plan.pdf

Speijer D, Hammond M, Lukeš J (2020) Comparing early eukaryotic integration of mitochondria and chloroplasts in the light of internal ROS challenges: timing is of the essence. *mBio* **11**: e00955-20. doi: 10.1128/mBio.00955-20

Sugimoto M, Oono Y, Kawahara Y, Gusev O, Maekawa M, Matsumoto T, Levinskikh M, Sychev V, Novikova N, Grigoriev A (2016) Gene expression of rice seeds surviving 13- and 20-month exposure to space environment. *Life Science Space Research* **11**: 10-17. https://doi.org/10.1016/j.lssr.2016.10.001

Suman S, Rodriguez OC, Winters TA, Fornace AJ Jr, Albanese C, Datta K (2013) Therapeutic and space radiation exposure of mouse brain causes impaired DNA repair response and premature senescence by chronic oxidant production. *Aging* **5**: 607-622. https://doi.org/10.18632/aging.100587

Surendran A, Lakshmanan M, Chee JY, Sulaiman AM, Thuoc DV, Sudesh K. (2020). Can polyhydroxyalkanoates be produced efficiently from waste plant and 68 animal oils? *Frontiers in Bioengineering and Biotechnology* **8**: 169. https://doi.org/10.3389/fbioe.2020.00169

Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic and biotic stress combinations. *New Phytology* **203**: 32-43. https://doi.org/10.1111/nph.12797

Suzuki T, Uruno A, Yumoto A, Taguchi K, Suzuki M, Harada N, Ryoke R, Naganuma E, Osanai N, Goto A, Suda H, Browne R, Otsuki A, Katsuoka F, Zorzi M, Yamazaki T, Saigusa D, Koshiba S, Nakamura T, Fukumoto S, Ikehata H, Nishikawa K, Suzuki N, Hirano I, Shimizu R, Oishi T, Motohashi H, Tsubouchi H, Okada R, Kudo, T, Shimomura M, Kensler TW, Mizuno H, Shirakawa M, Takahashi S, Shiba D, Yamamoto M (2020) Nrf2 contributes to the weight gain of mice during space travel. *Communications in Biology* **3**: 496. https://doi.org/10.1038/s42003-020-01227-2

Tepfer D, Leach S (2017) Survival and DNA damage in plant seeds exposed for 558 and 682 days outside the international space station. *Astrobiology* **17**: 205-215. https://doi.org/10.1089/ast.2015.1457

Tseng BP, Giedzinski E, Izadi A, Suarez T, Lan ML, Tran KK, Acharya MM, Nelson GA, Raber J, Parihar VK, Limoli CL (2014) Functional consequences of radiation-induced oxidative stress in cultured neural stem cells and the brain exposed to charged particle irradiation. *Antioxidants and Redox Signaling* **20**: 1410-1422. https://doi.org/10.1089/ars.2012.5134

Turker MS, Grygoryev D, Lasarev M, Ohlrich A, Rwatambuga FA, Johnson S, Dan C, Eckelmann B, Hryciw G, Mao JH, Snijders AM, Gauny S, Kronenberg A (2017) Simulated space radiation-induced mutants in the mouse kidney display widespread genomic change. *PLoS One* **12**: e0180412. https://doi.org/10.1371/journal.pone.0180412

Turroni S, Magnani M, KC P, Lesnik P, Vidal H, Heer M (2020) Gut microbiome and space travelers' health: state of the art and possible pro/prebiotic strategies for long-term space missions. *Frontiers in Physiology* **11**: 553929. Available: https://www.frontiersin.org/articles/10.3389/fphys.2020.553929

Uda Y, Spatz JM, Hussein A, Garcia JH, Lai F, Dedic C, Fulzele K, Dougherty S, Eberle M, Adamson C, Misener L, Gerstenfeld L, Divieti Pajevic P (2021) Global transcriptomic analysis of a murine osteocytic cell line subjected to spaceflight. *FASEB Journal* **35**: e21578. https://doi.org/10.1096/fj.202100059R

U.S.NRC § 20.1004 Units Of Radiation Dose. | NRC.gov , visited 11/4/2023.

