Space Biology Beyond LEO Instrumentation & Science Series

Science Working Group 2022 Annual Report

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“In the depths of space, a new world beckons
A lonely, dusty plain where no life has trod
But humans, bold and daring
See a future, bright and glowing

With their ingenuity and knowledge
They will forge a new path
And use the tiniest of creatures
To create a sustainable life

Microbes, small and mighty
Will be their trusty allies
Converting rock and dust to oxygen
And sustenance for all to thrive

With this new technology
Humans will spread their wings
And journey to the moon and Mars
Where they will live and prosper

A new era has begun
Thanks to the power of the microbe
And humanity's unending drive
To explore and thrive in space.”

-Generated by AI from the prompt: Write a poem about humans using microbes to create a sustainable life support system enabling them to live and prosper on the moon and Mars

1. Introduction

1.1 Thriving in Deep Space: Biological research 2025-2035

Humans are poised to explore deep space: the realm of space beyond Earth's orbit. NASA will soon send humans back to the Moon with the Artemis program, and is developing programs to support crewed missions to Mars. Human exploration of such new environments demands fundamental research that can provide the knowledge necessary to ensure the safety of explorers and aid in the development of a sustainable presence in space. Accordingly, the Agency's Moon to Mars objectives[1] include three goals in the area of Human Biological Sciences (HBS-1, -2, -3), with the aim to "Advance understanding of how biology responds to the environments of the Moon, Mars, and deep space to advance fundamental knowledge, support safe, productive human space missions and reduce risks for future exploration." Advancing this understanding is a task that is both complex-- comprising diverse organisms, processes, and methods-- and difficult-- because the very aspects of deep space that we strive to understand are the aspects that make it hard to conduct research in that environment. This report of the Beyond LEO Instrumentation & Science Series Science Working Group (BLISS-SWG) represents input from a group of scientists from diverse disciplines within the space biology research and engineering community on the nature of the science and technology that can be used to achieve those aims.

1.2 Statement of Task

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 beyond LEO exploration. Specifically, the two aims of the BLISS-SWG, as stated in the charter, are:

1. To define the technical capabilities that should be sought in order to enable biological research beyond LEO; and
2. To report on the potential scientific gains of various experimental organisms in future research beyond LEO.

The BLISS-SWG published its first Annual Report in fall of 2021[2]; this report served the dual purpose of laying the groundwork for long-term planning and describing research priorities that could be feasible within the next two to five years. To define the science questions of interest, the members of the BLISS-SWG surveyed several strategic documents describing NASA's priorities for research in the space life sciences: the Decadal Survey in Biological and Physical Sciences 2011[3], the report of the previous Life Beyond Low Earth Orbit Science Working Group[4], the Space Biology Science Plan 2016-2025[5], and the Human Research Program Integrated Research Plan[6]. In summary, research Beyond LEO initially will be limited to predominantly autonomous experiments with organisms that can survive storage for 6-12 months.

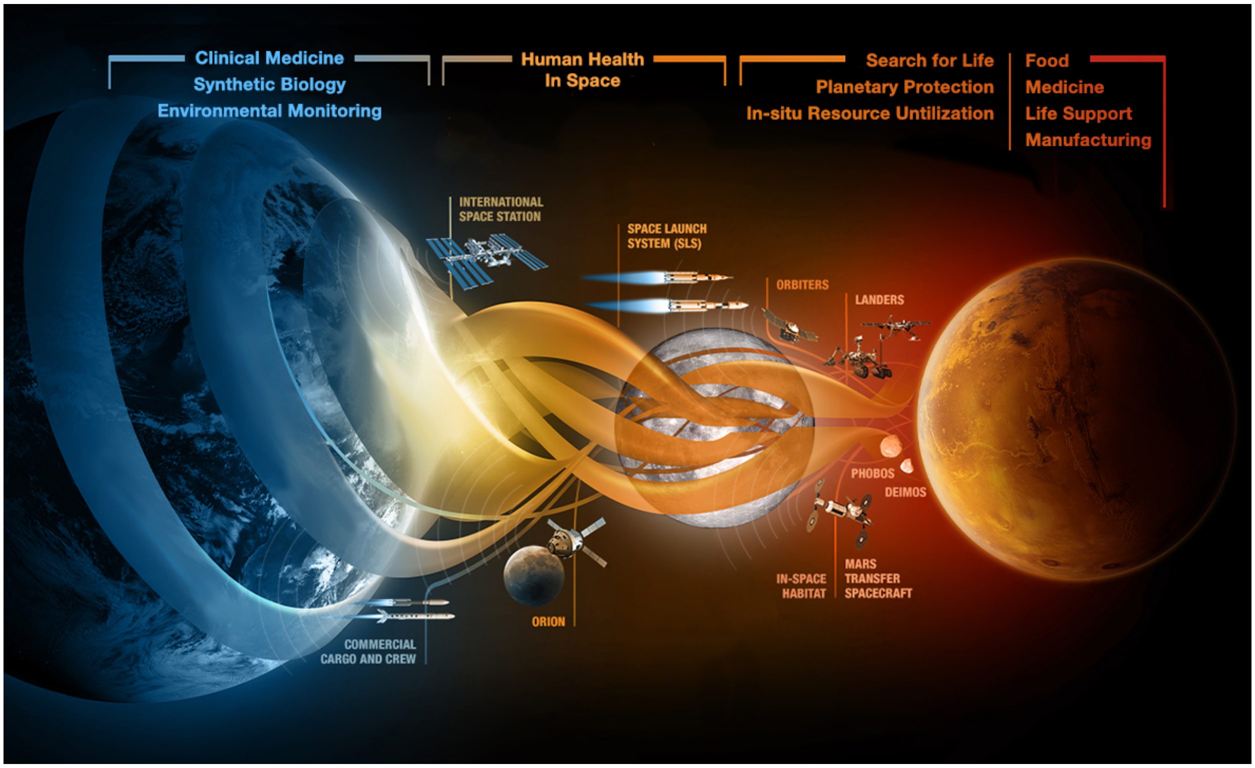

Figure 1: From Earth to LEO, and Moon to Mars, each environment is unique and offers opportunities to study how life responds to associated stressors. Vehicles and research platforms will be utilized to address areas of research to enable Thiving in Deep Space.

For 2022, the Space Biology Program asked the BLISS-SWG to furnish a report discussing science questions and capability for a longer timeline of 15 years. In addition, the BLISS-SWG was specifically asked to consider the role of Artificial Intelligence and Machine Learning (AI/ML) as tools in space research. The 2022 report therefore adopts much of the same structure as the 2023 report, addressing the same science questions but extending further into the future and considering recent advances of AI/ML as well as additional future needs. For example, we expect that within five years hardware could be developed to support complex cell systems, such as organ-on-a chip, and habitats deployed for the study of multicellular organisms, such as *C. elegans* and *tardigrades*. Within the next 15 years, experiments with astronaut involvement and sample return or real-time omics sample analysis in BLEO will be required.

To avoid duplication, the 2022 SWG report omits the in-depth background sections provided in the 2021 report on the lunar and deep space environments and the associated unique technological constraints; for that information, we refer readers to last year's report. However, the members of the 2022 SWG identified a few new areas for which they chose to provide comments in this year's report: building synergy across disciplines, developing technology to benefit humanity on Earth, and building and sustaining a robust space science community. It is recognized that NASA should address these programmatic areas to effectively address the research questions described in the science section of the report.

Artificial intelligence refers to artificial systems capable of intelligence, broadly defined as the ability to perceive, represent knowledge, learn, plan, and ultimately to reason or problem-solve.

Machine learning is a subset of AI that utilizes algorithms able to learn through data and/or experience. Building predictive models from biological data and using them alone or in combination with simulation and human expertise has created new research opportunities. In this annual report, we focus on AI/ML in part because it is long overdue as an enabling capability for BLEO activities and has come to the forefront due to recent advances in capabilities that are now approaching widespread usability or on the verge of widespread societal impact[7]. No longer simply behind the scenes, these technologies are enabling automated driving, language translation, artistic and hyper realistic imagery (e.g., DALL-E2) and videos (e.g., deepfakes), and a host of applications not yet visible to consumers. Translation of AI/ML technologies into the specific applications needed for BLEO activities will require progress across ML methods, software, and hardware that underpin AI as discussed below in each section.

2. Platforms and Types of Missions for 2025-2035

With the Artemis program underway, platforms are available, or will be shortly, for conducting research in the the BLEO environment. Research has already been conducted in deep space free flyers and inside the Artemis I Orion vehicle. From 2025-2035, research will be conducted on Commercial Lunar Payload Services (CLPS) landers, on crewed future Artemis missions inside the Orion vehicle and deployed secondary payloads, in future free flyer spacecraft, within the Lunar Gateway, and in planned human-rated landers and lunar habitats. Crew tending and communications will continue to be limited factors for research, and need to be address with hardware with improved autonomy and AI/ML capabilities.

Current & Future Platforms

1. CLPS Lunar Landers

The Lunar Explorer Instrument for space biology Applications (LEIA) will be the first biological payload to utilize a CLPS lander. CLPS offers a data and power interface, but no conditioned environment for Biology. LEIA and other autonomous and fully-contained, cubesat-style payloads are most appropriate secondary payloads on these CLPS landers. No sample return will be available, and while operations during lunar night may be possible in the future, missions are currently planned for a single lunar day only.

2. Internal Artemis missions

The Orion capsule was utilized for a biological mission on Artemis I. Another is planned for Artemis II. While there is some payload capacity for these missions, space, crew time, and sample return are limited. No conditioned stowage is currently available as well. Internal payloads do have the benefit of exposure to a controlled environment similar to ISS, provided by the on-board ECLSS system to maintain the crew environment. Passive hardware with limited requirement for crew tending are most appropriate, though some power, data, and comm may be available in the future.

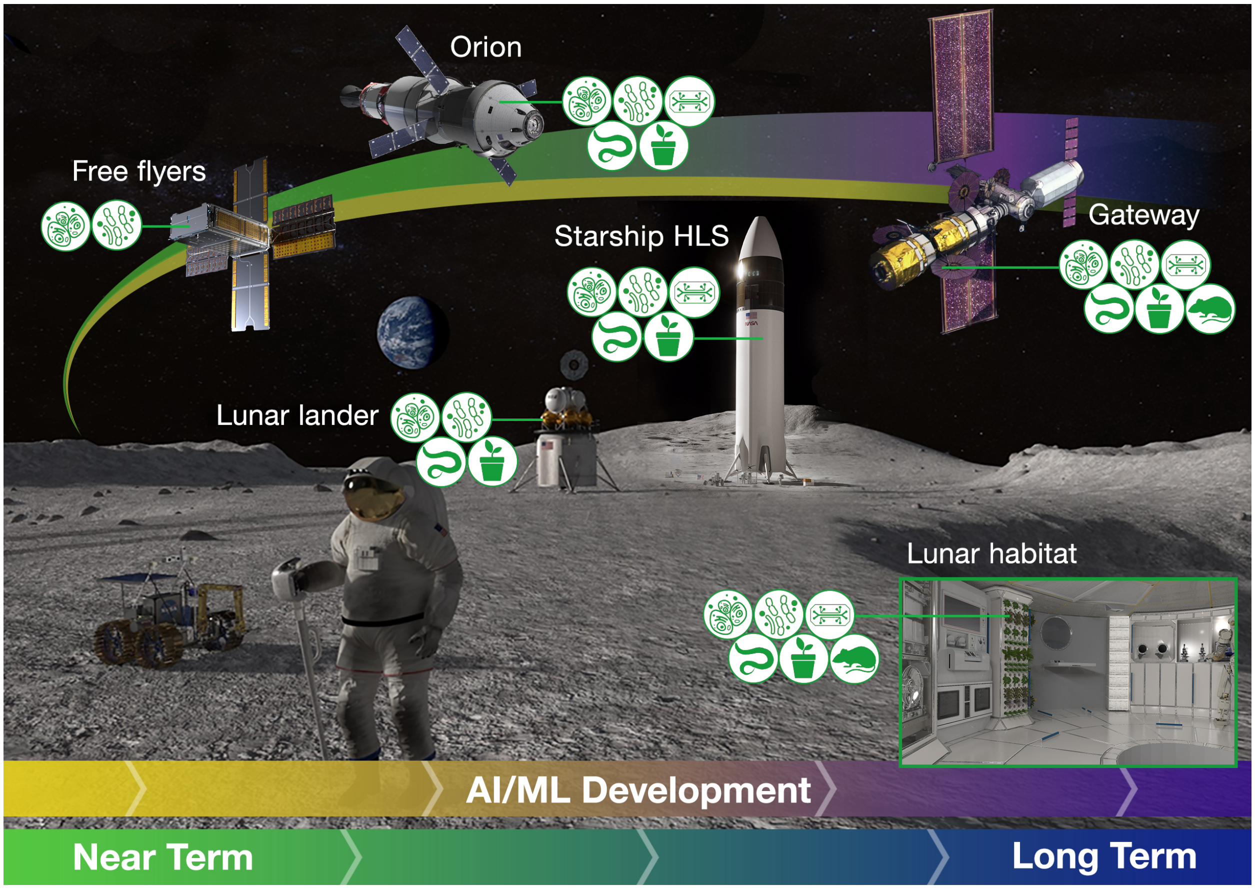

Figure 2: BLEO platforms will offer opportunities for biological payloads with increasing complexity.

3. Free Flyer missions

Artemis launches on SLS, as well as other launch vehicles, will have opportunities for secondary payloads to be deployed BLEO. This opportunity was utilized for several CubeSat missions on Artemis I, including BioSentinel, the first biological CubeSat mission BLEO. Free flyers offer opportunities for experiments in microgravity and unique radiation environments, depending on the orbit in which they are placed. LEO CubeSats have been fully autonomous, sealed, and have limited power and data transmission. This will continue to be the case in BLEO, with potentially even more limited data transmission though technology is improving. Since CubeSats rely on their own power and autonomous control, they are appropriate for hosting longer duration missions, anywhere from weeks to years.

4. Gateway

The Lunar Gateway is expected to be established in the mid to late 2020s through a series of launches resulting in the assembly of what could be the first space station beyond LEO and the first space station in a lunar orbit. Led by NASA, the Gateway, like the International Space Station, is expected to involve a wide range of international partners including the European

Space Agency, Canadian Space Agency, Japan Aerospace Exploration Agency, and a myriad of commercial partners.

While ECLSS may be available for Biology experiments, it may not be available while crew is absent. Sample return and crew tending will likely be limited if available. Experiments utilizing hardware from ISS, or similar capabilities, may have limited utility due to their heavy reliance on crew manipulation. Next generation hardware must include improved autonomy and AI/ML, even for payloads internal to Gateway.

There will likely also be opportunities for externally mounted payloads as well as small satellites deployed from Gateway. Like LEO biological cubesats flown to date, these will need to be fully autonomous and will likely have limited data transmission capabilities, but will be able to expose biology to a unique environment of microgravity combined with increased radiation.

