Research Campaign

Bioregenerative Life Support Systems: Coordinated Research into Organisms, Technology and Systems Integration

Primary Author: Jane Shevtsov, 731-432-6797, UCLA, jane.eco@gmail.com

Co-Authors:

Christine Escobar, Space Lab Technologies, LLC, chris@spacelabtech.com

Andrew C. Schuerger, Univ. of Florida, Gainesville, FL, chuerg@ufl.edu

Christophe Marcel Trouillefou, Université de Montpellier, christophe.trouillefou@gmail.com

L.K. Tuominen, Sole Proprietor, Cleveland, OH, lktuominen@outlook.net

Raymond Wheeler, NASA Space Life Sciences Laboratory, Kennedy Space Center, raymond.m.wheeler@nasa.gov

Co-Signers:

Nathan O'Neil, University of Florida, Gainesville, FL

Dragos G. Zaharescu, University of California Davis, CA

Oscar Monje, Amentum, Kennedy Space Center, FL

Münire Gül, Arke Biyoteknoloji and Arge A.Ş, Turkey

Introduction. An Environmental Control and Life Support System (ECLSS) for spacecraft satisfies the physiological needs of the crew by revitalizing the atmosphere, maintaining temperature and humidity, providing food and water, and removing wastes. As we travel further beyond low Earth orbit, the increased cost of resupply and resource constraints (e.g., volume, power, and crew time) will necessitate life support systems with higher efficiency, autonomy, and mass closure than the physicochemical (PC) systems in use today[1, 2]. NASA's technology roadmap states that self-sufficient life support systems are crucial for sustaining life on long-duration missions[3]. Several NASA needs assessments identify closed regenerative life support as an enabling technology for long-term sustained human exploration, including the Lunar Human Exploration Strategic Knowledge Gap (SKG III-J-3), Decadal Survey on Biological and Physical Sciences in Space Studies (DSBPS TSES6 and P3), NASA 2020 Technology Taxonomy (TX06.3.5), and Global Exploration Roadmap.

Just as on Earth, living organisms can provide multiple life support functions in space, by recycling waste products to generate O_2, water, and food. Living systems can reproduce and self-repair, allowing continuous functioning. Organisms, especially plants, also have a positive psychological impact on crew[4]. Space agencies have researched the use of plants for life support and supplemental food production for decades, making bioregenerative life support systems (BLSS) one of the most enduring themes for space life science research[5 - 16].

BLSS development, closure, and capacity must evolve with exploration mission duration, distance, and complexity, with a phased approach. Near-term missions will demonstrate key concepts and validate components in the space environment while ground analogs test integrated technologies. BLSS components can eventually integrate with more permanent habitation systems[17, 2]. Efficient and reliable space life support will require integration of biological and PC components into an engineered ecosystem that sustains the crew and itself. This paper discusses and recommends critical areas of research and development at organismal, system, and technology levels to realize space-viable biological systems for space life support.

I. Organism-Focused Questions

Organism-focused questions have received the most attention in US BLSS research in recent decades. However, important questions remain regarding integration of both plants and microbial decomposers into BLSS design. The space environment differs from the terrestrial one, with fractional gravity or microgravity, reduced atmospheric pressure, elevated radiation, and biological isolation. How various plant and microbial taxa respond to these changes, and which taxa can thrive under such conditions, are major questions.

