Topical: The case for a set of ‘best practices’ in regolith-based agriculture applied to bioregenerative food systems

Primary Author

Laura Elizabeth Fackrell, *Department of Astronomy and Planetary Science-Northern Arizona University* (l.e.fackrell@gmail.com)

Co-Authors

Rafael Loureiro, *Department of Biological Sciences- Winston-Salem State University*

Andrew Palmer, *Department of Ocean Engineering and Marine Sciences, Department of Biomedical and Chemical Engineering and Sciences, Aldrin Space Institute - Florida Institute of Technology*

Abstract

Food security during high-risk, long duration missions necessitates the application of fail-safes which may be better served through the integration of multiple, complementary methodologies that source food production. Regolith-based agriculture (RBA) has the potential to serve as an essential component of such multi-method food systems. Here we emphasize the potential of RBA as a viable food system approach while making a case for the development of a set of ‘best practices’ in RBA research to achieve a TRL that is mission viable.

Introduction

The success of crewed space missions hinges upon several mission-critical components, including access to a safe, sustainable, and sufficiently nutritious food supply [1]. The development of bioregenerative food systems (BFS) has been suggested as the most cost-effective approach for planetary colonization and resupply missions [2–8]. Current food production systems are adequate to support present day missions within low Earth orbit (LEO), but they are insufficient to meet the future demands associated with deep space exploration [4,8] Existing BFS such as VEGGIE and APH (Advanced Plant Habitat) employ chiefly hydroponic strategies that have proven viable in the microgravity environment of the International Space Station (ISS) [9]. Such systems are essential for long duration space travel aboard spacecraft. Still, additional in-situ resources (ISR) could potentially be leveraged at lunar or planetary sites through the application of Regolith-based agriculture (RBA) [10].

Though RBA has excellent potential as a component in lunar and planetary based food systems, efforts to develop and evaluate the viability of RBA vary widely in approach, the regolith simulants used, nutrients applied, growing conditions, plant selection, metrics for success, etc. This limits our ability to objectively gauge this approach's current technology readiness level (TRL), further impeding our ability to identify the changes needed to raise the TRL to mission-viable standards. Here we emphasize the potential of RBA as a viable food system approach while making a case for the development of a set of 'best practices' in RBA research to achieve a TRL that is mission viable.

Developing Sustainable Off-World Food Systems

The topic of food systems for long duration missions is often approached from an 'us vs. them' mantra that seeks to demonstrate which system is best above all others. However, to date, a study that thoroughly and directly compares different approaches to determine the most viable method is limited or nonexistent. Yet, the advantages and disadvantages of various food system approaches are often complementary. For example, degradation of nutrients in pre-packaged products can be supplemented by fresh food products, while shelf-stable pre-packaged options complement the short shelf-life of fresh food. The application of hydroponic methods for certain crops can reduce water requirements. In contrast, regolith-based methods are used to leverage the advantage of the plant-microbe interactions that can reduce the risk of pathogenic infection that can plague both hydroponic and soil-based systems [11]. The need for food security during high-risk, long duration missions necessitates the application of fail-safes which may be better served through the integration of multiple, complementary methodologies that source food production. RBA has the potential to serve as an essential component of such multi-method food systems, but the lack of coherence within RBA research limits our ability to develop RBA methods that meet mission standards.

Advantages of Regolith Based Agriculture

RBA leverages centuries of agricultural knowledge and the evolutionarily adapted relationship between plants, microorganisms, and soil to develop life support and food production. RBA can leverage the availability of sustainable approaches that require less fertilizer, reduced potential for certain pathogenic infections, provide additional avenues for nutrient cycling within closed systems to support life support functions, and provide more suitable conditions to promote beneficial interactions with microorganisms compared to other approaches such as hydroponics [12]. This last advantage is of particular note as RBA exploits millions of years of co-evolved plant-microbial associations significant for proper plant development and health.