5. Human-rated landers/Habitats

The Artemis program will leverage the Lunar Gateway and other lunar infrastructure to support a new set of human lunar landings and exploration campaigns in the late 2020s to early 2030s. In August 2022, NASA identified candidate regions for Artemis lunar landings [ref], which would be accessible via the "combined capabilities of the Space Launch System (SLS) rocket, the Orion spacecraft, and the SpaceX-provided Starship human landing system (HLS)." HLS is expected to have a full ECLSS system, which may be available for biological payloads. It may also have power and data available to hosted payloads, with a potential ability for payloads to be placed on the lunar surface.

3. Technology and data requirements

Living organisms will experience stressful conditions Beyond LEO. This will include exposure to high energy radiation, microgravity or partial gravity, and non-terrestrial chemical environments. Organisms used in experiments will require the capability to survive long-duration spaceflight, which will be possible due to advances in technology and/ or due to the biology of the organisms chosen for study. An explanation of each of these “stressors” and potential organism choices was provided in the 2021 report. This report will focus on experiments and technology required to study the impacts and mitigation strategies for BLEO stressor exposure, during the upcoming decade (2025-2035). Here, we provide a brief introduction of the technology required to perform experiments and analyze data in the unique constraints of BLEO, including experimentation and data analysis that involves limited astronaut involvement or that is entirely remote and autonomous. An understanding of how organisms adapt or fail to adapt to altered gravity, space radiation, and other stressors is critical for assessing short and long-term risks to astronauts and ultimately assessing the potential for human habitation in space, on the moon, or on other worlds, e.g., Mars.

3.1 Science Requirements, Current State of the Art, & Trends

Missions beyond LEO will expose organisms to microgravity and space radiation which can profoundly impact molecular signaling, biological processes and physiology. Results from space flown organisms as well ground-based organismal models suggest that exposure to the deep space environment will lead to alterations in metabolism, cell division, and cellular stress responses among others. Such findings can inform on the critical science requirements for the development of technologies that will further enable research in LEO.

3.2 Technology to support humans thriving in deep space

Experimentation approaches will need to adapt to the unique and diverse constraints of BLEO missions and environments. We highlight here several key experimentation trends in biotechnology for which substantial advancements are in progress here on Earth, and which we believe will be useful for BLEO biological experiments: synthetic biology, 3D printing, organoids, and artificial intelligence.

Much has already been written about the potential for synthetic biology to aid adaptation of organisms to extreme outer space environments[8,9]. Synthetically altered microbes have the potential to enhance the utilization of planetary resources, support human health and medicine, and even terraform planetary landscapes. The ability to generate organisms with specific capabilities on-demand may be possible through synthetic biological approaches. However, significant research is needed to investigate the possibilities and limitations of synthetic biology in space. New experimentation approaches should focus on expanding synthetic biology capabilities and testing them in BLEO environments.

Similarly, 3D printing holds promise in several domains to support BLEO exploration. For example, 3D printing of biomaterials has been proposed for facilitating regenerative medicine in space (e.g. on-demand organ and tissue printing using human cellular material)[10]. As this is far from reality, BLEO biological experimentation should focus on testing this technology in various environments. Additionally, 3D printing also holds a role in facilitating *in-situ* experimental design. If BLEO missions or habitats have 3D printing capabilities, lab equipment such as microfluidic chips can be manufactured in real-time in space[11]. Additive manufacturing of biological materials would remove the need to ship extra experimental components, and would open the possibility to design new experiments in space based on the output of previous experiments.

Many of the most informative biological experiments performed in space have involved live model organisms, such as rats, mice, worms, fruit flies, cell cultures and microbes. However, the duration of proposed BLEO missions may limit the use of traditional model organisms for experimentation. In some cases, technology should be developed to enhance our abilities to maintain model organism research in BLEO settings. Cell-based platforms, such as organoids or organ-on-a-chip, could also be leveraged, since these models can be seeded *in-situ* from cryopreserved cells stored for months or years. These platforms will also provide useful *in-situ* translational capabilities for medical purposes.

Due to the constraints of BLEO missions, much biological experimentation may need to take place with limited or no human crew involvement. Therefore, technology development should focus on automated experimental techniques, some of which will benefit from AI-powered direction. Earth-based labs have already automated many laboratory processes including biomarker quantification, biochemical and cell-based assays[12], aspects of library preparation[13], and cell culture[14]. These individually automated lab protocols can be incorporated into closed-loop "self-driving labs" which can be programmed to complete a full experiment autonomously. On Earth, self-driving lab technology is most mature in the field of chemistry[15–17]. These terrestrial successes should be used as templates to expand for space bioscience experimentation in BLEO.

Data analysis may need to be performed in BLEO, as returning omics datasets to Earth may be limited by bandwidth availability. Trained AI machines can also learn from experimental output and data analysis to design a new experiment, in a process known as generative experimental[18] design. Another possibility involves use of free-flyers on lunar cycling orbits[19], which would have expanded communication opportunities due to regular Earth flybys. These flybys also create the possibility for sample return.

3.3 Data Analysis Requirements

Autonomous experiments or "self-driving labs" will require *in-situ* data processing capabilities, with the choice to either communicate data down to Earth or analyze data *in-situ* to inform future experiments. The tradeoff in resources will need to be considered for mission design. A mission experiment that downlinks raw data eliminates the requirement for on-board analysis computing processors, but communication opportunities will likely be limited. On the other hand, a mission experiment that concludes with an automated data processing and analysis pipeline requires considerable on-board storage and analysis capabilities, but when tied into a self-driving lab interface could be used to inform future experiments.

The broader market for AI chips is growing rapidly, but translation of these nascent technologies into space compatible solutions is not a given. *One promising approach to accelerating in situ* data processing capabilities is neuromorphic computing. Neuromorphic chips significantly reduce the computational requirements for machine learning neural network training and inferencing, by representing models as analog circuits[20,21]. Low power requirements and radiation resilience make neuromorphic computing a promising avenue for *in-situ* data processing and analysis for BLEO experiments[22,23]. A current SBIR project is making progress on this technological requirement[24].

Automated remote data analysis can leverage several terrestrial AI/ML applications, but additional research and innovation is needed to fully realize these opportunities. Transfer learning is an ML technique in which a generalized model is trained on a data type with a large amount of data, then refined for a prediction task on a smaller, more specific dataset. Transfer learning holds great promise for enhancing our ability to analyze data generated *in-situ* in BLEO experiments. A generalized model can be trained on large Earth-based biological datasets (e.g. huge amounts of microbial genomic sequencing data), then refined to classify or identify outputs from experiments (e.g. functional sequence annotation in BLEO microbial sequencing analysis). It would be possible to develop a publicly available pre-trained "zoo" of models, one for each general biological task, then deploy each model for real-time prediction or classification in *in-situ* mission experimental settings. Since BLEO experimental data are likely to be unexpectedly different from the Earth-based training datasets, we can also take advantage of adaptive model architectures that are flexible to perform inference on datasets that are out-of-distribution compared to the original training data[25].

Federated learning is another ML technique with great promise for accelerating BLEO experimentation and data analysis. Federated learning trains a model using datasets that are physically separated (e.g. at two different hospitals, or on Earth and in space)[26]. The advantage of this approach is that combining data for training is not necessary to build a model that incorporates the predictive power of multiple available datasets. This means that it is not necessarily required to downlink full datasets to Earth in order to gain insights; rather, only the model weights are downlinked for training, reducing needed resources.

Beyond data processing, AI/ML methods are useful for any space instrumentation where automation is required and detecting faults is useful. Many AI/ML methods have been developed specifically for this application. However, further development is necessary to enable fault detection using space-compatible electronics and for integration of these types of systems into BLEO payloads. Integrating deep learning into scientific practice presents a complex challenge that must be addressed to enable human-in-the-loop and AI-driven design, discovery, and evaluation. Autonomous robotic control has the potential to take the outputs of machine learning models and conduct experiments on synthesis and testing lines, which can automate scientific workflows and optimize the operation of instruments.

Usually, however, the factors necessary to understand a phenomenon such as a biological phenotype or comprehensive response to an external stressor, cannot be captured by a single data type. Modeling complex systems requires using measurements that describe the same entities from different perspectives, scales, or modalities (e.g., images, sound readings, natural language sequences, chemical reactions, multi-omic signatures, chemical and genetic perturbations, behavioral response). Multimodal learning is concerned with studying how data generated by diverse distributions can be fused together for better performance[27]. Combining multiple data types can compensate for missing or unreliable information in any single data type, and multiple sources of evidence pointing to the same outcome are less likely to be false positives. Combining different types of data can create bridges between the molecular and organism levels[28] for modeling physical, chemical, or biological phenomena on a large scale. Further, as neural network models require large data for robust training, grounding models in knowledge[29] will be increasingly important to expand the use of machine learning models to settings where datasets are small or sparsely labeled. Scientific knowledge, such as rotational equivariance in molecules[30], equality constraints in mathematics[31], biological structure[32], and multi-scale organization of complex systems[33,34] can be incorporated into model to produce actionable outputs that can be meaningfully interpreted.

Standard supervised learning assumes that all test labels appear in the training dataset, often called closed-world learning. However, scientific discovery requires both identifying previously characterized classes of objects as well as recognizing novel classes that do not appear in any dataset collected so far. This setting, known as open-world learning, requires the model to identify novel classes not seen during training of the machine learning model[35]. However, many challenges remain unsolved towards achieving these goals, including advancing algorithms to identify novel classes, dealing with continual streams of high-dimensional multivariate data whose class distributions vary with time, and leveraging prior knowledge to facilitate characterization of novel classes[36,37].

Finally, AI/ML methods are in general less sensitive to distributional shifts between datasets or data types, compared to traditional statistical or bioinformatic methods. These methods are therefore valuable for analyzing data from different experiments or from multiple data modalities (e.g. multi-omics data or omics and phenotypic data together). Thus, regardless of whether analysis is taking place *in-situ* or on the ground, it will be important to invest in better understanding the types of AI/ML methods that are particularly useful for BLEO data analysis, and where AI/ML can most pertinently advance research goals.

4. LIFE Beyond LEO: Questions of importance for 2025-2035

Section A. How does the BLEO environment impact cellular functions?

Critical questions in this research area

In the 2021 report, important areas relevant to prokaryote, eukaryote, single and multicellular organisms living and surviving the BLEO environment were discussed. Three key questions that are still relevant for the 2025-2035 time period but were discussed extensively in the 2021 report include:

1. How does exposure to Beyond LEO increase oxidative stress in cells?
2. How does living beyond LEO alter DNA damage, DNA repair and DNA mutations?
3. What changes occur to the transcriptome of cells due to the BLEO environment?

Advances in technology will allow more in-depth studies to be performed. Below other critical questions are discussed and updated.

4. How does exposure to beyond LEO alter DNA structure?

Higher-order DNA structure, which includes nucleoids, nucleosomes, chromosomes and the 3D global genome structure is influenced by DNA binding proteins[2]. Prokaryote[38] and eukaryote[39] genomes are organized into chromosomal interaction domains (CIDs) and topologically-associated domains (TADs), respectively. These domains and the folding and positioning of DNA/ chromosomes in specific compartments or locations influences gene regulation[40]. In prokaryotes, actively transcribed genes are associated with the boundaries of the CIDs, and in eukaryotes, the TADs can alter the proximity of enhancers and gene promoters. Changes to the 3D genome structure have also been linked to human disease[41].

Nuclear elongation and changes in nuclear structure, seen by Hoechst dye staining, have been detected in osteoblasts exposed to microgravity[42]. 3D genome structural changes have also been detected with *Vibrio natriegens*[43] and *Klebsilla pneumoniae* grown under simulated microgravity[44] using genome-wide high resolution chromosome conformation capture (Hi-C). This latter experiment determined the chromosome structure was looser and DNA methylation decreased under simulated microgravity in *Klebsilla pneumoniae*.

Methylation of the DNA is known to be associated with altered gene expression, but it can also increase chromatin condensation and decrease DNA flexibility[45]. Since methylation patterns alter higher-order DNA structure, and DNA structure alters DNA damage-induction[46] and DNA repair[47,48], the role of DNA methylation and the DNA structure should be considered with respect to a cell's ability to function normally in the BLEO environment.

5. How does exposure to beyond LEO alter the epigenome?

The epigenome consists of modifications to the DNA and the eukaryotic histones: the DNA and histones can be methylated, while other epigenetic histone modifications include acetylation, phosphorylation, and ubiquitinylation. Specific modifications relating to DNA repair were discussed in the 2021 report and DNA methylation has been discussed above relating to DNA structure. DNA methylation is an epigenetic modification linked to transcription regulation and so data from experiments should be analyzed in combination with transcriptome data. DNA methylation is important across species and is particularly relevant for plant development and physiology[49]. Since plants are key to humans living beyond LEO, the DNA methylome should be investigated using plant cells or tissue from plants grown in BLEO.

Detection of base modifications including methylation can be done in situ using single molecule nanopore sequencing, previously performed on the International Space Station[50] and at lunar and Mars gravity during parabolic flight[51]. The basecalling process has now advanced to include detection of 5mC and 5hmC as standard, while many additional base modifications can also be detected using additional analyses. While nanopore sequencing itself has been done in space, *in situ* methylation analysis has not yet been done to our knowledge; in addition, additional work is required to automate the process of nanopore sequencing, which currently requires human operators. Current efforts to automate nanopore sequencing are underway[52,53], but numerous challenges remain including reagent and flow cell stabilization, complex sample preparation procedures, and relatively high DNA input concentration requirements.

Over the past few years, lactylation has been identified as a new histone modification that regulates transcription[54] and provides a link between transcription regulation and metabolism. Lactate, a product of glycolysis, is added at lysine residues on H2A, H2B, H3 and H4 by p300 and can be removed by HDAC1/3[55]. The modification has been identified in a number of species and is linked with disease states such as inflammation, tumorigenesis, and neuroinflammation in Alzheimer's disease[55]. The link with cancer, neurodegeneration and glycolysis means that histone lactylation should be studied in cells living in the BLEO environment.

6. How does exposure to Beyond LEO alter cellular metabolism?

As mentioned last year, many studies have used transcriptome data to identify potential changes in metabolism during spaceflight, high LET irradiation or simulated microgravity. These pathways include oxidative phosphorylation, lipid metabolism, carbohydrate metabolism, and anaerobic metabolism. Many of the metabolic changes are likely connected and could result in disease. It is hoped that by 2035, metabolomics could be studied by using probes to measure actual metabolites.