IA. Selecting and Combining Plants for Space Exploration

Plants are key to BLSS, as photosynthesis produces O_2 and removes CO_2 from the atmosphere. Because plant taxa differ in rates of photosynthesis, gas exchange, water consumption, nutrient use, and edible biomass production, selecting taxa for BLSS design is likely to be driven in large part by mission scenarios. Furthermore, diverse assemblages of species are likely to yield more robust system functioning, as well as healthier astronaut diets, than monocropping. In this context, NASA and the space life sciences research community could advance its mission by supporting research on the development of "constructed fit" plant communities for specific mission contexts, including (a) continued ecophysiological research, (b) crop selection and development, and (c) novel research exploring the functioning of species assemblages. During the next 10 years, missions to low Earth orbit and lunar exploration will allow *in-situ* research. Goal (a) requires Earth-based plant research to test the effects of Lunar and Martian environments (or other mission contexts) on the ecological function of selected taxa. Centrifuge or other partial gravity simulation studies that establish BLSS function for planetary surfaces will be particularly valuable. Historically, crop selection (b) has focused on small plants or 'dwarf' cultivars, sometimes with rapid life cycles[18 - 20], largely through conventional breeding and mutant screening. Crop screening criteria can expand to include robustness to spaceflight environment stressors and nutritional quality by taking advantage of genetic markers, gene editing, and genetic engineering[21 - 23]. We may also be able to engineer plants to produce other products, including building materials, pharmaceuticals, or high-value chemicals[24]. Assessment of which taxa function well together in space systems (c) continues to be a gap in space life sciences research. The Lunar Greenhouse Prototype project has advanced BLSS polyculture[25, 26]; however, studies of the performance of combinations of taxa and the ecological stoichiometry that maximize crop production efficiency and robustness are little studied. For example, use of crops with different growth forms, life cycle lengths, and nutrient acquisition strategies may improve light and nutrient use efficiencies, both critical factors for spaceflight [1]. Evaluating impacts of allelopathic chemicals could also inform compartmentation of incompatible crops[27].

IB. Microbial Environments

A healthy plant microbiome is key to BLSS performance, health, and stability, while an unhealthy microbiome can hinder plant growth and health. BIOS-3 and Biosphere 2 experiments showed significant effects of microbial communities on atmospheric dynamics, trace contaminant control, and plant disease resistance[28]. Beneficial microbes may require active assistance, while pathogens must be detected and mitigated to avoid crop loss[29]. The introduction, dispersal, and virulence of phytopathogens must be assessed within the International Space Station (ISS) as proxies for the same processes in future crewed missions to the Moon and Mars. Information is needed about the stability and viability of microbial communities under long term isolation and the effects of microbiome composition on crop health and performance[16]. The physiological, evolutionary, and ecological effects of stressors such as radiation, low gravity, and poor gas mixing on microbial communities must be understood to facilitate the design of microbiomes beneficial to plants and the BLSS ecosystem. Microcosm experiments in relevant analog or spaceflight environments will illuminate the effects of species diversity and composition on microbial community health and stability.

1C. Linking Nontraditional Food Sources with BLSS Design

A large portion of plant biomass grown for BLSS is inedible and can be put to new uses. Ideally, this biomass would diversify astronaut diets, enable nutrient cycling, and provide high value materials such as medicines, manufacturing materials, or energy feedstocks. For example, insects such as silkworms, mealworms, and crickets could become a resource-efficient protein source, as they take up little space and consume materials inedible to humans[30]. In many Asian, South American, and African cultures, insects are a major source of protein and micronutrients[31, 32]. Combining cellulosic biomass, regolith, and microbes (including fungi) to build soil and provide food is another example of sustainable resource reuse[33, 34]. Efforts at *in situ* resource utilization could also be integrated with cellulosic biomass reuse, creating opportunities for additive manufacturing. System-level research in diversion of plant biomass from the main producer - consumer - decomposer loop for such purposes is a prerequisite to defining criteria for sustainable long-term habitation in Lunar and Martian mission contexts. If diversion of inedible biomass for uses that sequester carbon are unsuited to mission context, it could instead be used as a feedstock for bacteria engineered to produce necessary or high value products. Cell culturing technology to produce meat in space is potentially valuable[35 - 37]. Aquaculture is another multifunctional BLSS strategy for Lunar and Martian missions. For example, duckweed (*Lemna sp.*) and the fern *Azolla* can potentially provide both food and wastewater purification function[38, 39]. Farming detritivorous fish would be another efficient way of producing food rich in micronutrients and protein[40]. Research on non-traditional organisms would expand options for BLSS design across mission contexts.

Recommendation: Research on interactions among plants, microbes and other organisms.