The rhizosphere, the zone immediately surrounding the roots, are areas of high microbial number and diversity, and metabolic activity. This region can support an upper limit of ≈10^11 microbial cells/gram of root tissue with as many as 10^4 different species [13,14]. These microorganisms are heterogeneously distributed throughout the rhizosphere creating spatial diversity while also changing over the life of the host [15–17]. RBA should mimic and/or preserve many of these critical host-microbial associations better than other growth strategies in which microbiomes are more limited, and spatial coordination is frequently lost [18]. Host plants and their microbial cohorts have co-evolved since the origin of terrestrial plants, and engineered microbiomes are already applied in terrestrial agriculture, and such techniques should be applicable to RBA as well [19,20].

The composition and behavior of this microbial community significantly impacts agricultural yields, disease resistance, nutrient utilization, nutrient uptake, and secondary metabolite production [14,16,21–25]. Nitrogen fixation, the assimilation of nitrogen gas into organic compounds, is one of the most prominent examples of the beneficial activities of bacteria and provides host plants with a ready supply of this vital macronutrient. Members of the root microbiome can also facilitate the solubilization of other essential nutrients critical for plant growth, such as phosphorus and potassium. Similar associations between plants and mycorrhizal fungi, which help solubilize phosphorus, provide secondary metabolites to improve disease resistance in host plants. Leveraging the microbiome to facilitate nutrient uptake for plants would increase the value of regolith as an in-situ resource by making it a more suitable substrate for plant growth. Substrate transformation would be driven by biological processes with minimal additional processing and should significantly improve yields in RBA systems.

As with other food system approaches, RBA also has disadvantages, including the need to process regolith to be a viable growth medium and potentially more significant space and water requirements than soil-less methods. However, one of the most important current limitations is a lack of consistency in RBA research that limits our ability to evaluate TRL and develop RBA approaches that are not as water or space heavy and establish the feasibility of processing regolith to process regolith be a viable growth medium component. Thus, the need for a set of ‘best practices’ to bring greater coherency and consistency to RBA research.

The case for a set of ‘best practices’

The lack of consistency used to develop and evaluate RBA in research limits our ability to provide methods that meet mission viable standards. This wide variety of approaches affects many aspects of the RBA from experimental design, measuring plant response, measuring microbial-plant interactions, selection of appropriate (or more often inappropriate) regolith simulants, etc. The following provides two examples that emphasize the need for a set of ‘best practices’ specifically for measuring plant response and selecting appropriate regolith simulants. Similar examples can also be cited for other aspects but are beyond the scope of the current work. This can provide a starting point from which established best practices are developed and applied to RBA research efforts.

Measuring Plant Response

A detailed and standardized description of the experimental design and tested parameters will steer RBA research towards consistent and comparable methods for testing plant responses.

Standardization will facilitate the accurate replication of experiments, facilitate the comparison of results amongst publications, and avoid artifacts due to uncontrolled variables. Variables important to record for the controlled plant environment include:

- Temperature (Celsius) - Sensors should register the temperature throughout the day. At a minimum, they should measure at least two times a day, i.e., during the light and dark periods.
- Air quality parameters such as atmospheric moisture (kPa), carbon dioxide (μmol^{-1}), and air velocity (m s^{-1}) should be measured hourly if possible, or at least two times a day, i.e., during the light and dark periods.
- Frequency, amount, and location (i.e., rooting zone or bulk area) of watering and any nutrients applied.
- Any pre-treatment of regolith simulant: (i) if it was sterilized or not (and a description of the sterilization method), (ii) the amount used in each tested sample, and (iii) any nutrient or other amendments made to account for leaching of nutrients after sterilization.

Variables important to record for plant response include

- Photoperiod (h) and photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}) should be measured at the start and the end of the experiment and every two weeks during the crop's growth cycle
- Germination rates (%) and later in seedling and adult growth rate using relative growth rate (RGR) or size standardized growth rate (SGR) depending on the kind of crop or if the tested crops will come to full term or be evaluated as microgreens.
- Growth throughout the samples' life-cycle, such as shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (RDW), and root dry weight (RDW).
- Stomatal conductance (mmol m^{-2} s^{-1}) and transpiration (E) alongside plant leaf area (LA) and specific leaf area (SLA), especially if there is an a posteriori phytohormonal analysis of the samples (e.g., stomatal conductance with leaf abscisic acid concentrations).