Mitochondrial dysfunction has been documented in multiple samples from spaceflight samples of humans and mice[56]. A reduction in transcription of nuclear encoded mitochondrial oxidative phosphorylation genes and evidence of oxidative stress during spaceflight suggests that ATP synthesis via oxidative phosphorylation may be impaired or inefficient. Under these conditions, ATP synthesis can occur by cytosolic glycolysis with lactate as the product[57]. Lactate levels in cells and the circulation is intertwined with glucose metabolism and oxidative phosphorylation, and since it can now also affect the epigenome, lactate is an important metabolite to reveal the energy status of cells, muscles and organs during spaceflight and on the lunar surface.

From blood samples collected during spaceflight, astronauts have increased total cholesterol and low-density lipoprotein (LDL), and decreased high-density lipoprotein[56], which supports changes in lipid metabolism. The major organ involved in lipid metabolism is the liver; fatty acids are used as fuel and triglycerides are exported as LDL. Ground-based HZE particle irradiation of mice[58] increased lipid in the liver, and simulated microgravity head-down bed rest of rhesus macaques[59] also showed an increased trend of lipid accumulation and liver inflammation.

Importantly, mice flown in space for 13 to 42 days also accumulate lipid droplets in their livers[60,61]. These alterations in lipid metabolism have been linked to the peroxisome proliferator-activated receptor alpha[60] (PPAR$\boldsymbol{\alpha}$), and PPAR$\boldsymbol{\alpha}$ is a transcription factor with target genes involved in glucose and lipid metabolism[62]. A major concern for lipid accumulation in the liver is non-alcoholic fatty liver disease (NAFLD), of which there are two types: non-alcoholic fatty liver (NAFL), where fat accumulates but there is no inflammation or injury, and non-alcoholic steatohepatitis (NASH), where fat accumulation, inflammation and injury occurs. Insulin resistance and hypertension are implicated in NAFLD[63] as is muscle wasting[64]; another alteration found in spaceflight. 75% of patients with type II diabetes have NAFLD, and metabolic syndrome is a high risk for progression to NASH. The disease is linked with a perturbation in metabolism of carbohydrates and fatty acids, resulting in a build-up of toxic lipids, stress and liver injury, fibrogenesis and progression to cirrhosis and potentially hepatocellular carcinoma[63]. Since spaceflight is associated with diabetogenesis[65] and lipid accumulation in the liver, astronauts on long missions could be at risk for NASH. NASH is also a risk factor for cardiovascular disease. Alterations to liver lipid metabolism and the increased risk of diabetes due to spaceflight, increases the priority of research into metabolic changes due to partial gravity and exposure to GCR and SPE.

Feasible research beyond LEO in the years 2025-2035

During this time period, it is possible that some sample return will be possible from the lunar surface or from Gateway and this will allow for more complex experimental techniques to be performed if autonomous techniques for sample processing and omics experiments are still being developed.

1. How does exposure to BLEO alter DNA repair and DNA mutations?

With the advancement of techniques to perform DNA sequencing autonomously, it will be possible to isolate DNA from a variety of cells on the lunar surface or in deep space and perform genome wide sequencing to identify mutations. The types of mutations can reveal information about DNA repair, as specific types of DNA lesions give rise to specific types of mutations[66]. Mutation frequency and mutation types could be determined in replicating and non-replicating single and multicellular organisms, as well as cells in culture. Mutations can drive evolution, or in the case of humans could lead to disease. It is likely that mutation frequency will be higher in the BLEO environment due to exposure to radiation. Altered gravity may contribute to a higher mutation frequency if DNA structure or DNA repair is altered. Mutation hot spots and fragile sites in human chromosomes have been identified and are linked with genetic diseases and oncogenesis[67–70]. Mutations in mitochondrial DNA are associated with coronary heart disease[71] and radiation exposure can increase cardiovascular disease[72,73].

Genome wide sequencing techniques, such as Linear Amplification-Mediated High-Throughput Genome-wide Translocation Sequencing (LAM-HTGTS), are available to detect the sites of DSBs and have been used to detect breaks induced by ionizing radiation[74]. Specific-types of sequencing such as OG-seq, click-code-seq, AP-seq and snAP-seq[66] have also been developed to detect oxidative DNA damage in the genome. Adapting these techniques to be autonomous will enhance our knowledge about DNA damage induction in cells in deep space. These types of techniques could be used to develop biodosimeters, as well as inform about the performance of radiation countermeasures and cellular DNA repair.

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

By 2025-2035, autonomous RNA isolation from cells and autonomous RNA-seq should be possible. Last year, experiments were described that were more a targeted approach to determine whether expression from single genes are altered by BLEO. With the ability to perform RNA-seq in the 2025-2035 time period, the transcriptome of cells can be interrogated, allowing changes in gene expression due to the effects of partial g and deep space radiation to be revealed. This will be very powerful and will help to understand how cells adapt to survive the stressors of deep space travel.

3. How does exposure to BLEO alter DNA structure and the epigenome?

Advances in microfluidics, autonomous PCR and DNA sequencing will open up possibilities of interrogating the genome's 3D structure. Adaptations of techniques such as Hi-C[39], could provide information about genome-wide DNA structure in prokaryote and eukaryote cells beyond LEO. More limited knowledge could be obtained about the positioning of specific target sequences within the nucleus of adherent eukaryotic cells using CRISPR-based live cell imaging[39]. Cells express a mutant Cas9 fused to a fluorescent protein that binds but does not cut the DNA. The mutant Cas9 is targeted to a DNA sequence using a guided RNA. Multiple sequences can be located using different fluorescent molecules and guided RNAs, and cells are imaged live. The advantage of this type of experiment is the ability to document changes over time.

Since DNA methylation can alter DNA structure and transcription, a combination of Hi-C sequencing, RNAseq and sequencing to detect DNA methylation, will enhance our understanding of how BLEO changes to DNA structure also alter gene expression. Current DNA methylation detection uses bisulfite sequencing, single molecule real time sequencing (SMRT) and Oxford nanopore technologies (ONT)[75]. SMRT and ONT have the advantage that they do not use harsh treatments of the DNA. ONT, similar to whole genome bisulfite sequencing (WGBS), can be used to analyze DNA from any species, where SMRT is usually used for bacteria. The disadvantage of ONT and SMRT is the need for a large input of DNA compared to WGBS. While these technologies currently require sample return, it is possible that they may be implemented in autonomous payloads in the future (e.g., Charoenboonvivat et al. 2022[52]).

As described in the 2021 report, methylated DNA and heterochromatin can be identified by live cell imaging using a fluorescent-tagged methyl CpG binding protein 2 (MeCP2)[76] and this could be combined with the CRISPR-live cell imaging to identify DNA sequences near methylated DNA regions. Nanopore-based identification of methylation is described above.

In the 2021 report, mintbodies (fluorescent-tagged antibodies produced in the cell) were discussed relating to imaging of H3 lysine 9 acetylation and H4 lysine 20 monomethylation using fluorescence microscopy[77,78]. The repertoire of fluorescent-tagged proteins to mark epigenetic modifications has been expanded to include split fluorescent molecules. One protein has a binding domain fused to part of the fluorescent molecule and another protein has a substrate domain fused to the other part of the fluorescent molecule. When they bind at the same site on the modification the two parts of the fluorescent molecule come together and the chromatin modification is marked by fluorescence. These systems are called BiFC sensors and have been developed for H3K27 methylation, H3K9 methylation, H4K8 acetylation and H3K9 acetylation[79]. Hopefully in the next 10-15 years similar tools will be developed for the histone lysine lactylation modifications. With the use of fluorescence microscopy, chromatin modification changes could be followed in live cells on the Moon or Gateway.

ChIP-seq could also be used to identify DNA regions with specific chromatin modifications if sample return is possible or if sample processing has progressed to perform this technique[80]. Droplet microfluidics for single cell ChIP-seq has already been developed[81]. ChIP-seq will allow a number of different histone modifications to be studied, including lactylation.

4. How does exposure to Beyond LEO alter cellular metabolism?

Recent analysis of omics data from mammalian cells and tissues flown in space has determined that lipid pathways are dysregulated and differences are seen in animal tissues, but not cell lines in culture[56]. This is likely explained by the interaction of the metabolic activity of different organs in animals and humans. Cell culture models or Organ-on-a Chip could be used to simulate metabolic disturbed states by altering the medium surrounding the cells; allowing the physiological state found in astronauts/ a disease state to be studied autonomously in cells in culture in space. It is likely that cell culture studies will occur on the Moon or in deep space prior to animal studies due to the need for astronaut involvement in animal experiments. Humans with diabetes have elevated blood glucose, and humans with NAFLD have elevated blood levels of fatty acid due to the breakdown of triglycerides in adipose tissue[63]. For diabetes, the glucose level in the cell culture medium could be increased, and for NAFLD, fatty acids could be added to the medium. The combination of the space environment and the disease model could be assessed. The effects on cell viability of experiments on Gateway or the lunar surface could be autonomously monitored using dyes such as Alamar Blue and compared to normal gravity either by the same experiment being performed on Earth or by the use of centrifuges on Gateway. Loss of oxidative phosphorylation and increased use of cytosolic glycolysis could be monitored by cellular levels of lactate. Cells can be modified to express the genetically encoded metabolic indicator for lactate (GEM-IL)[82], which changes in fluorescence when bound to lactate. A fluorescent probe has recently been reported that can detect lipid droplets, mitochondria and lysosomes[83] and is able to detect mitochondrial damage during apoptosis. There is also a lipid droplet fluorescent probe (lip-YB) that can detect NAFLD in mice[84]. Metabolic changes in cells can therefore be monitored by fluorescence microscopy. It is hoped that in the next decade more probes will be developed to detect different metabolites and metabolic changes during exposure to the lunar and deep space environment.

Model organisms relevant to science goals in this area

By 2025-2035, it is hoped that technology will be advanced to support mammalian/ plant cell culture in deep space, on the Moon or Mars. Examples of important mammalian cell types/ cultures needed to address the questions in this section will include, but not be limited to, muscle, bone, liver, endothelial cells, cardiomyocytes and neuronal cells. The use of differentiated versus replicating cultures should be considered as autonomous maintenance of a replicating culture requires more advanced technology. The majority of cell types in a human/ animal/ plant also exist in a differentiated state and hence, experiments using differentiated cells will be highly relevant. Organoids and organ-on-a chip will also be important to study cells with 3D structure or with multiple cell types interacting as found in humans and animals. Even though prior to 2025 the focus will be on single and multicellular organisms for experiments in BLEO, experiments in 2025-2035 will still require single and multicellular organisms as technology will have advanced and allow more complex and omics-type experiments. Understanding prokaryotes and simple eukaryotes will still be important to life Beyond LEO. With astronaut involvement on the Moon or in orbit around the Moon, it should also be possible to perform experiments on circulating cells from crew members. This in-situ analysis of changes to circulating cells will provide information about changes in BLEO rather than the stresses of returning to Earth, and will prevent the need for storage of frozen samples and the potential loss of samples during the mission or return to Earth.

Technology needs for this science area, 2025-2035

- Key areas of development for AI/ML needed in this science area are: AI-assisted robotics for automated experiments; integration of pre-trained AI/ML models for real-time inference/prediction to reduce sample return (e.g. object detection via fluorescent microscope); AI/ML based analysis of integrated multi-omics data
- Development of standardized bioinformatic analysis methods to integrate multi-omics data (e.g. RNASeq, ChIPSeq and metabolomics): for example Multi-Omics Factor Analysis (MOFA)[85].
- Cell culture capabilities -robotics to maintain cells, differentiated cells - less culture time, organ-on-a chip, organoids
- Autonomous RNA and DNA isolation
- Autonomous RNA and DNA sequencing
- PCR
- Fluorescence microscopy
- Fluorescence measurements similar to a plate reader

Section B. How does the Beyond LEO environment impact microbes and microbial communities?

Microbes are essential for all life on Earth as they are critical for the effective cycling of nutrients and functioning of ecosystems. As humans continue to explore beyond LEO, it will be imperative to understand how the deep space environment impacts the health and function of microbes.

Critical questions in this research area

1. What are the impacts of the deep space environment on bacterial physiology?

Microbes are essential for the health and function of ecosystems, thus it is critical to understand how the effects of the deep space environment impacts on microbe physiology. Specific questions that need to be addressed include:

a. How does partial gravity, deep space radiation and the synergistic effects of these two environmental hazards impact microbes?
b. Do the radiation and gravity conditions of the Lunar surface impact the physiology of microbes?
c. How variable are these physiological responses across microbial taxa and within microbial communities?
d. Does the microbial response to these deep space hazards change over time? In other words, do some microbial taxa adapt to and/or evolve resistance to these unique environmental stressors?

2. What is the potential for pathogens to arise under deep space environmental conditions?

Pathogenic and mutualistic microbes share many common communication mechanisms often referred to as Microbe Associated Molecular Patterns (MAMPs)[86–88], yet it is not always known what triggers the transition from health to disease. With the unique environmental stressors and hazards of the deep space environment, several questions that need to be addressed include:

a. Does the expression of MAMPs change under different environmental conditions, such as deep space and Lunar hazards?
b. Do host responses to these microbial taxa and communities change under deep space environmental conditions?

3. What is the potential for microbial-based biotechnologies under deep space environmental conditions?

Microbes will serve as a valuable resource for the synthesis of medicines, foods, and materials for future space exploration. Several areas that need to be addressed include:

a. Can automation and artificial intelligence be used to ensure stability or health of the spacecraft microbiome?
b. Can the spacecraft microbiome be primed to promote human health? In other words can spacecraft-associated microbes be used as eukaryotic host probiotics?

c. Can microbial communities be used to remediate Lunar soils to improve or increase food production?
d. Can microbial communities grown under deep space environmental conditions be used as resources for novel and in-flight pharmaceuticals and products?

Feasible research beyond LEO in the years 2025-2035

All of the critical research areas could be conducted in the next 10 years given enough access to platforms beyond LEO. For example, genetically manipulable organisms, such as *E. coli, B. subtilis,* and *S. cerevisiae* can be engineered to express key products and then tested to see how the physiology changes under microgravity or radiation environments.

Additionally, microbial communities, such as biofilms and nutrient-cycling microbial mats are amenable to autonomous monitoring with minimal human intervention to assess how the interactions between microbes and spacecraft materials are impacted in deep space environment conditions.

Model organisms relevant to science goals in this area

Although single microbial species will be valuable for the production of key drugs and products during spaceflight, most microbes in spacecraft and life support systems will exist as communities of interacting taxa. Therefore, it will be important to use a range of microbial model systems to more fully understand the impact of the deep space environment on microbial life.

Technology needs for this science area, 2025-2035

- Although most of the technologies to grow microbial communities and taxa in space currently exist, improved technologies for the regular and autonomous monitoring of taxa would be needed.

Section C. How does the BLEO environment impact the physiology of multi-cellular animals?

Multi-cellular animals are essential means to study physiological changes in specific tissues as well as organism-level phenotypes . As humans continue to explore beyond LEO, it will be imperative to understand how the deep space environment impacts the health and function of animals.