II. Integration-Focused Questions

BLSS systems are by definition ecological systems[28]. System integration-oriented research questions encompass the architecture, control, stability, and efficiency of integrated BLSS components. In the US, system-level studies were largely abandoned around the turn of the century, although research continues to advance in China (Lunar Palace) and, to a somewhat lesser degree, Russia (BIOS-3) and Europe (MELiSSA). Critical research areas for the next decade include the costs and benefits of closure, improved nutrient recycling methods, and stability and control in various configurations and at different scales.

IIA. Degree of Closure

Decisions about life support system design are largely driven by equivalent system mass (ESM) over the mission duration[41], reliability constraints[42], and the feasibility of resupply from Earth. Closure is the portion of expended substance that is recycled (and not stored) per unit time, reflecting the ability of a BLSS to continue functioning without resupply. Regenerative technologies that increase closure may reduce BLSS ESM over time, but their complexity and low maturity may decrease reliability. The tradeoff between closure and reliability may be countered by the resilience of biological components afforded by their ability to reproduce and self-regulate. The costs and benefits of increased closure depend on mission duration and distance from Earth, which drive the ease and cost of resupply. Future missions will require continuous reassessment of cost-benefit ratios of closure for each consumable resource (e.g., oxygen, nitrogen, or water) as technologies improve over time. Closure cost-benefit analysis requires models of BLSS behavior. Improvements in nutrient recycling will increase closure.

IIB. Nutrient Cycling

BLSS experiments thus far have not fully closed the nutrient loop between food production and waste mineralization. For near-term missions, plant nutrients can be supplied with fertilizer. For longer missions, this will become costly and impractical, so nutrients need to be recycled from wastes such as urine, feces, and inedible plant biomass. The most effective means of recycling inedible biomass remains unclear. Microbial bioreactors (e.g. aerobic or anaerobic digestors) and plant-microbe ecosystems are proven, safe waste oxidation methods. Bioreactors that retain bioavailable N forms are especially attractive due to efficiency gained by avoiding denitrification/fixation[44]. Human feces and inedible plant biomass can both be recycled through thermophilic composting, producing a rich plant growth medium[45 - 47]. Development of safe and effective composting methods suitable for space, including supplemental pathogen reduction methods (e.g., irradiation or bacteriophages) should be investigated. Plant growth media should support nutrient recycling. Composting or other forms of decomposition (e.g. pyrolysis[48]) are only useful if the nutrients made available by the process return to plants. A range of coupled plant growth and waste recycling technologies should be co-developed, including artificial soil from in-situ regolith. Urine is a key challenge in meeting human and plant needs in a BLSS, as it is nutrient-rich but presents a particular challenge for recycling because its relatively high sodium levels can harm plants. Most plants actively restrict sodium uptake, creating a problem for recycling urinary sodium. However, some salt-tolerant plants and seaweeds accumulate higher amounts of sodium and are consumed in some cultures. Mesophilic halophiles have been used in to treat a waste stream and separate salt and biomass[49], and their biomass can be turned into a biochar substrate for plant media[50].

IIC. Stability and Control

The main difference between a space habitat BLSS and an Earth ecosystem is the rapid material cycling rates imposed by the constrained habitat volume, which affect stability. In a regenerative life support system, the stability and controllability of ecosystems are critical to reliability and resilience for crew survival, health, and performance. Fundamental research areas include 1) the definition and quantification of stability, 2) the identification, optimization, and control of factors contributing to stability and 3) development of mathematical and physical models to simulate and test control of BLSS systems.

In a space habitat, the critical state variables are those that keep the crew alive and healthy (e.g., CO_2 and O_2 partial pressure, air quality, and availability of nutritious food and potable water). The system composition or configuration can change to maintain the desired state of habitability. In an engineering context, this dynamic conception of stability might be described as resilience or robustness[51]. Any mass lost from the system, either through leakage or becoming unusable, must be replaced to avoid eventual system failure. Systems of differential equations describing mass flow between components allow stability analysis and quantification but the desired states and potential disturbances warrant further definition.

After defining stability in the context of space habitats, the next questions are (a) what features ensure stability and (b) what control parameters can be used to achieve it? Many ecologists theorize that species diversity enhances stability[52, 53]. Engineers tend to say stability requires accurate and prompt control of processes maintaining material cycles[54]. Literature indicates that the answer remains unclear[51, 55, 56]; however, research should combine modern ecology with engineering principles and develop ways to control ecosystem parameters[57 - 59].

