Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Nutrient Recycling is a Critical Process in Closed Ecological Systems

Frieda B. Taub

taub@uw.edu

University of Washington

Natalie E. Stillwell

University of Washington

Rachel H. Kasbohm

University of Washington

Morrison B. Helton

University of Washington

Article

Keywords:

Posted Date: November 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11763168/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Permanent space colonies will approach being Closed Ecological Systems; recycling of essential elements will minimize transport from Earth: Food to Metabolism to Wastes to Recycle to Food, etc. Marine Closed Ecological Systems have been reported to remain photosynthetically active for decades. In contrast, our freshwater, chemically defined Closed Ecological Systems tend to run down, and grazers die out in about a year. Our 259 mL Closed Ecological Systems, consisting of 3 species of green algae (*Ankistrodesmus*, *Scenedesmus* and *Selenastrum*), the grazers (*Daphnia magna*), and unidentified microorganisms, have been studied under a variety of conditions. Our earlier studies have shown that high nutrient concentrations, including those associated with algal cultures, harm *Daphnia*. In an initial experiment, we tested lower concentrations of algal suspension (10%, 5%, and 1%); lower concentrations reduced chlorophyll a (an estimate of algal abundance) but did not statistically change the persistence of *Daphnia* populations. In general, the systems declined, which we assume was caused by nutrient limitation. For the following observations, supplements of C, N, P, C + N, N + P, and C + N+P were tested after the initial algal-*Daphnia* interactions (day 38). The introduction of C and P had no significant effect. The addition of N had mixed effects; if *Daphnia* grazing was low (3 replicates), chlorophyll a increased significantly; if *Daphnia* grazing was high (2 replicates), chlorophyll a remained low. Adding N together with C, P, or C + P all resulted in increases in chlorophyll a. **Despite variability, some N compounds appear to fail to recycle and persist in forms unavailable for algal regrowth**. Although it is often assumed that sewage treatment or artificial wetlands recycle all complex molecules into those needed for food production, evidence suggests otherwise. The European Space Agency developed a recycling system that began with anaerobic digestion of human waste, with eventual food production. In contrast, the National Aeronautics and Space Administration has tended to store or dispose of waste but is currently studying waste recycling. Improved waste recycling methods will greatly benefit Earth's ecosystems.

Introduction

This paper focuses on the demonstrated inadequacy of long-term nutrient regeneration and the responses to added nutrients in our freshwater ecosystems. The experiments test the hypothesis that our artificial, simplistic communities will recycle nutrients indefinitely to support continued photosynthesis, grazing, and nutrient recycling—three essential ecosystem properties. Textbooks suggest that ecosystems have infinite recycling capacity, but this assumption must be robustly tested for long-term, regenerating life-support systems. To date, we have not confirmed the hypothesis that nutrient recycling is complete in our systems.

Future colonies on the Moon and Mars will eventually approach being Closed Ecological Systems (CESs) because resupply from Earth will be costly; therefore, recycling essential elements will be critical. In our CES experiments reported here, recycling appears to be incomplete. Fertilizers will be necessary to grow food on Martian colonies, and human waste products are a potential source of the essential elements. In an ideal ecosystem, all the elements are recycled, and energy is gained by photosynthesis or anaerobic

processes and balanced by entropy loss. Human waste products constitute a significant daily output that cannot be avoided; they have been quantified by NASA (NASA-STD-3001 Technical Brief). NASA and the ESA have developed different approaches to their role in extraterrestrial life support systems. NASA has tended toward storage or release of waste products (into space or to burn in the atmosphere) but is now researching recycling; the ESA has developed MELiSSA, which starts with anaerobic processing of wastes to be transformed into food production via various processes (Hendrickx, De Wever et al. 2006). On Earth, we assume essential elements are recycled, although in practice we augment intensive farming with transported fertilizers. In addition to water recycling, permanent colonies will require recycling of elements.

The book and movie “The Martian” by Andy Weir highlighted the importance of using human waste products to produce food (Weir 2014). Mark Watney, an astronaut who was accidentally abandoned on Mars, used stored fecal material, his fresh fecal material (for its live microbes), and food scraps to augment Martian regolith and grow the potatoes he brought for a research project. “The worse it smells, the better things are going. That’s the bacteria at work!” (p. 13).