Critical questions in this research area

1. How does the space environment impact the following aging and lifespan factors and mechanisms? Can any of these changes lead to disease and/or frailty?

Microgravity and ionizing radiation are profound stressors to the organism. Bone loss[89,90], immune dysregulation[91,92], neurovestibular changes[93], cardiovascular deficits[94–96] among others have been reported in space-faring humans. These changes resemble aspects of aging on Earth.

- Oxidative stress responses
- Telomere dynamics
- Stem cell dysfunction
- Autophagy
- Immune response
- Cognition

2. How does the space environment impact circadian signaling? What are the consequences of any such alterations to organisms, tissues and cell populations?

The rotation of the Earth along its axis generates a defined daily light-dark cycle that affects the activities, behavior, cellular signaling and physiology of organisms. Circadian rhythms are found in numerous molecular, cellular and organ-system processes as well as behavior which can be entrained by light-dark cycles[97]. Perturbations in the timing of light exposure and the presence of conflicting cues such as shift work and irregular feeding times may disrupt these rhythmic processes. Some of the effects of light-cycle perturbations and shift work include altered sleep-wake cycles and poor sleep quality[98,99]and cardiovascular deficits[100] among others.

Space habitation in low Earth orbit is characterized by altered light:dark cycles. ISS crew experience sunrise to sunrise intervals spanning 1.5 hours. Further, the spaceflight experience is characterized by periods of intense activity (e.g. vehicular maneuvers and EVA's) that resemble shift work. Future deep space missions, lunar and planetary habitation (e.g. Mars) also will expose mission crew to altered light:dark cycles and episodes of heightened activity for even greater periods of time. As such, additional studies are needed to address current knowledge gaps in circadian biology in space, especially with regard to host-associated microbiomes. There is extensive evidence to suggest that symbioses exhibit daily rhythms that impact both host and symbiont[101,102].

3. How does the space environment impact reproductive fitness?

Exposure to microgravity and ionizing radiation can lead to reduced sperm counts and motility in animal models. In human males, parabolic flight can lead to reduced sperm counts and motility[103]. In addition, data modeling suggests an increased risk for early menopause in females who receive the equivalent doses of radiation from a Mars mission[104]. Additional studies are needed to establish the underlying mechanisms for the decline in reproductive fitness associated with spaceflight.

4. Do clinically relevant substances impact disease progression differently during exposure to the space environment?

Astropharmacy is an emerging area of research as there is a[105] growing recognition that not only do pharmacodynamics and kinetics change in response to spaceflight[106], but also due to the fact that providing a safe and effective pharmacy in flight is critical to mission operations[107]. Personalized approaches to astropharmacy are also being considered[108,109]. As we move beyond LEO further foundational research on how pharmacodynamics and kinetic change beyond LEO will be important for making operational decisions about appropriate medications and doses. Additionally, being able to produce medications on demand will be important[110] and it may be that synthetic biology approaches[111] should be considered for such on demand production. Therefore, developing such capabilities should be a priority for animal health research.

Feasible research beyond LEO in the years 2025-2035

Previous investments on the development of hardware for yeast, nematode and fruitfly model systems and the relative simplicity of their maintenance make these organisms highly amenable for use in biological research beyond LEO. Although expected to be challenging, rodent research beyond low Earth orbit should not be overlooked, especially given the large investment in and return on investment from rodent research in LEO.

Development and incorporation of non- and emerging-model organisms for studies BLEO.

Historically, space biology and biological studies in LEO have focused on the use of traditional model organisms (i.e., mice, *Drosophila melanogaster*, *Caenorhabditis elegans*). Although the benefits (i.e., short generation time, genetic tractability and resources, relevance to human health) of these model systems that make them so attractive for terrestrial studies also make them appealing for LEO studies, as our studies move deeper into space it is prudent to consider if additional models might provide attractive features both in terms of their fundamental biology, but also in terms of logistical considerations for doing science in BLEO.

One example of an emerging-model system which warrants consideration for BLEO studies is the tardigrade. Tardigrades, or water bears as they are colloquially known, are a group of microscopic animals renowned for their ‘extremotolerant abilities,’ that is, the ability to tolerate a number of abiotic stresses including: desiccation, freezing, high temperatures, anoxia, and particularly germane to space biology studies, high levels of ionizing radiation, microgravity, and even prolonged exposure to the vacuum of outer space.

While a prolonged presence in space has been found in many traditional model systems to have myriad detrimental effects, the use of tardigrades or similar extremotolerant organisms (e.g., rotifers, brine shrimp) combined with traditional model systems promises to reveal not just how organisms succumb to the rigors of space flight, but also how some of these rigors can be overcome or coped with.

As a comparative model system, tardigrades again offer some advantages. The phylum tardigrada is one of three members of the panarthropod clade, which includes better known model systems such as *D. melanogaster*. Furthermore, panarthropoda belong to a larger group of animals known as ecdysozoans, which include nematode worms such as *C. elegans*. These phylogenetic relationships place tardigrades between two of the best studied invertebrate genetic model systems – fruit flies and round worms – making them an ideal comparative system. It should be noted that tardigrades can often be cultured robustly both in liquid culture or on agar plates, such like *C. elegans*, and as such many of the culture hardware developed for other invertebrate model systems should be portable to tardigrades.

Tardigrades offer additional benefits typical of model systems: short generation times, sequencing resources – soon to include transcriptomes from tardigrades cultured on the International Space Station and sequenced for successive generations, existing culture hardware for space biology experiments, but in addition provide a key advantage for BLEO studies, which is the ability to be placed in a state of suspended animation. This ability to enter a cryptobiotic or suspended animation state is triggered by stress (e.g., desiccation) and does not require the use of exogenous preservatives, such as glycerol. Once in a cryptobiotic state tardigrade can remain viable for years or even decades. This approach would be a major advantage for experiments BLEO where long-term storage of specimens could be need both before initiation of experiments, but also to ensure the safe recovery of viable samples and would simplify logistics that would be difficult or impossible of systems such as *Drosophila* that require continuous culture due to a lack of viable long-term storage methods.

Other animal models not traditionally used for space biology that warrant consideration for BLEO studies include termites (nutrition and agricultural waste remediation) grasshoppers (nutrition and food security), planaria (regeneration), and hydra (stem cell aging).

Priorities

1. Develop rodent research capabilities

Currently, the majority of hardware development for the study of biology beyond LEO are geared towards simple model organisms such as yeast and invertebrates. The rationale for this prioritization is the significant technological challenges of developing habitats for mammalian models. Given the greater hurdles in the development of rodent research habitats, it is even more paramount that such efforts are initiated earlier in the planning process of BLEO research.

2. Improved hardware for fruit fly/nematode habitat designs

Currently, the majority of hardware available for fruit fly/nematode experiments in LEO require astronaut intervention for operations. Further, the vast majority of such hardware requires sample return in order to obtain results. For example, in the latest call for experiments on Artemis II both solicited fly and nematode experiments are dependent on sample return and the solicited fly experiments are dependent on astronaut intervention for operation. For non-crewed and non-sample return scenarios, automation and data return via telemetry are required. While options exist and/or are in development/planning consideration should be given targeted solicitation of proposals that raise the Technology Readiness Level (TRL) of fruit fly/nematode habitats to ensure such habitats are available prior to solicitation of hypothesis driven experiments that utilize these habitats BLEO.

Technology needs for this science area, 2025-2035

- Applications of AI/ML in this science area include raising the TRL of AI-assisted robotic animal habitats; automating animal experimentation and analysis to decrease sample return necessity; using trained AI vision models to perform wellness checks (e.g. rodent habitat video); using trained AI models to perform behavioral and physiological analysis.
- Needed improvements to fruit fly habitat designs
- Needed improvements to nematode habitat designs
- Habitat systems to accommodate rodent studies

Section D. How does life Beyond LEO impact plant development and physiology?

Background

Plants are a vital and valuable component of bioregenerative life support systems (BLSS) for long duration space missions. This section is to provide an over-arching and relatively near-term objective (2025-2035): How to most efficiently grow crops on the moon and in deep space, first in experimental testing then in serious crop growing in association with an occupied lunar habitat. There are several questions associated with this objective utilizing spaceflight and/or lunar-based platforms.

Critical questions for 2025-2035 in this research area

1. 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. Gateway, if available, will provide a valuable deep space science testbed. Potential simulated g-levels on Gateway would be able to facilitate full spectrum studies of different g levels in deep space. So far, intermediate g levels have been simulated in the laboratory and on ISS, finding, for example, lunar gravity impacting root growth physical and chemical parameters in a similar manner to microgravity and Mars gravity impacting in a similar manner to Earth gravity[112–114]. Results are consistent with, but not proven by, very preliminary results of experiments on the far side of the moon[114]. Knowledge gained from studies using intermediate g levels are too limited to develop reliable countermeasures and AL/MI based prediction tools for plant response and performance during deep space explorations. Indeed, Javier Medina[115] has said “the most important effort should be invested in incorporating plants to the coming initiatives of deep space exploration. The cis-lunar space considered for the Gateway project should be efficiently used for incorporating plants (both model and crop species) to a research platform that will be a fundamental tool to fill this gap in knowledge[4]. This aspect will be crucial for further exploration initiatives, such as an extended mission to Mars.” Local inertial acceleration (“gravity”) not only impacts plant development directly but also indirectly by creating conundrums for humans who want to water them – subject of the next section.

Altered gravity also impacts secondary metabolite production. Ideally, entire plants should be edible. This might be accomplished by a combination of genetic manipulation and low-g effects on the lignocellulose system of stems and roots. Hypocotyls synthesize less lignin when sprouting in orbital low gravity; the effect of lunar gravity on this process should be pursued. A project (“*Arabidopsis*-GRO”) is currently underway to characterize the molecular details of the response of *A. thaliana* lignin pathways to orbital space flight. It is also thought that reduced lignin synthesis leaves more aromatic precursors available for increased secondary metabolite and protein synthesis, potentially adding to nutritious value, flavor and resistance to microbial and fungal attack. Thus, tomato stems, for example, might be made more tender, tastier and more nutritious, or at least more compostable (if they don't become resistant to decomposition due to increased secondary metabolites).

2. 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. This is still the most challenging issue in off-earth plant cultivation. 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's Passive Orbital Nutrient Delivery System (PONDS) prototype and XROOTs have completed a series of pioneer experiments to demonstrate hydroponic and/or aeroponic water/nutrient delivery approaches to support plant growth. Both approaches achieved some milestones, but experienced some challenges under true microgravity conditions. How Lunar and Martian gravity levels could help mitigate these challenges encountered in microgravity is still at the theoretical and computational stage since a true partial g environment is needed for such evaluations. At about 1/6th g the lunar surface would be an ideal venue for exploring this question.

A lunar settlement might use regolith as porous root-zone media to minimize equipment, upmass and energy. Seed germination tests with lunar regolith and lunar regolith simulant results[116,117], as well as root zone aeration by oxygen producing polymers[118], have yielded encouraging results and need to be explored further. The possibility of using lunar regolith represents a huge technological saving and could eliminate long-term trade studies among the above-mentioned, sometimes competing, technologies. It appears that some toxic effects could be mitigated by an aqueous rinse of the root-zone regolith before planting. Broader investigation of this application of regolith should have a high priority in early lunar surface research.

3. How do plant-microbe interactions affect plant quality and growth in space (microbiome, beneficial microbes, as well as pathogens)?

More than a dozen bacterial genera and a wide variety of fungi are plant pathogens. On the other hand beneficial microbes can promote plant growth, increase resistance to pathogens and reduce the need for fertilizer input. Therefore, they would be valuable additions to increase plant productivity in space. Ground based studies are needed to develop minimal consortia to supplement growth media in space. Beneficial microbial strains will need to be carefully vetted to ensure safety and efficacy, and more studies are needed to understand the response of plants in space to opportunistic pathogens. Zinnia plants growing in Veggie hardware on the ISS were more susceptible to *Fusarium* infection when their roots were under hypoxia and excess water[119]. 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. Additionally, some bacterial pathogens were found to be more virulent in space[120] which could increase the risk of plant disease. Data from current and historical Microbial Tracking studies and microbial community analyses of plant grow-outs on orbit could provide valuable insights in spatial and temporal evolvement within a confined environment for AI/ML modeling. Indeed, tracked microbes collected from Veggie have already been evaluated for such properties. Earth-grown plants are assisted by plant growth-promoting bacteria (PGPB), which gain their nutrition/carbon from plant exudates and provide the plant with signals that manipulate plant hormones, interfere with ethylene production, fix atmospheric nitrogen (for legumes), and produce resistance to potential pathogens. Soil bacteria are also capable of solubilizing critical plant nutrient minerals, such as phosphorus and iron, by producing siderophores. Some 21 bacterial species were harvested from the Veggie module, propagated in the laboratory and tested for probiotic activities. Five isolates were positive for siderophore production, five for phosphate solubilization, five for indole production, nine in ACC deaminase activity, and six showed strong anti-fungal activity[121]. Additionally, four species of *Methylobacteria* isolated from ISS have been characterized genetically as PGPB's, and three of these are considered novel species[122], demonstrating the power of genomic analysis and artificial intelligence. .

Preparation for space travel beyond LEO is a very good reason to aggressively pursue this question to understand the impact of long duration exposure to the deep space environment on interactions among individual beneficial microbes/opportunistic pathogens, plant microbiome, plant hosts, and the environment microbiome. This includes studies of pathogenic and commensal microbial responses (genotypic, molecular genetic, metabolomic and phenotypic) with or without host plants. Even though LEO and ground based facilities (confined environment) will be the primary platforms for research on this question, BLEO Microbial Tracking and environmental and plant microbiome monitoring are essential and may become a built-in feature of "routine" lunar crop production.

4. What are the effects of different radiation levels on plant quality and growth in space?

The numerous published findings that 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[123] were summarized in last year's report. In deep space, GCRs present as an extremely low dose background radiation with maximum accumulative GCR dose at milligray range for a 10-day exposure and a total dose rate around 0.4 mGy/d at the lunar surface[124]. Protons released from a large SPE pose a more significant impact than GCRs. On the other hand, there is evidence that a single GCR thin-down hit can destroy multiple cells in the seed embryo leading to developmental anomalies during sprouting. *Arabidopsis* seeds (under 1 atm) have been exposed to the Stratosphere (36-40 km) environment above Antarctica in a 30-day high altitude balloon mission. In a parallel experiment, seeds were exposed to 40 cGy GCRs 1 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 (& developmental aberrations) were also revealed in these GCR or Stratosphere exposed seeds. with the GCR exposure generating 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[125]. Heritable epigenetic changes were also detected in rice seeds following space flight[126].