Control systems regulate environmental parameters (like temperature and air flow) that drive biological processes (like photosynthesis) and keep life support parameters within an appropriate range. The regulation of biological processes, commonly practiced in growth chambers and bioreactors, must be applied on a whole-system level to achieve environmental stability within the habitat. Developing such control systems will allow biological systems to be reliably integrated with technological ones. Process control and architecture optimization (e.g., component integration and species selection) require high fidelity BLSS models (both mathematical and physical prototypes). Such models can help life support system designers understand the effects of residence time, buffering capacity, cycling rates, and trace contaminant buildup on system behavior and stability[60]. In addition, designers must understand system behavior across a variety of configurations and scales, so small scale systems designed for near term missions can evolve into larger scale systems for space settlements[28]. Incorporation of detailed process models into environmental control algorithms can also improve system stability. Open source BLSS modeling tools and digital twin development (virtual models integrated with the physical system and sensor networks) will go far to facilitate such studies.
Recommendation: Prioritize research on BLSS modeling, stability and control.

III. Technology Questions

A "combined approach of fundamental research, hardware development, and operational testing is required to achieve reliable bioregenerative life support systems"[17] and integration with spacecraft habitats. Technological research goals in the coming decade include miniaturizing and ruggedizing autonomous monitoring and control hardware and building test facilities to support long term, relevant environment testing of integrated BLSS and PC systems.

IIIA. Reliability and Robustness of Autonomous Monitoring and Control Systems

The reliability of biological components is limited by the reliability of the hardware and software that regulates their environment (e.g., temperature, light, or air flow). The need for such robustness increases with mission duration and distance from Earth due increased cost of failures and their prevention (e.g., mass of spare parts) and reduced ability to abort to Earth. Autonomous monitoring and control systems with state of the art robotics, sensors, machine learning, and artificial intelligence can improve BLSS reliability, efficiency, and resilience by detecting and responding to off-nominal events. Novel sensing technologies continue to emerge in the fields of optics, spectroscopy, electro- and biochemistry, electromagnetics, multi-omics, and biosensing that can monitor organism and ecosystem health and stress. Desirable sensors are compact; non-invasive; low power; respond rapidly; and operate with little to no crew time or consumables (e.g. reagents)[29]. Terrestrial advances in autonomous controlled environment agriculture (CEA) are outpacing space technology. However, terrestrial systems are often too massive, too energy intensive, or intolerant of the space environment. Research and development is needed to minitiarize and ruggedize terrestrial CEA robotics, sensors, and data handling systems for high levels of radiation and CO_2, fluctuating pressures, and variable gravity[29]. In particular, microgravity or partial gravity compatibility remains a significant challenge for reliable fluid control. Finally, validated system performance models are needed to inform hardware architecture design and component selections for improved reliability.

IIIB. Test Facilities for System Integration and Relevant Environment Testing

A cross-cutting need in BLSS research is high fidelity testbeds that can simulate the conditions that would be encountered in space, prior to flight testing. Relevant environment test facilities with tight closure, recirculated air, and pressure control allow 1) studies of gas exchange and balance; 2) testing of biological component integration and interoperability with existing PC technologies or other spacecraft systems; 3) assessment of component arrangements, such as spacing for succession planting and multi-cropping to improve volume efficiency; and 4) long-duration studies of system stability and sustainability under increasing levels of closure. International BLSS integrated test facilities include ESA's bioregenerative test bed for the MELiSSA Project[61], Russia's Bios 3 facility[62], Japan's Closed Ecological Experiment Facility[63], German Aerospace Center's (DLR) food production analog (EDEN-ISS)[64], and China's Lunar Palace test facility. Lunar Palace recently completed a one year long "human in the loop" test[30, 65]. NASA had a large, closed plant production chamber with connected waste processing capabilities, called the Biomass Production Chamber[16], but this facility was decommissioned ca. 2000. Currently, NASA has no full scale, closed integrated test facilities for bioregenerative life support research. Because component-level testing cannot provide an understanding of emergent system-level properties, development of a flight-ready, first-generation, space-based BLSS module for deployment in Earth or Lunar orbit by 2032 is critical to advancement of the above research priorities. Technological improvements in automation and AI afford rapidly accelerated modeling and hypothesis testing via machine learning and more adaptive control systems for dynamic, coupled processes. Now is the time to merge biological and ecological knowledge gained since the 1960s with massive sensor networks and computational models that will allow us to rapidly improve design, function, monitoring, and control of BLSS in space.