Early in the space race, the need for regenerative life support systems was recognized. For example, Boeing Company supported a 1959-61 research project using freeze-dried human fecal material to grow fish (tilapia) (Taub 1963, Taub 1965). The plan was to use human waste as the nutrient base of an algal-fish food chain, but the fish ate the human fecal flakes directly. Most early ecological efforts focused on using algae, usually *Chlorella*, to convert human CO_2 to O_2 (Taub 1974). As it became obvious that total regenerative systems would not be needed for many decades, ecological interest waned and is now being reinvigorated.

NASA earlier showed interest in simple CESs, supporting the studies of Claire Folsome and Joe Hanson that led to the shrimp-containing Ecospheres and the University of Hawaii sealed ecological systems. (Hanson and Press 1981, Hanson 1985, Folsome and Hanson 1986). The commercial EcoSpheres™ supported the shrimp for up to 8 years in our laboratory and up to 11 years in others, supporting the concept that these sealed ecosystems had complete nutrient recycling. See discussion for a more detailed comparison of our freshwater and their dilute marine system.

It must be recognized that most experimental aquatic microcosm experiments are open to the Earth’s atmosphere with free exchange of CO_2 and O_2. Systems designed to work on the Moon or Mars will not have that option and must be designed with atmospheres compatible with humans and food production systems. Wastes, such as fecal material, uneaten food, and urinary solids, will either have to be stored (out of sight and out of mind) with fertilizers shipped in indefinitely, or recycling systems will have to be adequate to grow future meals with processed wastes.

Other ecological CESs, with defined microbiota, include those of Seppe Kuehn, his students, and colleagues. Of particular interest is a study that examined the maintenance of carbon recycling in closed microbial communities in which some communities approached stability during a 50-day experiment

(Astacio, Prabhakara Kaumudi et al. 2021, Astacio 2023). Other experiments include CES studies (Moran, Graham et al., Rillig 2015, Rillig and Antonovics 2019, Gu, Zhang et al. 2024, Hosoda, Seno et al. 2024, Li, Liu et al. 2026).

Biosphere 2 used constructed wetlands to convert wastewater and condensates to irrigate its intensive agricultural areas and produced potable water with an additional two-stage filtration (Nelson 2020). A UV sterilizer was built but was not used during Biosphere 2 because the crew was healthy. Additional Biosphere 2 studies are covered in the discussion.

The International Space Station (ISS) has developed processes for waste treatment but depends largely on food delivery from Earth. NASA had earlier developed the Biomass Production Chamber for the growth of plants suitable for long-term isolation from Earth (Wheeler, Mackowiak et al. 1996) but discontinued that facility as being far ahead of its need.

A problem we have not seen addressed is the relatively few elements needed to grow crops, e.g., Hoagland's Nutrient Solution (Zoroddu, Aaseth et al. 2019) and the requirements of the human body (Zoroddu, Aaseth et al. 2019). Especially critical is the need for K (but not Na) in plants, whereas animals require Na to be more abundant than K. The extraterrestrial systems will need to solve these problems.

Our experimental technique differs in that it uses a few well-studied species rather than naturally occurring aquatic communities. Each of these species has more than a thousand publications and has survived in our chemically defined media of various compositions for decades. In contrast, most micro/mesocosm studies use local, undefined water sources and organisms, making non-repeatability difficult to identify. Our current studies are less well-defined than earlier "gnotobiotic" studies (i.e., all species, including microbial, are identified (Taub 1969a, Taub 1969b, Taub 1969c)). Samples of our old microcosms are saved for future DNA analysis, should funding become available.

Our laboratory has been studying the properties of freshwater CESs consisting of three species of green algae, the grazer Daphnia magna, and unidentified microbes in 259 mL sealed bottles (200 mL liquid, 59 mL gas space). Earlier studies demonstrated gaseous distribution within closed systems (e.g., most of the O_2 is in the gas phase) (Taub and McLaskey 2013). Lower nutrient concentrations resulted in lower pH and O_2 concentrations and allowed longer persistence of the grazers, to hundreds of days (usually less than 365 days), but not indefinitely (Taub, McGrath-Flinn et al. 2025). High concentrations of N, 0.125 mM, were beneficial to *Daphnia* in open systems but toxic in closed systems (Taub, McGrath-Flinn et al. 2025).

In general, our experiments have shown algal blooms that are reduced by *Daphnia* grazing. Over time, the *Daphnia* population decreases and dies out; the chlorophyll may increase or remain low, leading to an eventual decrease. Although the *Daphnia* produce ephippia (sexual eggs), we have not seen evidence of their hatching later in the systems. Earlier published studies demonstrate this tendency of the systems to "run down" over months (Taub 2019, Taub, McGrath-Flinn et al. 2025).