Chronic gamma-irradiation of *A. thaliana* through its 54-day life cycle was found to modify several biochemical and growth-related end-points[127], except at the lowest dose rate studied, which was approximately 1.9 mGy/day. In this study, progeny seeds germinated at the normal rate. This dose rate is approximately the highest experienced to date in human space flight[128] and several times the 0.4 mGy/d reported for the lunar surface and the 0.3 mGy/d in lunar orbit as derived from last year's BLISS report[124]. If the effects of GCR and low-LET space radiation are considered separately it is seen that the impact of low-LET (protons, electrons) can be neglected except in possible cases of solar proton storms, while coronal GCR hits on seeds slightly reduce germination and induce developmental anomalies.

While studies 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 could be done, the relative significance of radiation risk to crop value should be assessed in the context of other risks.

5. How can legging strategies be used to both monitor and maximize plant quality and growth in space?

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. 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), day length (14 d), and regolith composition (basalt, pyroxene, olivine). Data to date indicate that a truly major question to be resolved is whether to use ambient or artificial light or a combination thereof. In other words, the conclusion drawn from the research in all the areas encompassed in this report must be combined with engineering restrictions to create the optimum strategy for food crop production on the moon while extrapolating toward similar achievement on the surface of Mars.

6. 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 et al., 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[129,130]. Air and water supplies must originate from earth. Prototype growth chambers are being used nationwide in educational programs, but these pay very little attention to atmospheric pressure requirements. Most spaceborne plant research to date has been performed on spacecraft with earth-like atmospheric conditions – one atmosphere (101 kPa), 20% (21 kPa) O_2. On the moon a fully-enclosed long cylindrical growth chamber will be required, as internal pressure must be held against space vacuum. Appropriate levels of CO_2 will be required, and this might be provided by a small bleed from a human habitation module, where residents are exhaling CO_2. To conserve resources a human habitation module is likely to be at less than earth-atmosphere conditions, such as lowered total pressure (55 kPa, 0.54 atm?) and O_2 at or below 21 kPa. Crop plants will have to be selected on the basis of low-pressure tolerance. Earth-based testing of plant growth under reduced pressure should be continued. Early experiments showed that wheat could be grown at pressure as low as 10 kPa (0.1 atmosphere), with 0.2 atmosphere being satisfactory and offering reduced leakage from the module, less N_2, easier O_2 management and possible optimization of gas mixtures[131]. Lettuce growth at 67 and 33 kPa while holding O_2 at 21 kPa and CO_2 at 0.12 kPa was reduced up to 30% but anthocyanin synthesis increased[132]. Hypobaria does not alleviate the need for ethylene control in the closed module[133]. There seem to be various choices: use a low-pressure bleed of gas from a nearby human habitation module with or without recycle, deposit a pressurized mixed-gas supply dedicated to the plant growth chamber, or grow crop plants within the human habitation module. The last choice, if regolith is to form root matrix, requires bringing regolith into the habitation module, which could be a serious safety problem. A trade study between growing plants in a lunar greenhouse and growing them within a (low-pressure) human habitat is needed. Furthermore, hypobaria also constitutes a unique stress and more studies are needed to enable plants to adapt and thrive under these unfamiliar environmental conditions.

7. What plants and novel organisms should be used and or developed for food production and BLSS in space?

Nutritious value. An ideal crop plant for lunar campers should supply edible nutrients from all of its components (high harvest index): roots, stems, leaves, fruits (a 10-day aeroponic beet?). Radish, carrot and turnip are the most conspicuous candidates, with lettuce and kale being a close second. But these are low in carbohydrate, so it will be necessary to accommodate potato[134] grain species in the form of dwarf rice and wheat. A partial list of plants grown in space to date includes several varieties of lettuce, radishes, peas, zinnias, dwarf wheat and sunflowers. “Veggie” has successfully grown a variety of plants, including three types of lettuce, Chinese cabbage, mizuna mustard, red Russian kale and zinnia flowers. The Advanced Plant Habitat is an enclosed and automated chamber with cameras and more than 180 sensors that are in constant interactive contact with a team on the ground. Its water recovery, atmosphere content, moisture levels and temperature are all automated. It has a broad spectral range of colors of LED lights. A recycle principle is needed to deal with stem and root waste, such as composting in a human waste reservoir. A rotating composter has been studied at the University of Arizona. Ideally, entire plants should be edible. This might be accomplished by a combination of genetic manipulation and low-g effects on the lignocellulose system of stems and roots mentioned above.

Additionally, space efficiency and nutrient content are two critical selection criteria, therefore, microgreens could be the excellent choice. Cyanobacteria or unicellular algae have been proposed for recycling oxygen from CO_2 and providing food at the end of their cycle; however, palatability issues will need to be solved by further research for the feasibility of crew consumption.

9. Multi-stressor effects (combined effects).

It is clear that plants in nature are exposed to multiple stresses 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[135,136]. This was discussed in detail last year.

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.

10. 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. Only a limited number of crop plants can produce edible material within the 14-day lunar day. Extending the lunar day would be a technological challenge. The intense solar ultraviolet light will damage crop plants. This will dictate the composition of a lunar greenhouse. If it is inflatable with a transparent roof, for example, the material will need a high UV extinction coefficient and will also need to reflect at least 50% of the visible (PAR) spectrum. Alternatives include construction of a cylindrical one-way mirror admitting sunlight by day and reflected diurnal LED illumination by night using power from the habitat, or integrating the greenhouse with the habitat and using habitat light. The latter introduces the problem of regolith hazards to crew in the habitat if regolith is to be used for ISRU root tray simplification.

Feasible research beyond LEO in the years 2025-2035

In the absence of sample return and limited options for downstream analysis, only some of the listed questions can be feasibly addressed in the near term. Some of these are listed below.

1. What are the effects of different g levels on germination, tropisms, secondary metabolite production and food quality?

In the context of the above questions, only lunar gravity would be explored. Other variables (pressure, illumination, moisture) will still be challenging for lunar surface experiments. If Gateway becomes available, it will be a valuable science test bed in deep space with micro g and potential simulated g-levels to facilitate full spectrum studies of different g levels. Also see #9.

2. How can root zone water, nutrient and O2 provision be optimized for plant quality and growth in space?

The selection process involving porous media vs hydroponic vs aeroponic approaches might be resolved by testing the suitability of fresh lunar regolith as root zone media with artificial gray water. This combination would reduce upmass for a lunar settlement and could be tested by depositing a permanently pressurized growth chamber at a specified depth into the regolith and using solar-powered environmental controls and image and data telemetry. In addition, how effective lunar gravity can mitigate the challenges encountered in microgravity for root zone water delivery and O_2 provision and support full plant growth cycle can be tested on the lunar surface. Hydroponic and aeroponic water/nutrient delivery technologies can also be tested as well.

3. How do plant-microbe interactions affect plant quality and growth in space (microbiome, beneficial microbes, as well as pathogens)?

Hypocotyls synthesize less lignin when sprouting in orbital low gravity; the effect of lunar gravity on this process should be pursued. A project ("*Arabidopsis*-GRO") is currently underway to characterize the molecular details of the response of *A. thaliana* lignin pathways to orbital space flight. As noted above, testing of selected microbes' abilities to mobilize nutrients in lunar regolith could lead to improved root matrix development. If Gateway becomes available, these questions can be investigated in deep space with micro g and potential simulated intermediate g-levels. BLEO microbial tracking and environmental and plant microbiome monitoring are essential. Also see #9.

4. What are the effects of different radiation levels on plant quality and growth in space?

The 0.4 mGy/d reported for the lunar surface and the 0.3 mGy/d in lunar orbit as derived from last year's BLISS report are similar to dose rates experienced aboard ISS[128]. With so much of this radiation due to galactic cosmic particles it will be useful to convert these dose rates to mSv/d based on a scientific RBE or legislated Quality Factor when such becomes available, given the results of GCR exposures mentioned above. In some scenarios the lunar greenhouse is buried under a certain thickness of regolith, which would absorb a significant fraction of incoming ionizing radiation. However, radiation protection of plants need not be the primary reason for a regolith cover for crop growth modules. Facilities outside and inside Gateway will provide long-duration exposure platforms for plants and other biological organisms and for characterizing the deep space radiation field and its impact on biological systems.

5. How can legging strategies be used to both monitor and maximize plant quality and growth in space?

Initially a small number of food crop plants should be selected for study. A remote means of scooping regolith into a growth chamber will be needed. Pressure and composition of an artificial atmosphere needs to be optimized and supplied by a pressure bottle. Solar-powered environmental controls and image and data telemetry, with real-time remote control from earth will optimize operations. Light control will require a combination of heavily filtered sunlight supplemented (during lunar night) by battery-powered LED illumination. It may turn out that using ambient light is not a good choice if, for example, the habitat is placed in a lava pit or cave or is fully insulated with a cover of regolith. As habitation plans evolve, a major trade study will be needed between using ambient and LED light. In any case the latter will be necessary during lunar night. Crew time spent on farming should be minimized owing to responsibilities for other tasks and risk of exposure to regolith nanoparticles. Multisensor automation has been developed for the Advanced Plant Habitat (APH) currently on ISS.

6. How does atmospheric composition and pressure affect plant quality and growth in space?

In the 2025-2035 time frame progress can be made in several ways. Factorial-design experiments can begin on earth using selected gas mixtures at, say 55 and 21 kPa. A sparse-matrix version of such studies could be implemented on ISS and Lunar Gateway. A scaled-down greenhouse on the lunar surface would be the next logical step. These results would lead to a selection from the choices of gas supply strategies mentioned above.

7. What plants and novel organisms should be used and or developed for food production and BLSS in space?

Research will also be needed to generate crop cultivars with improved traits either by breeding/selection or genetic engineering. Traits of interest include high harvest index, the ability to withstand stress, enhanced plant performance under unfavorable conditions, resistance to pathogens/pests and improved nutritional content. LEO and ground based studies will be the primary research efforts for this question during 2025-2035. However, understanding mechanisms underlying the responses of plants to the BLEO environment using model organisms, microgreens, and well-established crop model plants will inform important strategies, including genetic engineering strategies.

9. Multi-stressor effects (combined effects).

In summary, plants (including seeds) and associated microbiome 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, low O_2, and altered air pressure) during BLEO explorations. To date, combined effects have been studied much less in crop plants and other candidate biology for deep space BLSS than in model organisms, so further ground studies will need to precede nearly all BLEO experiments designed with a high level of multiple-stress consciousness. Taking the preceding sections together and in BLEO context, multi-stress testing by canvassing the above-mentioned variables will be a priority for BLEO biology and exploration.

10. What are the comparative effects of ambient vs. built-environment (LED) illumination on photosynthesis and tropisms?

It may turn out that using ambient light is not a good choice if, for example, the habitat is placed in a lava pit or cave or is fully insulated with a cover of regolith. As habitation plans evolve, a major trade study will be needed between using ambient and LED light. In any case the latter will be necessary during lunar night.

Model organisms relevant to science goals in this area

Model organisms (*Arabidopsis*, moss species, algae and cyanobacteria), microgreens, and model crop species are good candidates for investigations including seed storage, survival, and full plant growth. Multigenerational studies may be also feasible when platforms and hardware are available. It should be possible, but perhaps not necessary, to conduct experiments with differentiating cell cultures for guidance in a synthetic biology approach.

Technology needs for this science area, 2025-2035

- Applications of AI/ML in this science area: Synthetic biology and gene editing can take enormous advantage of computational biology, driven by AI. Feeding plant health parameters to the “digital farmer” can minimize human attention to crops, especially those growing under conditions unsuitable for human habitation. Several robotic gardening systems are commercially available and can optimize planting, weeding, and other activities. Additional capabilities desired but not yet available include a robust in-situ microbial tracking and monitoring capability. Machine-based responses to the complicated data set from the monitoring system will be an inevitable engineering feature in the 2025-2035 time frame.
- In situ high-throughput analysis and diagnostic tools and bioinformatics capability. Biochemical capabilities to perform on-board or in-habitat analyses of biochemical pathways, especially enzyme activities associated with aromatic

amino acid, lignin, and secondary metabolite pathways could inform further variety selection and/or genetic manipulation.

- Plant growth chamber and other hardware required for BLEO plant research either on the Gateway (near-term, using both facilities outside and inside the Gateway, during manned and unmanned periods) or the lunar surface (2025-2035).
- Lunar greenhouse technology development, such as using minimum-energy gas-phase pressure and composition and calculated combinations of processed wastewater (nitrogen, sodium, potassium) and lunar regolith (micronutrients). Should be able to replace active root trays with regolith and create acre-level enclosed lunar greenhouse systems separate from habitation modules. Gas supply from Earth will be a major challenge.

Section E. How does the BLEO environment impact interactions between microbes and their eukaryotic hosts?

"Life improves the capacity of the environment to sustain life. Life makes needed nutrients more readily available. It binds more energy into the system through the tremendous chemical interplay from organism to organism" – Frank Herbert, Dune[137]

Microbes have played a pivotal role in the evolution of eukaryotes. Symbiotic interactions with microbes have driven major innovations in eukaryotic physiology and enable eukaryotic hosts to recognize and respond to changes in the host's environment. A major gap in our understanding is the impact that environmental perturbations, such as the absence of gravity or elevated radiation levels, have on host-microbe interactions. Thus, for humans to continue to work and live in the space environment, research is needed on how symbiotic interactions are initiated, established and maintained.

Critical questions in this research area

1. How do changes in the space environment alter the initiation and persistence of beneficial interactions with microbes?

It is unclear how different taxa within a host microbiome respond to environmental perturbations, such as the absence of gravity. Specific questions that need to be addressed include:

a. Do the synergistic effects of microgravity and radiation alter the dynamics and interface between microbes and the animal and plant cells with which they associate?
b. Are there distinctive thresholds of gravity or radiation to initiate changes to microbial or host physiology?

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

As humans move Beyond LEO, the types and intensities of environmental stresses will change, therefore several key questions will need to be addressed to ensure the long-term health of plants, animals and fungi living in the space environment. Key question may include:

a. If a taxon goes extinct within a host microbiome during long-duration spaceflight is there the potential for the host to lose that functional capability?
b. Would extirpation of that taxon result in an impairment to host health?
c. Is there sufficient functional redundancy to compensate for the loss of a given taxon under a range of environmental perturbations in the space environment.
d. Do perturbations to the host microbiome increase risk for disease to the host? In other words do normally mutualistic, or commensal microbes, become pathogenic?

3. How can changes to the host microbiome be mitigated or controlled during long-duration space travel?

Assuming spaceflight does causes changes to the microbiome in terms of composition or changes the developmental timeline of host-microbe interactions several additional questions need to be addressed:

a. Are probiotics effective after the stress of long-duration spaceflight to maintain microbiome health?
b. What is the shelf life of probiotics and are most beneficial host-associated microbes amenable to being manufactured into a probiotic?
c. Is there a regular exchange of spacecraft microbiome and eukaryotic tissues? If so, does the space environment evolve typically beneficial symbionts towards pathogenic states?