References

1. Drysdale, A.E., S. Maxwell, M.K. Ewert, and A.J. Hanford. 2000. Systems analysis of life support for long-duration missions. Soc. Automotive Eng. Tech. Paper 2000-01-2394.
2. Douglas, G. L., Wheeler, R. M., & Fritsche, R. F. 2021. Sustaining Astronauts: Resource Limitations, Technology Needs, and Parallels between Spaceflight Food Systems and those on Earth. Sustainability, 13(16), 9424.
3. Kliss, Mark. Understanding the NASA Ta6: Human health, life support, and habitation systems technology roadmap. 46th International Conference on Environmental Systems, 2016.
4. Bates, S., V. Gushin, G. Bingham, A. Vinokhodova, J. Marquit, and V. Sytchev. 2009. Plants as countermeasures: A review of the literature and application to habitation systems for humans living in isolated or extreme environments. Habitation 12(1): 33-40.
5. Myers, J. 1954. Basic remarks on the use of plants as biological gas exchangers in a closed system. J. Aviation Med. 25: 407-411.
6. Pilgrim, A. J., and S. P. Johnson. 1962. Investigation of selected higher plants as gas exchange mechanisms for closed ecological systems. Boeing Co, Seattle WA.
7. Ward, C.H, S.S. Wilks, and H.L Craft. 1963. Use of algae and other plants in the development of life support systems. Amer. Biology Teacher 25: 512-521.
8. Taub, F.B. 1974. Closed ecological systems. *In*: R.F. Johnston, P.W. Frank, and C.D. Michener (*eds*.) Ann. Rev. Ecology Systematics. Annual Reviews Inc., Palo Alto CA: 139-160.
9. Olson, R. L., E. A. Gustan, and T. J. Vinopal. 1984. CELSS transportation analysis. Advances in Space Research 4.12: 241-250.
10. Kliss, M. and R.D. MacElroy. 1990. Salad machine: A vegetable production unit for long duration space missions. SAE Tech. Paper 901280. Williamsburg, VA, USA.
11. Salisbury, F.B., J.E. Gitelson, and G.M. Lisovsky. 1997. Bios-3: Siberian experiments in bioregenerative life support. BioScience 47: 575-585.
12. Ferl R, Wheeler R, Levine HG, Paul AL. 2002. Plants in space. Current opinion in plant biology 5(3): 258-263.
13. de Micco, V., Aronne, G., Joseleau, J. P., & Ruel, K. 2008. Xylem development and cell wall changes of soybean seedlings grown in space. *Annals of botany*, *101*(5), 661–669, https://doi.org/10.1093/aob/mcn001
14. Wheeler, Raymond M. 2010. Plants for human life support in space: from Myers to Mars. Gravitational and Space Research 23 (2).
15. Ruyters, G., and M. Braun. 2014. Plant biology in space: recent accomplishments and recommendations for future research. *Plant Biology* 16: 4-11.
16. Wheeler, R.M. 2017. Agriculture for space: People and places paving the way. *Open Agriculture* 2: 14-32.
17. Wheeler, R. 2009. Roadmaps and Strategies for Crop Research for Bioregenerative Life Support Systems. *NASA Technical Memorandum*, 214768.
18. Massa, G.D., N.F. Dufour, J.A. Carver, M.E. Hummerick, R.M. Wheeler, R.C. Morrow, T.M. Smith. 2017. VEG-01: Veggie hardware validation testing on the International Space Station. Open Agriculture 2: 33-41.