Vasavada AR, Paige DA, Wood SE (1999). Near-surface temperatures on mercury and the moon and the stability of polar ice deposits. *Icarus* **141**: 179-193. https://doi.org/https://doi.org/10.1006/icar.1999.6175

Versari S, Longinotti G, Barenghi L, Maier JA, Bradamante S (2013) The challenging environment on board the International Space Station affects endothelial cell function by triggering oxidative stress through thioredoxin interacting protein overexpression: the ESA-SPHINX experiment. *FASEB Journal* **27**: 4466-4475. https://doi.org/10.1096/fj.13-229195

Villacampa A, Ciska M, Manzano A, Vandenbrink JP, Kiss JZ, Herranz R, Medina FJ (2021) From spaceflight to Mars g-levels: adaptive response of *A. thaliana* seedlings in a reduced gravity environment is enhanced by red-light photostimulation. *International Journal of Molecular Science* **22**: 899 https://doi.org/10.3390/ijms22020899

Vogel J, and Luisi BF (2011) Hfq and its constellation of RNA. *Nature Reviews Microbiology* **9**: 578-589. https://doi.org/10.1038/nrmicro2615

Volkmann D, Baluska F (2006) Gravity: one of the driving forces for evolution. *Protoplasma* **229**: 143-148. https://doi.org/10.1007/s00709-006-0200-4

Voorhies AA, Ott CM, Mehta S, Pierson DL, Crucian BE, Feiveson A, Oubre CM, Torralba M, Moncera K, Zhang Y, Zurek E, Lorenzi HA (2019). Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. *Science Report* **9**: 9911. https://doi.org/10.1038/s41598-019- 46303-8

Wamelink GWW, Frissel JY, Krijnen WH, Verwoert MR, Goedhart PW (2014). Can plants grow on Mars and the Moon: a growth experiment on Mars and Moon soil simulants. *PLoS One* **9**: e103138. https://doi.org/10.1371/journal.pone.0103138

Wang J, Zhang R, Zhang Y, Yang Y, Lin Y, Yan Y (2019) Developing a pyruvate-driven metabolic scenario for growth-coupled microbial production. *Metabolism Engineering* **55**: 191-200. https://doi.org/10.1016/j.ymben.2019.07.011

Wheeler RM, Fitzpatrick AH, Tibbitts TW (2019) Potatoes as a crop for space life support: effect of CO_2, irradiance, and photoperiod on leaf photosynthesis and stomatal conductance. *Frontiers in Plant Science* **10**: 1632. https://doi.org/10.3389/fpls.2019.01632

Whipps WL (1988) Mycoparatism and plant disease control. In: *Fungi in Biological Control Systems*, Ed. Burge MN, pp. 161-187, Manchester University Press.

Wielgoss S, Barrick JE, Tenaillon O, Cruveiller S, Chane-Woon-Ming B, Medigue C, Lenski RE, Schneider D (2011) Mutation rate inferred from synonymous substitutions in a long-term evolution experiment with *Escherichia coli*. *G3 Genes Genomes Genetics* **1**: 183-186. https://doi.org/10.1534/g3.111.000406

Wilson JW, Ott CM, Höner zu Bentrup K, Ramamurthy R, Quick L, Porwollik S, Cheng P, McClelland M, Tsaprailis G, Radabaugh T, Hunt A, Fernandez D, Richter E, Shah M, Kilcoyne M, Joshi L, Nelman-Gonzalez M, Hing S, Parra M, Dumars P, Norwood K, Bober R, Devich J, Ruggles A, Goulart C, Rupert M, Stodieck L, Stafford P, Catella L, Schurr MJ, Buchanan K, Morici L, McCracken J, Allen P, Baker-Coleman C, Hammond T, Vogel J, Nelson R, Pierson DL, Stefanyshyn-Piper HM, Nickerson CA (2007) Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proceedings of the National Academy of Science USA* **104**: 16299-16304. https://doi.org/10.1073/pnas.0707155104

Yang Y, Lin Y, Wang J, Wu Y, Zhang R, Cheng M, Shen X, Wang J, Chen Z, Li C, Yuan Q, Yan Y (2018) Sensor-regulator and RNAi based bifunctional dynamic control network for engineered microbial synthesis. *Nature Communications* **9**: 3043. https://doi.org/10.1038/s41467-018-05466-0

Zandalinas SI, Mittler R, Balfagon D, Arbona V, Gomez-Cadenas A (2018) Plant adaptations to the combination of drought and high temperatures. *Physiology of Plants* **162**: 2-12. https://doi.org/10.1111/ppl.12540

Zhang Y, Moreno-Villanueva M, Krieger S, Ramesh GT, Neelam S, Wu H (2017) Transcriptomics, NF-kappaB pathway, and their potential spaceflight-related health consequences. *International Journal of Molecular Science* **18**: 1166. https://doi.org/10.3390/ijms18061166

Zhang Y, Richards JT, Feiveson AH, Richards SE, Neelam S, Dreschel TW et al. (2022) Response of *Arabidopsis thaliana* and mizuna mustard seeds to simulated space radiation exposure. *Life* **12**: 144. https://doi.org/10.3390/life12020144

Zhou M, Callaham JB, Reyes M, Stasiak M, Riva A, Zupanska AK, Dixon MA, Paul AL, Ferl RJ (2017) Dissecting low atmospheric pressure stress: transcriptome responses to the components of hypobaria in *Arabidopsis*. *Frontiers in Plant Science* **8**: 528. https://doi.org/10.3389/fpls.2017.00528