Results

Experiment 1: Reducing algal culture input.

In Experiment 1, Fig. 1, we evaluated the effects of adding progressively reduced amounts of algal cultures (10%, 5%, and 1% of the inorganic media) to the CES to determine how reduced algae and nutrient concentrations would affect Chl a and *Daphnia* interactions. The nutrient carryover from the algal cultures is significant and detailed in the Supplemental Information. The calculated carryover from the algal cultures for N is 0.045, 0.024, and 0.005 mM, and for P is 0.00364, 0.00190, and 0.00040 mM. The C carryover from the algal cultures is unknown because the algal cultures received CO_2 from air.

Experiment 2: Supplementing nutrients after an initial start.

The addition of N (when grazing was low) and N with another element demonstrated significantly greater Chl a and *Daphnia*; we concluded that N was becoming limiting as the CESs aged. Adding C or P alone did not stimulate Chl a or *Daphnia* populations. The most obvious explanation is that some stable N-containing organic compound is being sequestered and is not available for algal regrowth. Chitin exoskeletons of the *Daphnia* may be one form, but it is not likely to be the only form because Experiment 1 (ungrazed) did not have any *Daphnia* present in half the treatments. Given aerobic conditions, it is unlikely the NO_3^- was converted to N_2. Even with the added elements, all the *Daphnia* were dead by 334 days.

Discussion

Our simple CESs can support measurable Chl a for more than a year, but Chl a decreases over time unless NO_3^- alone, or a mixture of NO_3^-, PO_4^{-3}, and $NaHCO_3$, is added. The *Daphnia* populations are much more variable. There seems to be an interaction between the algae and *Daphnia*; if the *Daphnia* can crop down the algae early (during the first week), they can persist longer. If the algae grow very quickly, they can kill *Daphnia* and escape grazing. This seems to be why the NO_3^- added treatment was not significantly different than the control: upon adding NO_3^-, massive algal blooms occurred in three of five replicates and *Daphnia* all died; in two of the five replicates, the *Daphnia* increased, and Chl a was vastly reduced. We are researching this further; high pH and high O_2 appear to be associated with the *Daphnia* mortalities, and we have some evidence that this occurs in lakes.

Our initial attempts to add microbes from a natural lake (Lake Washington, Seattle, WA, USA) did not reliably increase nutrient recycling (Taub, McGrath-Flinn et al. 2025). We have a few cases where Chl a increased late in the experiment, but replicates do not agree; in at least one case, Chl a is high in a control bottle with no Lake Washington addition or other manipulations, such as opening the microcosms or adding microbes intended for aquariums. Samples have been saved in case funding for DNA analyses becomes available. It is possible that microorganisms capable of converting organic nitrogen are present in our systems but are typically outcompeted by other microbes. Denitrification is

an additional possibility (Liu, Valentine et al. 2026), as well as influences from carbon sources (Carlson, Lui et al. 2020). Alternately, freshwater systems may differ from marine communities by having incomplete nutrient recycling, depending on inputs (from upstream or precipitation) for essential nutrients and depositing unregenerated organics onto the sediment or downstream.

An earlier Closed Ecological Systems study using natural assemblages or stable aquarium communities was titled “Some Patterns in Post-Closure Ecosystem Dynamics (Failure)” (Maguire Jr. 1980). Waste-stabilization-pond biota supported ostracods (the only visible organisms) for about a year in 125 mL, 250 mL, and 1250 mL containers, but persistence was shorter in 12.5 mL systems. Small microcosms (12.5 mL) from a Colorado ephemeral pond or an established aquarium tended to have shorter active crustacean populations than 125 mL systems, with 25% still having animals at the 400-day end of the experiment. Without comment, his chart shows close to 100% active crustacea in the Colorado ephemeral pond communities in 125 mL systems at the 400-day end. Following publications suggested that designing functional closed ecological systems would be extremely difficult (Botkin, Golubic et al. 1979, Botkin, Maguire Jr. et al. 1979)

The shrimp-containing ecosystems were previously sold as novelties by EcoSphere™ and currently as eccosmo™ (info@ecosphere-Europe.com). In our laboratory, the shrimp have lived for at least eight years, and anecdotally for 11 years, although the shrimp do not reproduce in these CES. The small marine systems are ~ 300 mL, contain ~ 20% gas space, dilute seawater, algae, some filamentous algae, a physical structure of either dead or “artificial Gorgonia” on which the shrimp graze or rest, $CaCO_3$ pellets, and magnets for cleaning the glass. Some of the original U of Hawaii CESs (without shrimp) are said to be on display at Space Analog for the Moon in Arizona and assumed to be photosynthetically active. Many older publications from the University of Hawaii and commercial novelties supported the supposition that natural ecological systems were self-contained and could perpetually recycle essential nutrients.