19. Bugbee, B., and G. Koerner. 1997. Yield comparisons and unique characteristics of the dwarf wheat cultivar “USU-Apogee.” Advances in Space Research 20(10): 1891-1894.
20. Williams, P.H. and C.B. Hill. 1986. Rapid-cycling populations of *Brassica*. 232(4756): 1385-1389.
21. Wang, J., Wei, L., Zheng, T., Zhao, X., Ali, J., Xu, J., and Z. Li. 2014. Simple sequence repeat markers reveal multiple loci governing grain-size variations in a *japonica* rice (*Oryza sativa* L.) mutant induced by cosmic radiation during spaceflight. *Euphytica* 196: 225-236.
22. Mahadev Shelake, R., Pramanik, D., and J-Y. Kim. 2019. Evolution of plant mutagenesis tools: A shifting paradigm from random to targeted genome editing. *Plant Biotechnology Reports* 13: 423-445.
23. Im, Y.J., Killens, R., Lee, A., Ji, M., Lowder, C., Grunden, A.M., and W.F. Boss. 2010. Redesigning plants for spaceflight and beyond: Transfer of genes from *Pyrococcus*. Gravitational and Space Research 23(2): 39-48.
24. Haveman, N., Settles, M., Zupanska, A., Graham, T., Link, B., Califar, B., Callaham, J., Jha, d., Massa, G., McDaniel, S., Parmar, C., Tucker, R., Wheeler, R. 2021. Elevating the Use of Genetic Engineering to Support Sustainable Plant Agriculture for Human Space Exploration. A Topical White Paper for Submission to the Biological and Physical Sciences in Space Decadal Survey 2023 - 2032.
25. Kacira, M., Giacomelli, G.A., Patterson, R.L., Furfaro, R., Sadler, P.D., Boscheri, G., Lobascio, C., Lamantea, M., Wheeler, R.M., and S. Rossignoli. 2011. System dynamics and performance factors of a Lunar Greenhouse prototype bioregenerative life support system. *Acta Horticulturae* 952: 575-582.
26. Patterson, R.L., Giacomelli, G.A., Hernandez, E., Yanes, M., and T. Jensen. 2014. Poly-culture food production and air revitalization mass and energy balances measured in a semi-closed lunar greenhouse prototype (LGH). Tucson, Arizona: 44th International Conference on Environmental Systems, 13-17 July 2014. ICES-2014-167.
27. Stutte, G.W. 2006. Process and product: Recirculating hydroponics and bioactive compounds in a controlled environment. HortScience 41(3): 526-530.
28. Escobar, C.M. and J.A. Nabity. 2017. Past, present, and future of closed human life support ecosystems - A review. *Intl. Conf. Environ. Systems* ICES-2017-311.
29. Escobar, C., Altaf, N., Barker, R., Bhuiyan, M., Correll, M., Fritsche, R., Humphrey, S., Jaiswal, P., Lantin, S., Larkin, E., Price, A., Tabetah, M., Toma, C. 2021. Research Campaign - Artificial Intelligence for Autonomous Space Plant Production. A Research Campaign White Paper for Submission to the Biological and Physical Sciences in Space Decadal Survey 2023 - 2032.
30. Fu, Y. L. Li, B. Xie, C. Dong, M. Wang, B. Jia, L. Shao, Y. Dong, S. Deng, H. Liu, G. Liu, B. Liu, D. Hu, and H. Liu. 2016. How to establish a bioregenerative life support system for long-term crewed missions to the Moon and Mars. Astrobiology 16(12) DOI:10.1089/ast.2016.1477.
31. Baiano, A. 2020. Edible insects: An overview on nutritional characteristics, safety, farming, production technologies, regulatory framework, and socio-economic and ethical implications. *Trends Food Sci. Technol.* **100**, 35–50.
32. Dobermann, D., Swift, J. A. & Field, L. M. 2017. Opportunities and hurdles of edible insects for food and feed. *Nutr. Bull.* **42**, 293–308.