Feasible research beyond LEO in the years 2025-2035

Addressing these key questions regarding the impact of BLEO on host-microbe interactions can be addressed using the following technologies and approaches:

- Examine synergistic effects of micro-, Lunar- and hypergravity gravity conditions and deep space radiation to assess effects on host-microbe colonization and persistence.
- Conduct space biomanufacturing of probiotics under microgravity conditions to assess shelflife and effectiveness
- Use organ-on-chips (e.g. gut epithelium) to monitor the onset of bacterial colonization in animals under deep space conditions
- Use of microfluidics to assess bacterial colonization of the plant microbiome (e.g. rhizosphere, phyllosphere, endophytes) under deep space flight conditions.
- Use of microfluidics to examine the effect of different microbiome compositions on *C. elegans* health/longevity BLEO versus on Earth.

Model organisms relevant to science goals in this area

- Organ-on-a-chip (e.g. gut epithelium, kidney, lung, nervous system) and cryopreservation of these chip systems for long-duration space missions.
- *Arabidopsis* - serves as an important model plant to examine the onset and development of the host rhizosphere. *Arabidopsis* seeds can be maintained in status for long duration time periods
- *Hydra* are essentially eternal cells with their stem cells that are in a constant state of renewal. Hydra form simplistic symbiotic associations with microbes and can serve as a valuable model for BLEO research.
- *Caenorhabditis elegans* are a model that enables genetic manipulation of both the host and microbiome.
- Rodents are a model that enables genetic manipulation of both the host and microbiome
- Crop plants that are well established and will be important food sources for crew will be recommended to examine host-microbe interactions

Technology needs for this science area, 2025-2035

- Improved cryopreservation of organ-on-a-chip technologies to facilitate the delay in activation.
- Better automated molecular biology techniques to conduct nucleic acid isolation, library prep and sequencing in the absence of human participation.
- Better autonomous BLEO cultivation, husbandry and observation capabilities for targeted host animals and plants.

- Better automated image analysis and AI-based tools to accelerate data extraction and identification of subtle phenotypes.

Section F. How does the BLEO environment impact evolutionary processes?

Exploration scenarios for BLEO in the 2025-2035 timeframe anticipate long-term human presence and activity in deep space, including on the surface of the Moon. The 2021 report of this working group highlighted both the potential sources for new genetic variation and the unique selective pressures of the BLEO environment. In particular, microbial evolution was emphasized as both a risk and opportunity for BLEO exploration and research. Maturation of Artemis and Gateway, along with CLPS lunar landers and other potential private efforts, allow for unprecedented access to the BLEO environment for scientific study. In this context, new challenges and opportunities arise that can help us better understand how Earth life adapts at the evolutionary level to spaceflight and non-terrestrial planetary environments.

Critical questions in this research area

1. How does spaceflight and the BLEO environment impact the evolutionary process?

The BLEO environment provides increased opportunities for the generation of genetic variation through increased environmental stresses, including:

a. Micro- and variable gravity,
b. The extreme built and non-terrestrial planetary environments of spacecraft, habitats, and the lunar surface,
c. Radiation,
d. Prolonged close contact between humans, environmental bacteria, commensals and pathogens in an artificial closed-loop life support system that is impacted by all the above factors.

2. What are the evolutionary selective pressures of the BLEO environment?

The factors listed above (gravity, radiation, built and planetary environments) also provide selective pressures on life. How does life adapt evolutionarily to the BLEO environment?

a. What is the evolutionary response to the selective pressures of reduced (lunar) gravity? What does this say about adaptation to Mars?
b. What are the evolutionary impacts of the non-terrestrial potentially selective factors, including built environments, regolith, gravity, altered day-night length and circadian disruption?
c. What are the mechanisms of observed selected genotypes, and what genes, pathways and processes are specifically selected?

3. Does selective pressure and/or exposure conditions of the on-orbit or BLEO built environment increase risk to crew relative to Earth environments or short-term LEO exposure?

a. How does the BLEO environment affect skin, gut and plant microbiomes on an evolutionary timescale? How is microbial virulence and biofilm formation impacted by long-term exposure to the BLEO environment?
b. How do the functional capabilities of microbial communities and microbe-host interactions change? Can examination of the functional capacity of these communities reveal new capabilities emerging? New pathogens that need to be monitored?

Feasible research beyond LEO in the years 2025-2035

Research in the 2025-2035 timeframe will benefit from improved access to space, advanced autonomous capabilities and increased hardware fidelity. This includes improved microfluidics, AI and machine learning, and new measurement technologies (e.g. sequencing, imaging, spectroscopy, etc.) Sample return will also be possible (though difficult) for select experiments depending on mission profiles for Artemis and CLPS. Sample return will allow for combined *in situ* and ground-based analysis. Comparative studies will be able to compare the effects of the BLEO environment to Earth, LEO (ISS), and the lunar surface. Research approaches may include screening using mutant libraries (selection), experimental evolution, competition studies. Experiments will include those designed to understand microbial evolutionary response to microgravity and deep space radiation. Built-environment and human and plant microbiome studies will advance knowledge and capabilities for long-term exploration. Platforms will include Artemis / Orion, Gateway, free flyers and CLPS landers. The upcoming BioSentinel[138,139] CubeSat and Deep Space Radiation Genomics (DSRG)[140] payload, scheduled to fly with Artemis I, highlight the types of experiments that are possible now; in the future, improved automation will lead to additional in situ analysis capabilities, such as sequencing to detect evolution[52].

Model organisms relevant to science goals in this area

As listed in the 2021 report, organisms of scientific interest include model bacteria relevant to human health and exploration scenarios that also have flight heritage (e.g., *Bacillus, Escherichia, Deinococcus, Pseudomonas, Salmonella*), as well as small eukaryotes (e.g., tardigrades and nematodes). Human health studies that examine microbiome-associated bacteria, specifically human microbiome (skin, oral, gut), such as human commensals including *Staphylococcus epidermidis and Enterococcus faecalis* are particularly relevant. Organisms that may play roles in nascent bioregenerative life support and food/pharmaceutical production capabilities, including cyanobacteria, green algae and bacteria with functional ecosystem roles (e.g. N-cycle bacteria) are relevant. Yeasts, including *Saccharomyces* (nearly 50% of essential yeast genes have functional human homologs[141] and filamentous fungi have relevance as fundamental research platforms, and with respect to human health and food / biopharmaceutical production. Research can also explore small plants with the potential for multi-generational and plant-microbe interaction studies (e.g., *Azospirillum* with algae, *Brassica* cultivars, *Arabidopsis*).

Technology needs for this science area, 2025-2035

Applications of AI/ML in this science area - autonomous execution of complex experiments with real-time troubleshooting capability, analytical methods for data analysis.

- Growth or metabolism detection via smart gas detectors, and optical detection, including optical density, absorbance, fluorescence, biosensors (fluorescent, electrical or optical), spectroscopy (fluorescence, luminescence, Raman, UV, IR, etc)
- Improved reagent storage techniques for analytical methods requiring chemical or biological reagents such as biological single molecule nanopore “strand” sequencing
- Solid-state nanopore sequencing and in situ basecalling of biopolymers, specifically nucleic acids.
- Chemical monitoring - mass spectroscopy, imaging (metabolic markers, gene expression), microelectrode sensors for gasses and molecules.
- Microfluidics technology that can support complex in situ experimental procedures, autonomously, including chemostat capability, sub-culturing/passaging, extractions and sample processing, staining, and growth maintenance and manipulations of organisms.
- Rad hard processors and experimental hardware
- Capability to support long-term studies autonomously.
- Capability for sample return under some scenarios (e.g. Gateway, or Lunar habitat/lander).

Section G. How does the Beyond LEO environment impact biotechnological processes, and how can they serve science, engineering, operations, and habitation in the Beyond LEO environment?

Introduction

Biotechnological processes harness cellular and biomolecular activities to provide services and generate products. The scope and capability of terrestrial biotechnology is growing rapidly (citation 1, citation 2), and this trend can be applied to beyond LEO missions in a multitude of ways: engineered microbes as part of life support systems; improved fidelity and lifetime organ chips; just in time drug production; high density data storage using DNA; and astronaut health monitoring by multiplex analysis of biomolecules, are just a few examples of how rapid advancements in biotechnology could help extend our reach into the solar system.

Deployment of biotechnologies will require an understanding of how the beyond LEO environment impacts their functions and outputs. Along these lines, the upcoming LEIA experiment (Lunar Explorer Instrument for space biology Applications) will deliver strains of yeast capable of producing carotenoids - an important human nutrient - to the lunar surface in the 2026 time-frame. This will be a major milestone in the investigation of biotechnological processes beyond LEO, and will provide important information on the impact of the deep space environment on the biosynthesis of products. While growth, metabolic, and production rates will be measured, payload limitations mean that many aspects of this model biotechnological process will remain uninvestigated. Moreover, this payload represents a small window into the rapidly developing biotechnology landscape. Thus, there is great potential for follow-on studies.

While our 2021 report was focused on near term opportunities (up to 2025), here we look further out to define the critical questions to be answered in the 2025 - 2035 timeframe, and what payload capabilities would be necessary to investigate them.

Critical questions in this research area in the years 2025-2035

1. How does the BLEO gravity environment affect biotechnological processes?

As discussed in the 2021 report, biotechnological processes involve growth; growth requires nutrient cycling; nutrient cycling is impaired by lack of buoyancy driven convective mixing. Beyond this, there are additional concerns with reduced gravity that only become especially 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 beyond LEO gravity environment. Thus it is important to understand how we can manipulate fluid and gas exchange to produce bioreactors that optimize performance of biotechnological systems in weightlessness and in reduced gravity.

2. How does the BLEO radiation environment affect biotechnological processes?

As discussed in the 2021 report, biotechnological processes must deal effectively with the radiation environment beyond LEO, which could impact both the biotic and abiotic components of a process. For example, the 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. Thus, it is important to understand how the radiation environment will impact biotechnological processes and what mitigations will be necessary.

By ~2025 this question will have been addressed in limited ways by the BioSentinel and LEIA missions, which will deliver *S. cerevisiae* to a heliocentric orbit and the lunar surface, respectively, and report upon the impact of these radiation environments on this important biomanufacturing chassis. Furthermore, the LEIA payload will feature a strain of yeast engineered to produce carotenoids. The Deep Space Radiation Genomics (DSRG)[140] payload on Artemis I will also expose yeast to deep space and facilitate additional in-depth analysis via samples returned to earth, including 6000 deletion mutants and 6000 overexpression mutants. Nevertheless, important questions will remain unanswered such as the impact of the beyond LEO radiation environment on the long term stability and performance of microbial strains and biotechnological processes.

3. How can biotechnological processes best utilize BLEO resources?

As discussed in the 2021 report, biotechnological processes that operate at a certain scale may greatly benefit from utilizing beyond LEO resources as inputs. The 2021 report discussed the potential for utilizing resources present in lunar regolith and the science and engineering questions that need to be tackled to enable this. Not discussed were resources available for use in other beyond LEO environments such as on Mars or asteroids. Mars offers a number of alluring resources such as water ice, abundant gaseous CO_2 and various minerals within the regolith. As activity pivots from the Moon to Mars, projects will be needed to understand how best to ingest these resources into biotechnological processes, and to in-turn engineer and deploy these technologies.

4. How can biotechnological processes serve science, engineering, operations, and habitations in the BLEO environment?

Not discussed in the 2021 report is the question of how biotechnology may serve science, engineering, operations, and habitations in the beyond LEO environment. While it is essential to understand how biotechnology may be impacted by the beyond LEO environment, this must not be decoupled from an evaluation of how biotechnology could best serve our beyond LEO goals. The application of new synthetic biology approaches is enabling a more rapid pace of biotechnological innovation, and the places where biotechnology is applicable continues to grow. Engineered microbes can report upon conditions within a host, generate useful products, and provide life support services; Biomolecules can serve as data repositories; Synthetic organoids and organs can serve as research platforms and medical resources. A program for identifying technologies that could best serve activities beyond LEO and maturing them will help accelerate their adoption.

5. How could biotechnology impact the beyond LEO environment? What are the planetary protection implications?

Not discussed in the 2021 report is the question of how biotechnology processes may impact the beyond LEO environment. Unless processes are self-contained or operate within an entirely closed-loop mission scenario, they will have some impact upon their environment. This is increasingly true for processes which rely upon inputs taken from the beyond LEO environment (*e.g.,* water from the lunar surface, CO_2 from the Mars atmosphere). Most biotechnological processes generate some degree of waste, both biological and otherwise, which will need to either be recycled or disposed of. How these inputs and outputs impact the beyond LEO environment and how justifiable these impacts are become important questions that should be answered before processes are deployed at scale.

Feasible research beyond LEO in the years 2025-2035

By ~2025 at least three missions will have measured the response of *S. cerevisiae* - a premier biotechnological chassis - to the beyond LEO environment. BioSentinel will first take yeast into a heliocentric orbit, LEIA will deliver yeast to the lunar surface, and DSRG will grow yeast in deep space and return samples to Earth. Thus research in the 2025-2035 should build off of these milestones.

Below we highlight high priority areas of research that could be accomplished in the 2025-2035 time-frame. These areas were identified in part by selecting a handful of technologies for deployment beyond LEO by 2040, and then identifying the knowledge of technology gaps that may need to be filled for this to occur.

1. Bioreactor development and validation

Biotechnological processes are tightly integrated into abiotic hardware that facilitates controlled growth and metabolism of cells and cell consortia, and it is important to begin developing and testing the performance of standard and customized bioreactors in the beyond LEO environment. Thus in this timeframe, automated bioreactor payloads demonstrating control of parameters required for standard biotechnological processing (temperature, gas, light, sensors, etc) should be deployed to Gateway, free flyers, and the lunar surface. We note that while many microbial processes are compatible with standardized bioreactor geometries and capabilities, other processes such as tissue and organ production, by 3-D printing, for example, will require orthogonal bioreactor development beyond LEO.

2. Development and testing of advanced monitoring and control technologies

Space operations can have severe personnel constraints and increased requirements for stability and fidelity that may necessitate the development or adoption of advanced technologies, especially including AI/ML, for the control or monitoring of biotechnological processes. For example, optogenetic control of organisms could facilitate control of biotechnological processes in space compatible formats.

A step in this directly will occur through the upcoming Biological Exploration 2 (BioX2) payload under development[52], which aims to advance technology readiness of technologies required to automated detection of evolution to quantify the selective pressure of microgravity and/or radiation on microbial model systems (e.g., *B. subtilis*).

3. Development and testing of automated payloads to couple biotechnologies to *In-situ* Resource Utilization (ISRU) technologies

Certain technologies at certain scales could require locally sourced material inputs to justify their inclusion in a mission scenario; If a kg of input material must be delivered from Earth to generate a kg of product then the biotechnological process is not valuable. Thus it will be important to begin to identify and develop ISRU technologies that can harvest extraterrestrial resources and input these into a biotechnology process. These resources include lunar regolith, CO_2 in the Martian and spacecraft atmospheres, lunar ice, and plant waste. In addition, technologies for the recycling of biotechnology waste will need to be developed for them to fit into a closed-loop mission architecture.