One difference between the CESs we research and the EcoSpheres is the life history of the grazers. The Hawaiian red shrimp (*Halocaridina rubra*) used in the EcoSpheres do not reproduce in these systems and live many years, unlike the *Daphnia* in our systems, which carry eggs after a week and can have multiple broods per month. Assuming the shrimp shed their carapace, it is likely eaten by the shrimp, returning essential nutrients to the system. Their feeding mechanism has been described: “primarily a microphagous herbivore with chelipeds that remove food materials from the surface of the crust, and maxillae that transfer the loosened plant and cyanobacterial fragments to the mouth” (Bailey-Brock and Brock 1993). They are unusual in that they can survive at least a week in anaerobic conditions, tolerate a wide range of salinities, and subsist on a wide variety of foods (Havird, Henry et al. 2014, Havird, Santos et al. 2014, Havird, Vaught et al. 2015, Havird, Brannock et al. 2022). They are reared by numerous individuals and are scheduled for an ESA space flight experiment (P.T.T.R.I Program, “SCAMPI: A shrimp odyssey”, by the Spring Institute).

In contrast, *Daphnia magna* is a filter feeder of small, suspended algae, bacterial cells, and detritus. They will hover over settled algal cells to resuspend and consume them, but they are not reported to physically scrape biofilm. Their lifespan is generally 30–60 days; they can reproduce at about 7–10 days of age. Females can produce asexual eggs that yield female offspring if food is available, with the number of eggs being related to food availability (8–32 young/brood is not uncommon). Embryological development occurs in the female's transparent carapace; egg development takes 4 days at 20 C, and once the young are released and ready to feed, the female sheds her carapace and ovulates a new clutch of eggs. Under unfavorable conditions, a female can produce males, mate, and produce ephippia—resting eggs that can persist for decades. Culture methods and chemical sensitivities have been extensively studied because this species has been used for decades to test toxicity responses of aquatic organisms, e.g., American Society for Testing Materials International (ASTM) and European Organization for Cooperation and Development (OECD). *Daphnia* are also considered important ecological grazers of phytoplankton and a major food source for young fish. More than 2000 publications on *Daphnia* research have been published. Our stock has been reared on a chemically defined medium with these three green algae species for at least 25 years. It is a "lab rat" for aquatic studies.

For Lunar and Martian LSS, Artificial Wetlands have been suggested for waste processing based on their successful use in Biosphere 2 and later development (Nelson, Tredwell et al. 2006, Nelson and Wolverton 2011, Nelson 2020). In general-purpose systems, it was suggested that root crops and uncooked greens such as lettuce not be planted in constructed wetlands because constructed wetlands are normally not disinfected, but tree crops and flowers are unlikely to contain pathogens. More research is needed to provide evidence that wetlands decompose all organic chemicals or at least make necessary elements available for growing foods. The question of pathogen transmission must also be addressed if there are contributors who may provide pathogens, or microbes that mutate to be pathogenic.

The European Space Agency developed MELiSSA, using thermophilic anaerobic bacteria as a first step in processing wastes to eventually produce food, thus completing the cycle (Hendrickx, De Wever et al. 2006). The complete cycle involves numerous separate steps, each quite complex on its own, but the conversion of waste product to the raw materials for eventual food production seems to be the most complex (Lissens, Verstraete et al. 2004, Steinberg, Kronyak et al. 2017).