33. Gros, J.B. and Lasseur, Ch. and Tikhomirov, A.A. and Manukovsky, N.S. and Kovalev, V.S. and Ushakova, S.A. and Zolotukhin, I.G. and Tirranen, L.S. and Karnachuk, R.A. and Dorofeev, V. Yu. 2005. Testing Soil-like Substrate for Growing Plants in Bioregenerative Life Support Systems. Advances in Space Research 36(7): 1312-1318.
34. He, W., Liu, H., Xing, Y., Jones, S.B. 201. Comparison of Three Soil-like Substrate Production Techniques for a Bioregenerative Life Support System. Advances in Space Research 46(9): 1156-1161.
35. Benjaminson, M.A., Gilchriest, J.A. , Lorenz, M. 2002. In vitro edible muscle protein production system (MPPS): Stage 1, fish. Acta Astronaut. 51: 879–889. https://doi.org/10.1016/S0094-5765(02)00033-4
36. Stephens, N. ., Di Silvio, L. ., Dunsford, I., Ellis, M ., Glencross, A. ., Sexton, A. 2018. Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in cellular agriculture, Trends Food Sci. Technol. 78: 155–166. https://doi.org/10.1016/j.tifs.2018.04.010.
37. Bernard, M., Przybyla, C., Laurand, X., Rollin, B., Gasset, E., Dutto, G., Averseng, J., Dusseau, L., Unch, LA. 2019. Vibration on fish Embryo (LAUVE) Project, Grenoble Newsp. Week - Exobiol. https://gnsw.sciencesconf.org/266306 (accessed February 10, 2020).
38. Liu, X., Min, C., Xia-shi, L. & Chungchu, L. 2008. Research on some functions of Azolla in CELSS system. *Acta Astronaut.* **63**, 1061–1066.
39. Escobar, Christine; Escobar, Adam. 2017. Duckweed: A Tiny Aquatic Plant with Enormous Potential for Bioregenerative Life Support Systems. *47th Int. Conference on Environmental Systems.*
40. Fry, Jillian P., Mailloux, Nicholas A., Love, David C., Milli, Michael C., Cao, Ling. 2018. Feed conversion efficiency in aquaculture: do we measure it correctly? *Environ. Res. Lett.* **13** 024017.
41. Levri, J. A., Vaccari, D. A., and Drysdale, A. E. 2000. Theory and Application of the Equivalent System Mass Metric. SAE Technical Paper No. 2000-01-2395.
42. Jones, Harry, and Michael Ewert. 2010. Ultra Reliable Closed Loop Life Support for Long Space Missions. 40th International Conference on Environmental Systems.
43. Todd and Josephson. 1996. The Design of Living Technologies for Waste Treatment. Ecological Engineering 6: 109-136.
44. Smith, D., Garland, J. & Rector, T. 2006. A Novel Membrane Bioreactor for Spacecraft Water Recycling. in Earth & Space: 1–6, American Society of Civil Engineers Conference, doi:10.1061/40830(188)46.
45. Chynoweth, D. P., Owens, J. M., Teixeira, A. A., Pullammanappallil, P., and Luniya, S. S. 2006. Anaerobic digestion of space mission wastes. Water Science and Technology 53 (8): 177–185, doi: 10.2166/wst.2006.248.
46. Abhishek S. Dhoble. 2014. Design and operation of an anaerobic digester for waste management and fuel generation during long term lunar mission. Advances in Space Research 54(8): 1502–1512, doi: 10.1016/j.asr.2014.06.029.
47. Pickett, M. T., Roberson, L. B., Calabria, J. L., Bullard, T. J., Turner, G., and Yeh, D. H. 2020. Regenerative water purification for space applications: Needs, challenges, and technologies towards ‘closing the loop’. Life Sciences in Space Research 24: 64–82, doi: 10.1016/j.lssr.2019.10.002.