4. Integration of biotechnology potential with other space life sciences research domains

A key advantage to some biotechnological processes is that genome editing can be utilized to reprogram the process to generate a new product. This enables scenarios such as “on-demand pharmacies” where cells are engineered to produce a target therapeutic, and downstream processing might exploit the advantages of low-gravity crystallization. This application would require tight integration with other space life sciences research domains related to astronaut health monitoring and intervention. Thus it will be important to begin developing cohesive frameworks through which astronaut health data could be used to identify targeted therapeutics that are generated by engineered living systems.

5. What support infrastructure and technologies will biotechnological processes require

A key milestone for biotechnological processes used beyond LEO will be the transition from single use demonstrations to processes that reliably add value (science, engineering, life support, food, health, etc.). This will require supporting capabilities such as maintenance, sterilization, etc. It will be important to understand which Earth technologies will work well beyond LEO and whether alternative technologies will need to be developed. Will there be an increased role for UV sterilization? New technologies for storage of production hosts? New technologies for storage of labile molecules or whole organisms?

6. How could biotechnology impact the lunar environment? What are the planetary protection implications? Could biotech impact the lunar environment?

Biotechnological processes will have some impact upon their extraterrestrial environment, both due to the resources they consume and the wastes they generate. How these inputs and outputs impact the beyond LEO environment and how justifiable these impacts are constitute important questions that should be answered before processes are deployed at scale.

Model organisms relevant to science goals in this area

The upcoming LEIA experiment (Lunar Explorer Instrument for space biology Applications) will deliver a strain of yeast engineered to produce carotenoids by ~2025. Biotechnology research in the 2025-2035 timeframe should build upon this milestone by continuing to develop our understanding of how the classic microbial factories relied upon for terrestrial biotechnology (e.g. *S. cerevisiae*, *E. coli*) respond to the beyond LEO environment, and by beginning to extend our knowledge to emerging models (which will grow in terrestrial importance and could have specific advantages in beyond LEO scenarios). Examples include synthetic microbial consortia, and even fully synthetic microbes.

Section H. How can the Beyond LEO environment be utilized synergistically to benefit the space biology, physical sciences, and astrobiology programs?

Introduction

Research needs in space biology are driving that field towards increased automation, whether to make measurements not previously possible, or to optimize crew time. Similar trends are underway for space research in the physical sciences. Research needs identified in other sections of this report include advancing microfluidics, cultivation, sensing, and characterization tools, somethimes blendedwith AI/ML. At the same time, a wide range of instruments and technologies are in development, have been developed, or have flight heritage as part of NASA's planetary science programs. Although space biology and physical sciences are supported by the NASA Division of Biological and Physical Sciences (BPS), these programs have synergies with instrumentation developed for astrobiology and planetary science missions. Here, we explore potential synergies between these programs that could be developed further in the BLEO environment, including opportunities for cross-feeding, technology development and advancement, interdisciplinary investigations, and other interdisciplinary mission activities.

Critical questions in this research area

1. How can NASA investments in the domains of human and robotic exploration benefit programs outside their original applications?

For example, how can instruments intended to assist in the search for life help inform crew health and safety, and support *in-situ* resource utilization and vice versa. The mineral history of the moon (and of all bodies in the solar system) has always been relevant to the understanding of the potential for the origin of life. Research into regolith at multiple sites and multiple depths yields data useful in a wide variety of categories including ISRU, habitability, planetology, bio-risk, and astrobiology. Combined planning for the acquisition and the analysis of samples across disciplines should begin in an early timeframe.

2. How can limited mission opportunities advance the goals of both exploration and science?

Broad sharing of analytical data obtained from lunar and planetary environments across disciplines should be planned well in advance of mission design. Secondary payload opportunities can be used to advance diverse NASA mission goals, for example MOXIE on Mars 2020. This can be extended to a wide range of future NASA planetary science and human exploration missions.

3. What specific challenges are common across different domains and can be addressed through synergistic study?

The Moon is a nitrogen desert. This aspect is a challenge for Lunar agriculture and ISRU and a potential mystery for pre-biotic evolution on Earth[142,143].

Space biology and astrobiology instruments have similar requirements for autonomous operation and data reduction to facilitate limited data budgets. Thus, AI/ML capabilities will be required in both domains. For example, a life detection instrument onboard a Europa lander mission could easily generate more data than the entire mission data budget. Thus automatic data processing will be essential, just as it is in many BLEO applications. In addition, autonomous decision-making and fault detection are required for autonomous instrument operations as well as exploration capabilities.

4. How can NASA programs evolve to facilitate synergistic opportunities?

With common technology needs, what technology transfer opportunities exist and how can these be enabled? What programmatic flexibility, such as joint funding, can accelerate group across different areas? What educational programs can create cross fertilization between BLEO, physical sciences, astrobiology, planetary science, and other areas?

Feasible research beyond LEO in the years 2025-2035

- Storage of biological or organic materials as components of space instruments or part of biotechnological processes. During the latter portion of this period chemical reagents, potentially including enzymes and microbes, will be selected and instrumented for the detection of biosignatures (on Mars or icy moons). Given the cost of upmass, it would be recommended to identify reagent and instrument needs that overlap in biotechnology, crew health, cell and molecular biology, space agriculture, and the search for extraterrestrial life.

Model organisms relevant to science goals in this area

- Cyanobacteria as nutrient sources, agents of terraforming, role in evolution. The astrobiology community arguably has more cyanobacteria experts than the agriculture/nutrition and space biology communities. Interdisciplinary dialogues might lead to imaginative applications of this family of robust organisms to nutrition, ISRU and habitability goals.
- Space-craft microbes - organisms that readily colonize the spacecraft could be used as models for material science projects to ascertain what efforts are needed to minimize damage to key materials used in spacecraft life support and habitats.
- Inclusion of non-traditional model organisms - Model organisms (*e.g. E. coli, C. elegans, Arabidopsis*) provide valuable insight into biological responses to BLEO conditions but not be fully representative, thus a continued effort to expand model organisms in all facets of biological and physical science research will be recommended.
- Synthetic biology using a range of target organisms for the production of pharmaceuticals, specialized food crops, or microbes to optimize use of in situ resources (e.g. remediation of Lunar regolith).

Technology needs for this science area, 2025-2035

- Applications of AI/ML in this science area include identifying areas where AI-assisted automated experimental systems can be built to benefit cross-disciplinary research purposes; identifying AI analysis techniques in each discipline that can be repurposed for additional analyses in other disciplines to avoid repeating development work; and potentially identifying research questions where it would be useful to build a multi-modal

AI model with data from space biology, astrobiology, and physical sciences to create a combined predictive model of an environment.

- How do life detection, monitoring of human and spacecraft health, and planetary protection concerns align technology development needs, in particular as related to microbial activity assessment and mitigation, for both Planetary Science and BPS missions?

Section I. How can technology/ knowledge from research in the Beyond LEO environment be utilized to benefit humanity on Earth?

The technologies needed for humans to venture and to thrive beyond LEO overlap significantly with those needed to address critical challenges of life on Earth. Here we focus on one common challenge: sustainability. Given today's challenges of feeding and housing the population of Earth, many of whom do not have clean water, adequate nutritious food, reliable electricity, and a safe habitat – the lessons of how to thrive with minimal resource requirements, a necessity for spaceflight missions, should find application on the ground. Acknowledging that budgetary constraints for space missions are vastly different than those to provide necessities on Earth, path-finding developments can nonetheless provide an advanced starting point for low-cost terrestrial technology implementations.

Living on another world is inherently unsustainable: we will not build semiconductor manufacturing facilities beyond Earth anytime soon, yet modern life and space exploration depend upon them. On the other hand, supporting crew beyond LEO does require providing clean, reliable drinking water onboard, and a small remote village has the same need, albeit with a far smaller budget. Sustainability, in particular closing the loop between waste products and resource needs, is critical to beyond-LEO activities; their enablement can offer lessons or a technology starting point, with adjustments likely required for scale and/or cost, for sustainable terrestrial activities.

In the manner that the US Department of Defense has driven improvements to building energy efficiency, renewable resource usage, and, recently, moves towards decarbonization[144], beyond-LEO activities could deliver extensively validated technologies to improve the efficiency of resource utilization on Earth. Variants of technologies to be used in space to recycle air, water, and carbon can be applied to industry and consumer needs on Earth. While we should not imagine this process to be a one-way street, the problems of developing solutions for the space environment can stimulate new ways to look at challenging problems by, in some cases, "solving the harder problem first." In the context of space, this means simultaneous constraints including environmental operating conditions and stringent performance requirements due to the impact of failure. Here we expand upon the potential benefits of beyond-LEO activities for humanity by considering potential benefits in each focus area covered in this report (Table I.1).

Table I.1. Potential Broader Impacts of Beyond LEO Research for Humanity

Section	Relevant Areas	Potential Outcomes Benefiting Humanity
A. Cellular functions	Characterization of cellular damage from space radiation; discoveries relating environment and metabolism to the epigenome.	Countermeasures for DNA damage; improved understanding of how basic cellular mechanisms contribute to disease processes.
B. Microbes and microbial communities	1. Microbes are critical for the health of all life on Earth. The impact of microgravity and radiation on microbes may lead to new insights into interactions between microbes and their environment.	1. Improved knowledge of microbial-environment interactions can be applied to understand the built environment on Earth; novel pathways or products may be identified in space that can be used for terrestrial applications.

	2. Certain microbes can be a hindrance to health on Earth or in space. Resistance to antimicrobial agents (drugs) is altered by the unique space environment, as is microbial virulence; understanding such changes is important to managing infections in crew and utilization of microbes as a resource.	2. Clues and guidance gleaned from BLEO studies of microbial response to antagonistic compounds may inform us on how to modify or develop new drug therapies to prevent unwanted microbes from thriving within an infected human host.
C. Physiology of multi-cellular animals	1. Study of mechanisms of human diseases such as cardiovascular or liver disease in space and development of countermeasures including protective compounds. 2. Understanding the physiological causes and management of the immune function degradation that results from space flight and non-terrestrial habitation. 3. Mitigating the adverse effects of ionizing radiation: countermeasures and mitigations are needed for spaceflight crew to cope with the unique BLEO radiation environment.	1. Improved understanding of disease initiation and progression in space could lead to preventative care on Earth. 2. Adaptation of whole-organism mitigations and countermeasures for the benefit of people on Earth who struggle with compromised immunity. 3. Adapting methods/approaches developed for space crew to terrestrial medicine could be an adjunct to radiation therapy: millions of people are treated with radiation (e.g., for cancer) and must deal with unintended, undesirable consequences.
D. Plant development and physiology	1. Understand the mechanisms of plant survival in harsh environments. 2. Plant studies in space environments demonstrate the value of genetic manipulation and inspire young generations via education outreach activities. 3. Develop sustainable crops for space exploration including dwarf cultivars.	1. Ability to improve plant growth under harsh conditions, especially relevant given climate change. 2. Increased pursuit of beneficial breeding and/or genetic manipulation by the research community. Increased imagination in plant research. Plant studies can be performed anywhere by anyone of all ages. 3. Dwarf cultivars, high-harvest index plants, lignin synthesis control, reduced water requirement, etc. are still important goals in sustainable food crop production on Earth.
E. Host-Microbe interactions	1. Improved understanding of plant-microbe interactions and their contribution to plant health in closed systems. 2. Improved understanding of built environment microbe-host interactions in tightly closed environments. 3. Use of automation and artificial intelligence for microbial tracking of indoor environments to prevent potential pathogens from colonizing eukaryotic hosts.	1. Improvement to sustainable approaches like vertical and indoor farming practices to provide higher crop yields under a range of growing and stress conditions. 2. Improved understanding of built environment could open up new avenues to address global health issues such as antimicrobial resistance. 3. Monitoring the microbial ecology of an environment using automated technologies could reduce spread of infectious diseases in diverse Earth settings such as hospitals, schools, and other built environments.
F. Evolutionary processes	1. Improved understanding of the evolutionary process in response to selective pressures not available or difficult to simulate on Earth, e.g., to predict or control how beneficial microbes or communities can be genetically optimized by guiding/encouraging evolution in helpful directions. 2. Automation developed for in situ analyses in the space environment.	1. Improved understanding of evolution in space environments may yield insights regarding evolutionary processes broadly, which could be applied to infectious disease/public health, synthetic biology, supporting the beneficial gut microbiome, improving biologically-based waste water treatment systems, etc. 2. Automation can be applied to Earth-based research on evolutionary processes to lower costs and enhance throughput.

G. Biotechnological processes	1. Bioproduced pharmaceuticals for deep space, with extreme requirements for simplified downstream processing. 2. Space biotechnologies developed for in-situ resource utilization (ISRU) such as fixing/scrubbing of CO_2. Development of closed-loop processes such as water recycling. 3. Biotechnological processes (e.g. *in-vitro* generation of replacement organs) could conceivably work better in, or take unique advantage of, the space environment. 4. Because of the unique constraints and requirements of the beyond LEO environment, there could be substantial improvements to personalized and precision medicine for astronauts.	1. Low-cost/shelf-stable/just-in-time production of pharmaceuticals or probiotics in a sustainable fashion for Earth applications. 2. Space ISRU applications such as CO_2 scrubbing could contribute to net-zero or net-negative carbon Earth economy and more sustainable resource usage. 3. In-space manufacturing using biotechnological processes could lead to direct benefit to humanity and the further development of a space economy. 4. Improved personalized and precision medicine in space could provide a model for similar advances on Earth.
H. Synergy with physical sciences and astrobiology	More rapid achievement of science goals and more rapid translation of space instrumentation into capabilities with Earth benefits	Greater impact of taxpayer dollars; faster progress towards societal goals supported through NASA-funded research.