NASA has largely depended on waste storage or disposal but has explored some methods of waste regeneration. A specialized space toilet has been developed to separate urinary and fecal waste; toilet paper, wipes, and gloves are stored in water-tight bags. To date, fecal waste is stored or disposed of in conditions that will burn up on reentry. Given the importance of water on the International Space Station (ISS), a membrane technology was developed to provide purified water; "today's coffee is tomorrow's coffee" (2020). NASA is developing a "Divergent Deployable Wastewater Treatment Facility" to treat feces, urine, and food waste separately because each has a different chemical composition. Ultimately, these will supply the nutrients for plant growth for a future diet, which may include algae (Drexler, Joustra et al. 2014, Fischer, Kloss et al. 2022, Degeurin 2026). NASA has experimented with converting

inedible tomato and pepper biomass into fertilizer for the next crop; simple leaching could release 70%, and dilute HCl released 85–95% of the required nutrients. They discussed the potential harmful effects of organic compounds from hydroponic systems that had been reported. Without recycling plant nutrients from waste, they estimated that 93 kg of fertilizer salts would be needed per person per year (Lunn, Stutte et al. 2017).

Conclusions

Our current hypothesis is that some essential nutrient, probably N, is not adequately recycled. In general, the systems have an algal bloom; if grazers are alive, the bloom is short-lived, and the population subsists on algal cell reproduction, although the standing stock (as estimated from Chl a measurements) is extremely low. In CESs with dead *Daphnia*, the algal bloom persists longer but eventually declines.

Since our freshwater CESs have the grazer population die out in about a year, are freshwater ecosystems less capable of recycling than marine ecosystems? Lakes and ponds eventually become bogs and terrestrial landscapes as sediments accumulate. Or are our highly synthetic ecosystems lacking essential recycling capabilities? Even when we added raw Lake Washington water, hoping its microbes would provide recycling, we did not observe increased nutrient regeneration (Taub, McGrath-Flinn et al. 2025). We are currently studying CESs with greater quantities of Lake Washington sediment and water added, as well as aquarium bacteria and anaerobic sites, to determine whether they extend the duration of *Daphnia* populations. One of our findings is that lower nutrient concentrations are more beneficial to *Daphnia* populations than higher ones; higher N and P concentrations tend to cause algal blooms associated with high pH that is stressful or lethal to *Daphnia*. This is an active area of study, with potential application to algal blooms, and will be presented in other publications soon.

Improved sewage treatment may be one of the most important contributions of space research to Earth's ecology (2019, 2020, Degeurin 2026). The purification of "grey water" (non-fecal wastes) has the potential to significantly reduce water needs. The ability to purify water from sinks, showers, and water fountains for reuse within the building is being used in the Netherlands; for example, toilet water goes to traditional wastewater facilities, while other water is purified and reused within the building (The National Plan of Action to Save Drinking Water (NPvA)). Water-scarce states in the USA are considering similar purification systems to reduce water demands. Researchers have developed an iThrone that requires no water or electricity inputs but uses urine to produce electricity to dry feces (2019). In remote, underdeveloped areas, this could reduce disease transmission. Studies are also underway to recycle wastes to grow algae (Drexler, Joustra et al. 2014) and potentially biofuels.

It is unsafe to assume that all ecological systems will automatically recycle all organics into nutrients available for the next cycle of primary production. Our systems apparently fail to make at least some N compounds available for the next cycle of photosynthesis. The University of Hawaii CES has not been tested for photosynthetic activity for decades, and the Ecospheres™ lack reproducing grazers. It is

unsafe to assume that current sewage treatment plants completely degrade organics, because significant quantities of biologically active chemicals such as hormones, pharmaceuticals, illegal drugs, and persistent organics are routinely released into receiving waters. Sewage treatment plants were initially designed to reduce Biological Oxygen Demand (BOD) and later modified to reduce P releases.

Nutrient recycling on Earth's systems is far from perfect; we import fertilizers and pollute receiving waters. Although the Earth has supported ecosystems for eons, not all sites are complete within themselves—they distribute their wastes and receive essentials from other systems. As these waste-treatment problems are solved for Lunar and Martian life support systems, they will be enormous gifts for Earth's communities. The cost/benefit ratios are very different for Lunar and Martian colonies than for established cities on Earth, and these differences will drive research and development of novel techniques that will benefit Earth.

Methods

The media are T82 (algal growth) and B/4 (*Daphnia* rearing) (S1.1 and S1.2). Medium T82 meets all of the needs for the algal species used; B medium has additional trace metals and vitamins required by the *Daphnia* and is usually used at ¼ concentration (S1.2)

The three species of green algae, *Ankistrodesmus* sp*., Scenedesmus obliquus*, and *Selenastrum capricornutum,* are grown on chemically defined Medium T82 plus air (for CO_2), $NaNO_3$ = 0.5 mM and KH_2PO_4 = 0.04 mM (S1.2)

The grazers are *Daphnia* magna, reared in chemically defined medium B/4, the algal mixture, and additional vitamins.