48. Aman Kumar, Ekta Singh, Rahul Mishra, Sunil Kumar. 2022. Biochar as environmental armour and its diverse role towards protecting soil, water and air. Science of the Total Environment 806: 150444. https://doi.org/10.1016/j.scitotenv.2021.150444
49. Joan Colón, Aaron A. Forbis-Stokes, Marc A. Deshusses. 2015. Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries. Energy for Sustainable Development 29: 57-64.
50. Latini, A., Bacci, G., Teodoro, M., Gattia, D. M., Bevivino, A., and Trakal, L. 2019. The Impact of Soil-Applied Biochars From Different Vegetal Feedstocks on Durum Wheat Plant Performance and Rhizospheric Bacterial Microbiota in Low Metal-Contaminated Soil. Frontiers in Microbiology 10: 2694, doi: 10.3389/fmicb.2019.02694.
51. Holling, C.S. 1996. Engineering Resilience versus Ecological Resilience. In National Academies of Engineering (Eds). Engineering within Ecological Constraints. pp. 31.
52. Hooper, D.U., F.S. Chapin III, J.J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J.H Lawton, D.M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A.J. Symstad, J. Vandermeer, & D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75(1): 3-35.
53. Peterson, G., C.R. Allen, CS. Holling. 1998. Ecological resilience, biodiversity, and scale. Ecosystems 1: 6-18.
54. Gitelson, Josef I., and Genry M. Lisovsky. *Man-made Closed Ecological Systems*, Vol. 9, CRC Press, 2003.
55. Donohue, I., Hillebrand, H., Montoya, J. M., Petchey, O. L., Pimm, S. L., Fowler, M. S., Healy, K., Jackson, A.L., Lurgi, M., McClean, D., O'Connor, N.E., O'Gorman, E.J., Yang, Q. 2016. Navigating the complexity of ecological stability. Ecology letters, 19(9): 1172-1185.
56. Grimm, V., & Wissel, C. 1997. Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. Oecologia, 109(3): 323-334.
57. Ross, S. R. J., Suzuki, Y., Kondoh, M., Suzuki, K., Villa Martín, P., & Dornelas, M. 2021. Illuminating the intrinsic and extrinsic drivers of ecological stability across scales. Ecological Research, 36(3): 364-378.
58. Arnoldi, J. F., Loreau, M., & Haegeman, B. 2016. Resilience, reactivity and variability: A mathematical comparison of ecological stability measures. Journal of theoretical biology, 389: 47-59.
59. Grimm, V., Schmidt, E., & Wissel, C. 1992. On the application of stability concepts in ecology. Ecological modelling, 63(1-4): 143-161.
60. Nelson, M., Dempster, W. F., and Allen, J. P. 2013. Key ecological challenges for closed systems facilities. Advances in Space Research 52(1): 86–96.
61. Lasseur, C., J.D. Brunet, H. De Weever, M. Dixon, C.G. Dussap, F. Godia, M. Mergeay, D. Van Der Straeten, W. Verstraete. 2010. MELISSA: The European project of a closed life support system. Gravitational and Space Biology 23(2): 3-12.25
62. Tikhomirov, A., A. Degermendzhi, S. Ushakova, Y. Kudenko, N. Tikhomirova, and Nl. Motorin. 2007. Research in Bios-3, a closed controlled experiment facility of the Institute of Biophysics of the Siberian Branch of the Russian Academy of Science. In: Y. Tako, T. Tani, R. Arai, S. Nozoe, and Y. Nakamura. (eds.) Application of a Closed Experimental Systems for Modeling. Inst. Environ. Sci., Rokkasho, Japan. pp. 129-136.

63. Tako, Y., R. Arai, S. Tsuga, O. Komatsubara, T. Masuda, S. Nozoe, K. Nitta. 2010. CEEF: Closed Ecology Experiment Facilities. Gravitational and Space Biology 23(2):13-24.
64. Zabel P., Zeidler C., Vrakking V., Dorn M., and Schubert D. 2020. Biomass production of the EDEN ISS space greenhouse in Antarctica during the 2018 experiment phase. Front. Plant Sci. 11: 656, doi: 10.3389/fpls.2020.00656.
65. Yuming Fu, Zhihao Yi, Yao Du, Hui Liu, Beizhen Xie, Hong Liu. 2021. Establishment of a closed artificial ecosystem to ensure human long-term survival on the moon. bioRxiv 2021.01.12.426282; doi: https://doi.org/10.1101/2021.01.12.426282