In addition to the need to accurately track and report environmental and plant response variables, a multitude of questions need to be directly examined in RBA research, such as plant response in micro or partial gravity. This can be highly challenging but is critical to advancing science. In microgravity, these challenges start with the limited access to space flight-based experiments that in themselves are also limited in sample size, crew time, and power utilization, as well as the sometimes-inevitable indirect effects of the spaceflight environment [26]. In partial gravity, the severity of the lack of information is even more critical, especially when most published papers that touch on the subject of plants submitted to Moon or Mars simulated gravity don't use stimulants as their primary growth media [27,28].

Following these gaps in plant physiology assessment, regolith-based work allied with partial gravity conditions should focus on future experiments in this field. These combined elements directly influence the plant's physiology, reflecting its overall production and establishment success, thus requiring a detailed assessment of the factors responsible for a successful or failed crop choice.

Regolith Selection

An essential aspect of TRL in regolith-based plant systems is the evaluation of the viability of regolith as a growth medium and identifying feasible and appropriate measures for ameliorative treatments that resolve existing limitations. However, large volumes of regolith from the moon, Mars, or other relevant bodies are not available. Simulant regolith must be used

[29,30]. Regolith simulants used in agricultural studies need to reflect conditions relevant to plants and plant-associated microorganisms to provide reliable responses.

For the moon, various simulants exist and have been used for plant growth studies, with most reflecting the composition of regolith found in Lunar Highlands [e.g. 31–34]. For Mars, most studies use either the JSC-Mars 1a [35] or the Mars Mojave Simulant (MMS) [36]. Though these lunar and Martian simulants help establish preliminary efforts to demonstrate plants grown in regolith-like substrates, specific characteristics relevant to agriculture are not reflected in these commonly used stimulants. The next stage of determining TRL for RBA requires the development of simulants that reflect these agriculturally pertinent characteristics.

A summary of essential characteristics to replicate in general lunar regolith simulants can be found in Taylor and Liu, 2010 [37]. Of the multiple characteristics they discuss, some of those most relevant for agriculture include:

- Abundance of reduced iron
- Presence of nanophase metallic iron and agglutinate glass
- Particle size distribution
- Lack of hydrated alteration
- Soil strength or resistance to penetration
- Mineralogy and elemental composition
- Soil chemistry (e.g., pH).

Many of these characteristics are co-dependent: The soil chemistry will most likely be reflected if you accurately represent the mineralogy and redox characteristics. Recently developed lunar simulants such as the one constructed by the Exolith lab at UCF reflect most characteristics, including mineralogy, and provide a relevant study growth material. However, nanophase metallic iron and agglutinate glass are challenging to mimic, especially at large volumes, and are typically absent from almost all simulants. While some success in simulating agglutinate glass has been provided by Spray, 2010 [38]. it can still be challenging to produce on a sufficient scale for plant growth studies. Both have potential implications on the effects of plant roots, available iron, and plant/microbe interactions in the soil and thus are essential to mimic in lunar simulants used for RBA research.

Similarly, Mars soil characteristics should reflect agriculturally relevant factors similar to those listed for lunar regolith, but taking into account important differences including mineralogy, soil chemistry, soil salinity and mars-like salt profiles, abundance of nano-phase iron oxides, etc. Though the JSC-Mars and MMS simulants are roughly basaltic, they lack many of these characteristics including salts. Recently developed simulants from Exolith lab [29] and University of Georgia [30] more accurately reflect these characteristics. Selecting appropriate regolith simulants and/or detailing the simulant characteristics are essential to providing relevant results that can be compared and contrasted with other studies.

Conclusions

The need for a well-established protocol to test and validate RBA methods as a component that leverages in-situ resources in Off-world agriculture is of extreme importance for advancing this science and further discussion of its feasibility. The absence of data standardization only casts a shadow of disbelief in the true potential of regolith-based agriculture. It contributes to poorly defending its viability for future Off-world settlements. Given the high-risk nature of such off-world mission efforts, reliance upon a one-size-fits-all approach, even those with very well-established protocols and a series of good practices followed, limits the capacity and sustainability of food systems developed for long duration missions. A better understanding of how plants respond to being exposed to regolith, either by directly analyzing their physiology or their association with a potentially introduced microbiome, can only be achieved if the generated results can be compared and these same experiments replicated by the several groups that, across the globe, are making efforts to enrich the knowledge in this field.

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