To compose the media used in the experiment, solution B is diluted to B/4, and 100 mL of an algal mixture is added to each liter of B/4; thus, there is carryover of T82 medium of 100/1100, or 9.09% of the more concentrated T82 medium. The medium plus algal culture is separated into separate containers for additional additives; one phenolphthalein container is added per liter.

The microcosms are 8 oz (259 mL) bottles (Appendix) with tightly fitting screw-top lids with plastic rings (Appendix). They are filled with mixed algal cultures to 200 mL, and medium- to large-sized *Daphnia* are added. The gas space is approximately 59 mL.

Experiment 1 had 6 replicates per treatment (total 24). Experiment 2 had 5 replicates per treatment (total 40). Statistics were analyzed using an ANOVA and Tukey HSD test (S2.2).

Observations were made approximately weekly, noting the Chlorophyll a (expressed as algae) and the number of small, medium, and large *Daphnia* and ephippia (winter eggs of *Daphnia*). Algae are measured using in vivo fluorescence, with a Turner Design FluoroSense Handheld Chlorophyll Model # 2860 000 C and a Neewer ND4 filter.

Abbreviations

ANOVA Analysis of Variance

ASTM American Society for Testing Materials International

BOD Biological Oxygen Demand

CES Closed Ecological Systems (energetically open to light and heat exchange, materially closed to the atmosphere and other exchanges)

CO_2 Carbon Dioxide

C Carbon

Chl a Chlorophyll a

Cl Chlorine

ESA European Space Agency

H Hydrogen **HCl** Hydrochloric acid

HSD Honestly Significant Difference

K Potassium **KH_2PO_4** Potassium Phosphate (monobasic)

LSS Life Support System

MELiSSA Micro-Ecological Life Support System Alternative (ESA program)

Na Sodium **$NaHCO_3$** Sodium bicarbonate

N Nitrogen

NASA National Aeronautics and Space Administration

O_2 Oxygen (gas, or dissolved) **O** Oxygen

OECD European Organization for Cooperation and Development

P Phosphorus

Declarations

Author Contribution

F.T. planned the experiments and analyzed the results. N.S., R.K., and M.H. implemented the experiments. N.S. and R.K. performed statistical analysis. All authors contributed to writing, reviewing, and preparation of figures.

Data Availability

All data supporting this study's findings will be made available upon request at taub@uw.edu.

Funding Declaration

Funding for this research was provided by the University of Washington School of Aquatic and Fishery Sciences.

References

1. "An innovative toilet called the ‘iThrone’ shrinks and dehydrates poop — no plumbing or power required." from https://humanitariangrandchallenge.org/innovative-toilet-ithrone/.
2. "Boldly Go! NASA’s New Space Toilet Offers More Comfort, Improved Efficiency for Deep Space Missions." from https://www.nasa.gov/humans-in-space/boldly-go-nasas-new-space-toilet-offers-more-comfort-improved-efficiency-for-deep-space-missions/.
3. Astacio, L. M. d. J. (2023). Carbon cycling and functional redundancy in closed microbial ecosystems. Ph.D., University of Illinois at Urbana-Champaign.
4. Astacio, L. M. d. J., H. Prabhakara Kaumudi, Z. Li, H. Mickalide and S. Kuehn (2021). "Closed microbial communities self-organize to persistently cycle carbon." Proceedings of the National Academy of Sciences118(45): e2013564118.
5. Bailey-Brock, J. H. and R. E. Brock (1993). "Feeding, reproduction, and sense organs of the Hawaiian anchialine shrimp Halocaridina rubra (Atyidae)."
6. Botkin, D. B., S. Golubic, B. Maguire Jr., B. Moore III, H. J. Morowitz and L. B. Slobodkin (1979). Closed regenerative life support systems for space travel: their development poses fundamental questions for ecological science. (COSPAR) Life Sciences and Space Research, Pergamon Press. 17: 3–12.
7. Botkin, D. B., B. Maguire Jr., B. Moore III, H. J. Morowitz and L. B. Slobodkin (1979). "A foundation for ecological theory." Memorie dell'Istituto Italiano di Idrobiologia.
8. Carlson, H. K., L. M. Lui, M. N. Price, A. E. Kazakov, A. V. Carr, J. V. Kuehl, T. K. Owens, T. Nielsen, A. P. Arkin and A. M. Deutschbauer (2020). "Selective carbon sources influence the end products of microbial nitrate respiration." The ISME Journal 14(8): 2034–2045.
9. Degeurin, M. (2026). "NASA wasterwater system will turn human poop into plant food." from https://www.popsci.com/technology/nasa-wastewater-system-turns-poop-into-plant-food/.