Summary of Organisms

Organism / Sample Type	Considerations and Rationale	Section A. Cellular Functions	Section B. Fundamental Microbiology and Ecology	Section C. Multicellular Physiology
Cell Cultures	Allows mammalian and plant experiments without on smaller, less resource-intensive scale than whole-organism experiments. **As on Earth, cell culture enables insights into molecular/cellular mechanism sometimes not possible with whole organisms**	**Human cell types related to HRP risk gaps, e.g. muscle, bone. Differentiated cells, organ-on-a chip, organoids or slices of organoids. Disease models. Plant cells and tissue.**	Not applicable	organ-on-a chip, **organoids or slices of organoids**
Model Bacteria and Archaea	Model organisms come with extensive published understanding of organism characteristics, often with flight heritage and established experimental systems. Some models yield findings generalizable across all domains; others are domain-specific. Sufficient work can enable previously less-studied species/strains to be developed into new model organisms.	**Microbes with well-understood cell biology and processes in common with eukaryotes, e.g. *Escherichia coli, Caulobacter crecentus.***	**Common laboratory models for microbial physiology (*E. coli*, etc.).**	any organisms relevant to astropharmacy
Model Eukarya		Budding yeast (*Saccharomyces cerevisiae*) and other microbial eukaryote models; *Arabidopsis thaliana*	**Common laboratory models for microbial physiology (*S. cerevisiae*, etc.)**	yeasts; small animal eukaryotes, e.g. *Drosophila melanogaster, Caenorhabditis elegans*. **Rodent research capabilities should be developed. Tardigrades are being developed as a model system.**
Organisms Useful for Targeted Functions or Questions	Studies of specific species/strains, biological behaviors or processes of particular relevance to spaceflight and BLEO. Can include non-model organisms	Engineered organisms, e.g. with promoter-reporter constructs, fluorescent protein vector. **Cell cultures derived from specific individuals, e.g. crew members' circulating white blood cells**	Representatives of functional guilds of interest;. stress tolerant microbes. Engineered organisms for synthetic biology. **Diverse non-model organisms isolated from spacecraft / astronaut microbiomes and support systems.**	**emerging models for space biology, e.g. tardigrades. Also: brine shrimp, rotifers (extremophiles); grasshoppers (nutrition and food security), planaria (regeneration), hydra (stem cell aging)**
Co-Cultures	Well-defined model groups of a few different species/strains for studying the effects of the BLEO environment on interactions between organisms.	not yet in scope	Pairs or small groups of organisms with well-defined ecological interactions: symbiosis, commensalism, syntrophy, competition, predation. **Model plants with model microbes.**	Capture data on non-axenic cultures, e.g. nematodes and tardigrades fed in algae or bacteria; small multicellular animals with native microbiomes
Complex Communities	Complex, natural communities relevant to spaceflight whose reactions to the BLEO and extreme built environment that cannot be reliably predicted from reductionist approaches	not yet in scope	Synthetic model communities. Naturally-evolved communities (e.g. soils, microbial mats). Cell cultures of gut, skin, plant with associated microbes. Built space microbiome.	not yet in scope

Organism / Sample Type	Section D. Plant Development and Physiology	Section E. Host-Microbe Interactions	Section F. Evolution	Section G. Biotechnological Processes	Section H. Synergy across fields
Cell Cultures	Plant cell culture; **differentiating cell cultures**	Organs-on-a-chip **(e.g. gut epithelium, kidney, lung, nervous system)**	not applicable	**Mammalian and plant cell cultures for astropharmacy, cultured meat, other applications**	
Model Bacteria and Archaea	Pathogenic and plant growth promoting bacteria; cyanobacteria as alternative primary producer	Open. more applicable to co-cultures and complex communities	**model bacteria relevant to human health and exploration scenarios that also have flight heritage (e.g., *Bacillus, Escherichia, Deinococcus, Pseudomonas, Salmonella*)**	**"Classic microbial factories relied upon for terrestrial biotechnology" (e.g. *E. coli*)**	**Cyanobacteria as nutrient sources, agents of terraforming, role in evolution**
Model Eukarya	*Arabidopsis,* moss species, green algae including *Chlorella*, crop species (see below) as seeds and mature plants	*Arabidopsis*, Crop species (e.g. lettuce, mizuna, peppers) Hydra, Rotifers, C*hlorella,* ***C. elegans*, rodents**	**Green algae (i.e. *Chlorella*); Yeasts and filamentous fungi; small animal eukaryotes e.g. Nematodes and Tardigrades; Small plants e.g. *Brassica* cultivars, *Arabidopsis*.**	**"classic microbial factories" (e.g. *Saccharomyces cerevisiae*)**	**Synthetic biology organisms; new/ non-traditional model organisms**
Organisms Useful for Targeted Functions or Questions	pathogenic and plant-growth-promoting bacteria. **Crops with high harvest index or specific nutritional traits (radish carrot, turnip, potato, greens, dwarf grains). Strains engineered for low lignocellulose. Lineages previously exposed to space stressors (multigenerational studies)**	Probiotics for plants and humans. **crop plants**	**human commensals (*Staphylococcus epidermidis* and *Enterococcus faecalis*); BLSS organisms (cyanobacteria, green algae, bacteria); yeasts; filamentous fungi)**	**Microbes engineered for functions such as bioreporters or information storage; organisms for ISRU functions (e.g. CO2 fixation, biomining). Even fully synthetic microbes.**	**synthetic biology organisms, specialized food crops; new/ non-traditional model organisms**
Co-Cultures	plants with microbiomes	Hydra and algae. Model host-microbe symbiotic systems. Organ-on-a-chip (human cells co-cultured with specific microbes)	**small plants with the potential for multi-generational and plant-microbe interaction studies (e.g., *Azospirillum* with algae, *Brassica* cultivars, *Arabidopsis*)**	**synthetic microbial consortia 3-D printed organs**	**synthetic microbial consortia**
Complex Communities	Microbiomes of soils, plant, and built environment. May be experimentation or just tracking.	Gut- skin-, plant- and built-microbiome; Termites	**microbiomes of humans, plants, built environment**	**Potentially biofouling communities; ECLSS communities**	**spacecraft microbes**

Summary of Technology Requirements

Technical requirements are formulated to enable answering the seven designated top-level science questions plus potential benefit areas beyond Space Biology -Beyond LEO science

A. Cells: measure cell structural changes, cellular functions, mutation rates, gene expression, altered cellular metabolism, oxidative stress, DNA repair and damage
B. Microbes: characterize ecologies, phenotypes, and dynamics of microorganisms, microbial communities, microbial ecosystems
C. Animals: track physiological status and changes in multi-cellular systems
D. Plants: follow plant development, monitor plant physiology/function, plant-microbial ecosystems
E. Host-microbe: assess host - microbe interactions
F. Evolution: track the evolutionary process (nucleic acid sequencing and related technologies)
G. Biotechnology/synthetic bio: implement and monitor biotechnological processes including synthetic biology and precision fermentation
H. Synergy of SB with others: identify synergistic benefits to space bio (other than BLEO), astrobio, ISRU, and other NASA programs
I. Benefit to humanity: identify new SB-BLEO technologies' benefits to humanity

Matrix of enabling technology capabilities for the 7 Science Questions, plus Autonomy

Requirement / Capability (see additional details below)	**Need per Science Question**						
	A	B	C	D	E	F	G
1. Support stasis of biological samples	✓	✓		✓	✓	✓	✓
2. Support growth/metabolic activity	✓	✓	✓	✓	✓	✓	✓
3. Provide reagents, drugs, agonists, etc.	✓	✓	✓	✓	✓	✓	✓
4. Processing capabilities	✓	✓	✓	✓	✓	✓	✓
➢ Chemostat / passaging capabilities (multi-gen.)	✓	✓			✓	✓	✓

➢ Preparation for analyses	✓	✓		✓ (gray)	✓ (gray)	✓	✓
➢ Storage/fixation/preservation	✓	✓		✓	✓	✓	✓ (gray)
5. Monitor the ambient (radiation, illumination, etc.)	✓	✓	✓	✓	✓	✓	✓
6. Measure biological processes / outcomes	✓	✓	✓	✓	✓	✓	✓
➢ Populations	✓	✓	✓ (gray)	✓	✓	✓	✓
➢ Reporters (single, multiple)	✓	✓	✓	✓	✓	✓	✓
➢ Imaging	✓	✓	✓	✓	✓	✓	✓
➢ Spectroscopy	✓	✓	✓ (gray)	✓	✓	✓	✓
➢ Molecular parameters	✓	✓	✓	✓	✓	✓	✓
➢ Physiological parameters	✓	✓	✓	✓	✓	✓	✓
➢ Omic analyses	✓	✓	✓	✓	✓	✓	✓
7. Autonomous experimental capabilities	✓	✓	✓ (gray)	✓	✓	✓	✓
➢ Fixed, pre-defined operational sequence	✓	✓		✓	✓	✓	✓
➢ Responsive autonomous decision-making	✓	✓	✓ (gray)	✓	✓	✓	✓
➢ Onboard autonomous data analysis	✓	✓	✓	✓	✓	✓	✓

List of requirements and capabilities (from matrix above) with additional details

Many are common (Black ✓ in matrix above), or may apply at times (Gray ✓ in matrix above), to many or all classes of life science experiments.

1. Support stasis of biological samples: pre-launch storage until start of science operations

- provide methods and supportive environments for biological specimen stasis
- durations of days to months (delivery/integration, pre-launch, transit, deployment, commissioning/check-out)

2. Support growth/metabolic activity throughout science experiments in a **biocompatible** environment that provides:

- media/nutrients, (dissolved) gases; waste management
- physical containment
- illumination for plants, (micro)algae
- pH, ionic conductivity, temperature, pressure, humidity control
- through stages of growth, division, reproduction; multi-generational if required

3. Provide critical reagents

- drugs / agonists, stains/dyes
- reagents for analytical processes
- standards, controls, reagents to support analytical measurements
- at specified concentrations/doses

4. Provide processing capabilities

- sample prep for analytical processes
- homogenization, lysis, capture, clean-up, concentration, desalting, filtration, etc.

5. Monitor the ambient

- radiation (dose/spectrum/flux; ionizing and UV/visible)
- temperature, pressure, humidity, gases (esp. O_2, CO_2)

6. Measure the biological processes / parameters of interest

- from single reporters to multiplexed measurements to -omic analyses
- for monocultures or some/all members of communities/ecologies
- measurement frequency according to anticipated rates of change (and method)
- General and physical measurement parameters
  - Morphology, morphometry
  - Overall metabolic activity, metabolic products
  - Photosynthetic activity/efficiency
  - Cell/organism population, replication
  - Reproductive processes
  - Cell membrane physical integrity/morphology
  - Cell/organism/community size/morphology
  - Pairwise and multi-partner interactions between cells, organisms, proteins

▪ Molecular parameters

- DNA sequence (incl mutations), RNA expression
- epigenetic modifications e.g. DNA methylation
- protein expression & state (incl post-translation mods)
- metabolites / physiological indicators
- receptor / ion channel status/expression

7. Autonomous experimental capabilities

- May utilize emerging methods in artificial intelligence / machine learning
- Required in certain circumstances:
  - Uncrewed platforms / limited crew availability
  - Ground science team contact intermittent / of limited-duration
  - Data volume / downlink bandwidth limitations
- when following a fixed, pre-defined operational sequence:

    - anomaly recovery / resiliency still necessary, may involve ground analysis and uplink of sequence modifications
    - manual override of predefined sequences / conditions typically possible
- when experimental control uses fully autonomous, responsive decision making:
    - more complex / long-duration experiments may be feasible
    - developing control software & debugging is far more costly, complex
- Onboard autonomous data processing and analysis
    - simple lossless data compression
    - "quick look" abbreviated summaries of key measurements
    - autonomous generation of science results via sophisticated ML/AI methods
    - large data volumes may return to Earth on a longer timescale than processed results due to downlink bandwidth constraints

BLEO Feasibility and Technology Availability

- A significant portion of the technology needs identified above, along with many of their existing and potential solutions, have been described elsewhere
- Most of the above are feasible in the next 15 years, with constraints on organism types, measurement duration, extent of analysis (e.g., not -omics analyses will be limited in scope/extent)
- Existing technologies already practiced in space flight form a basis for meeting many of the above requirements (microscopes, bioreactors/culturing systems, environmental management systems, centrifuges, cameras, sensors, meters, analyzers, spectrometers, etc.)
- Minor, moderate, or extensive development needs exist for each capability
- As experiments are specified within each experiment class, specific hardware items can be mapped onto the experiment matrix

Building and sustaining the scientific community

As a new programmatic direction, conducting biology experiments beyond Low Earth Orbit (BLEO) requires maintaining a vibrant, diverse, and robust scientific and technical community for the longevity of the program. This effort must be multi-faceted, including increased focus on personnel recruitment and retention, facilitation of cross-divisional project teams (e.g. engineering, data sciences, and life sciences), emphasis on open science principles for data sharing and citizen science to maximize return on investment, and modernized code and software sharing abilities when possible.

Sustainability will require careful consideration of how to recruit and retain excellent scientists and engineers with unique capabilities to the program. Traditionally, space biology is predominantly driven by a bottom-up approach with principal investigators writing proposals to specific science questions. Alternative funding mechanisms focused on recruiting and retaining people could be considered (e.g. career development awards, such as utilized by other funders, or mission and payload team awards as used by planetary sciences). A key challenge, as with most science, is that junior investigators are particularly adversely impacted by breaks in funding and publication. In this regard, with NIH being a more reliable and larger source of funding for biomedical researchers, consideration should be given to how to make NASA more competitive for retaining investigators vs. losing them to NIH; a joint NIH/NASA career development path might be an interesting option.

An effort such as BLEO biological research will be most successful if we limit siloing of expertise, information and data silos. Lack of communication between engineers and biologists has often led to payload delays or failures, and lessons learned not being shared beyond an individual payload team. Sometimes cross-divisional communication and efforts have limited individual reward(s) and may therefore be de-incentivized. A top-down, science community driven, programmatic shift to encourage and allow cross-disciplinary collaboration when appropriate would greatly strengthen this effort. Such a shift could bring BLEO biological research in line with other BLEO researchers within the Science Mission Directorate (SMD). However, such a shift would pose a challenge to recruiting and retaining investigators vs. the, largely, bottom-up approach employed by NIH. Clearly there is a balance to be struck to enable top down, science community driven, BLEO “flagship missions” and bottom up, individual PI driven experiments. Drawing from other SMD BLEO work, a mission and payloads model that also incorporates data sharing and funding for data analysis may be an option.

Similarly, increased use of open science approaches can help improve recruitment, retention, and increase diversity. This includes continuing and expanding the current policies of data sharing, and also expanding the citizen science efforts and education, training and outreach.

Finally, a barrier to rapid software development and collaboration is the set of requirements surrounding publishing code. Most biological data analysis takes place with open-source libraries. In most research settings, making code open-source in real-time on GitHub is standard procedure and supports open science principles. Building and sustaining a productive and supportive scientific community surrounding the BLEO space biological efforts will benefit greatly from the ability to collaborate at a rapid pace.

“In the cold, dark depths of space
Where light does not reach
A small spacecraft drifts
In a lonely, endless reach

Inside its walls, life thrives
Microbes growing and multiplying
In the warmth and moisture
Of their self-made paradise

They live and breathe
And flourish and grow
In this tiny, closed ecosystem
They have come to know

But outside the ship, the void
Is all that can be seen
No stars or planets
No life, no green

Yet still the microbes thrive
In their lonely, confined space
A testament to life's resilience
In the most inhospitable place.”

-Generated by AI from the prompt: Write a poem about lonely microbes growing inside a small spacecraft in deep space

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