10. Drexler, I. L. C., C. Joustra, A. Prieto, R. Bair and D. H. Yeh (2014). "AlgaeSim: A Model for Integrated Algal Biofuel Production and Wastewater Treatment." Water environment research 86(2): 163–176.
11. Folsome, C. E. and J. A. Hanson (1986). The Emergence of Materially-closed-system Ecology. Ecosystem Theory and Application. N. Polunin, John Wiley & Sons Ltd.: 269–288.
12. Gu, P., X. Zhang, A. Chen, Q. Tian, J. Zhang, T. Li, X. Li and G. Wang (2024). "Microbes and nutrient shift in a Closed Aquatic Ecosystem (CAES) during four weeks of operation." Life Sciences in Space Research 42: 91–98.
13. Hanson, J. A. (1985). "Experimental Ecosystems Sealed in Glass." NASA Tech Brief8.
14. Hanson, J. A. and T. A. Press (1981). Lab Community Lives in an air-tight bottle. Fort Lauderdale News.
15. Havird, J. C., P. M. Brannock, R. M. Yoshioka, R. C. Vaught, K. a. Carlson, C. Edwards, A. Tracy, C. W. Twining, Y. Zheng, D. Chai, A. E. Wilson, N. G. Hairston and S. R. Santos (2022). "Grazing by an endemic atyid shrimp controls microbial communities in the Hawaiian anchialine ecosystem." Limnology and Oceanography 67(9): 2012–2027.
16. Havird, J. C., R. P. Henry and S. R. Santos (2014). "Taking their breath away: Metabolic adaptations to low-oxygen levels and salinity transfer in anchialine shrimps (Crustacea: Atyidae)." Integrative and Comparative Biology 54: E86–E86.
17. Havird, J. C., S. R. Santos and R. P. Henry (2014). "Osmoregulation in the Hawaiian anchialine shrimp Halocaridina rubra (Crustacea: Atyidae): expression of ion transporters, mitochondria-rich cell proliferation and hemolymph osmolality during salinity transfers." Journal of experimental biology 217(Pt 13): 2309–2320.
18. Havird, J. C., R. C. Vaught, D. A. Weese and S. R. Santos (2015). "Reproduction and Development in Halocaridina rubra Holthuis, 1963 (Crustacea: Atyidae) Clarifies Larval Ecology in the Hawaiian Anchialine Ecosystem." The Biological bulletin (Lancaster) 229(2): 134–142.
19. Hendrickx, L., H. De Wever, V. Hermans, F. Mastroleo, N. Morin, A. Wilmotte, P. Janssen and M. Mergeay (2006). "Microbial ecology of the closed artificial ecosystem MELiSSA (Micro-Ecological Life Support System Alternative): Reinventing and compartmentalizing the Earth's food and oxygen regeneration system for long-haul space exploration missions." Research in Microbiology 157(1): 77–86.
20. Hosoda, K., S. Seno, N. Murakami, H. Matsuda, Y. Osada, R. Kamiura and M. Kondoh (2024). "Synthetic model ecosystem of 12 cryopreservable microbial species allowing for a noninvasive approach." BioSystems 235: 105087.
21. Li, L., H. Liu, J. Ding, Y. Cai, P. Zhao, L. Zhang, B. T. Steudel, Z. Wang and Z. Chen (2026). "The failure and revival of closed microbial ecosystems: evidence from the shifts in microbial and chemical diversities." ISME Communications 6(1): ycag005.
22. Lissens, G., W. Verstraete, T. Albrecht, G. Brunner, C. Creuly, J. Seon, G. Dussap and C. Lasseur (2004). "Advanced anaerobic bioconversion of lignocellulosic waste for bioregenerative life support

following thermal water treatment and biodegradation by Fibrobacter succinogenes." Biodegradation (Dordrecht) 15(3): 173–183.

23. Liu, J., D. L. Valentine, A. Bourbonnais, D. T. Andersen, D. Bianchi, G. Brown, M. B. Cardenas, D. Fillion, C. Frey, K. M. Gosselin, A. Ji, F. S. Kinnaman, D. Lacelle, M. F. Lehmann, K. McPaul, J. Mullahoo, V. J. Orphan, A. Pellerin, E. Reji, E. D. Swanner, T. Treude, A. M. Seltzer and E. D. Young (2026). "Natural 15N15N abundances constrain fixed nitrogen loss." Science 394(6820): 207–211.
24. Lunn, G., G. Stutte, L. Spencer and M. Hummerick (2017). Recovery of Nutrients from Inedible Biomass of Tomato and Pepper to Recycle Fertilizer. 47th International Conference on Environmental Systems. Charleston, South Carolina.
25. Maguire Jr., B. (1980). Some Patterns in Post-Closure Ecosystem Dynamics (Failure). Microcosms in Ecological Research. J. P. Giesy Jr, Technical Information Center U.S. Department of Energy 1980.
26. Moran, J., L. C. Graham and M. Tikhonov "Emergent predictability in microbial ecosystems." Science 392(6794): eadr1440.
27. Nelson, M. (2020). "The challenge of managing water and nutrient cycles in a mini-world – the lessons from Biosphere 2." Institute of Ecotechnics.
28. Nelson, M., R. Tredwell, A. Czech, G. Depuy, M. Suraja and F. Cattin (2006). "Worldwide Applications of Wastewater Gardens and Ecoscaping: Decentralised Systems which Transform Sewage from Problem to Productive, Sustainable Resource."
29. Nelson, M. and B. C. Wolverton (2011). "Plants+soil/wetland microbes: Food crop systems that also clean air and water." Advances in Space Research 47(4): 582–590.
30. Rillig, M. C. and J. Antonovics (2019). "Microbial biospherics: The experimental study of ecosystem function and evolution." Proceedings of the National Academy of Sciences of the United States of America 116(23): 11093–11098.
31. Rillig, M. C., Antonovics, J., Caruso, T., Lehmann, A., Powell, J. R., Veresoglou, S. D., Verbruggen, E. (2015). "Interchange of entire communities: microbial community coalescence." Trends in Ecology & Evolution 30(8): 470–476.
32. Steinberg, L. M., R. E. Kronyak and C. H. House (2017). "Coupling of anaerobic waste treatment to produce protein- and lipid-rich bacterial biomass." Life sciences in space research 15: 32–42.
33. Taub, F. B. (1963). "Some ecological aspects of space biology." Amer. Biol. Teacher 25(6): 412–421.
34. Taub, F. B. (1965). "From wastes to resources. Activities report." Res. Devel. Assoc. 17(72–78): 72–78.
35. Taub, F. B. (1969a). "A biological model of a freshwater community. A gnotobiotic ecosystem." Limnology and Oceanography 14(1): 136–142.
36. Taub, F. B. (1969b). "Gnotobiotic models of freshwater communities." Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie 17: 485–496.
37. Taub, F. B. (1969c). A continuous gnotobiotic (species defined) ecosystem. Structure and Function of Freshwater Microbial Communities. J. Cairns Jr. Blacksburg, Virginia, Virginia Polytech. Inst.:

101–120.

38. Taub, F. B. (1974). "Closed ecological systems." Annual Review of Ecology and Systematics 5: 139–160.
39. Taub, F. B. (2019). "Why did carbon become the pseudo-limiting factor in aquatic closed ecological systems?" Life sciences in space research 20: 30–34.
40. Taub, F. B., K. M. McGrath-Flinn, N. E. Stillwell and R. H. Kasbohm (2025). "Behavior of simple closed ecological systems; lower nutrient concentrations allow longer persistence of grazer populations." Life Sciences in Space Research 44: 47–57.
41. Taub, F. B. and A. K. McLaskey (2013). "Pressure, O_2, and CO2, in aquatic Closed Ecological Systems." Advances in Space Research 51(5): 812–824.
42. Weir, A. (2014). The Martian, Crown Publishing.
43. Wheeler, R. M., C. L. Mackowiak, G. W. Stutte, J. C. Sager, N. C. Yorio, L. M. Ruffe, R. E. Fortson, T. W. Dreschel, W. M. Knott and K. A. Corey (1996). "NASA's Biomass Production Chamber: A testbed for bioregenerative life support studies." Advances in Space Research 18(4/5): 215–224.
44. Zoroddu, M. A., J. Aaseth, G. Crisponi, S. Medici, M. Peana and V. M. Nurchi (2019). "The essential metals for humans: a brief overview." Journal of inorganic biochemistry 195: 120–129.

Figures

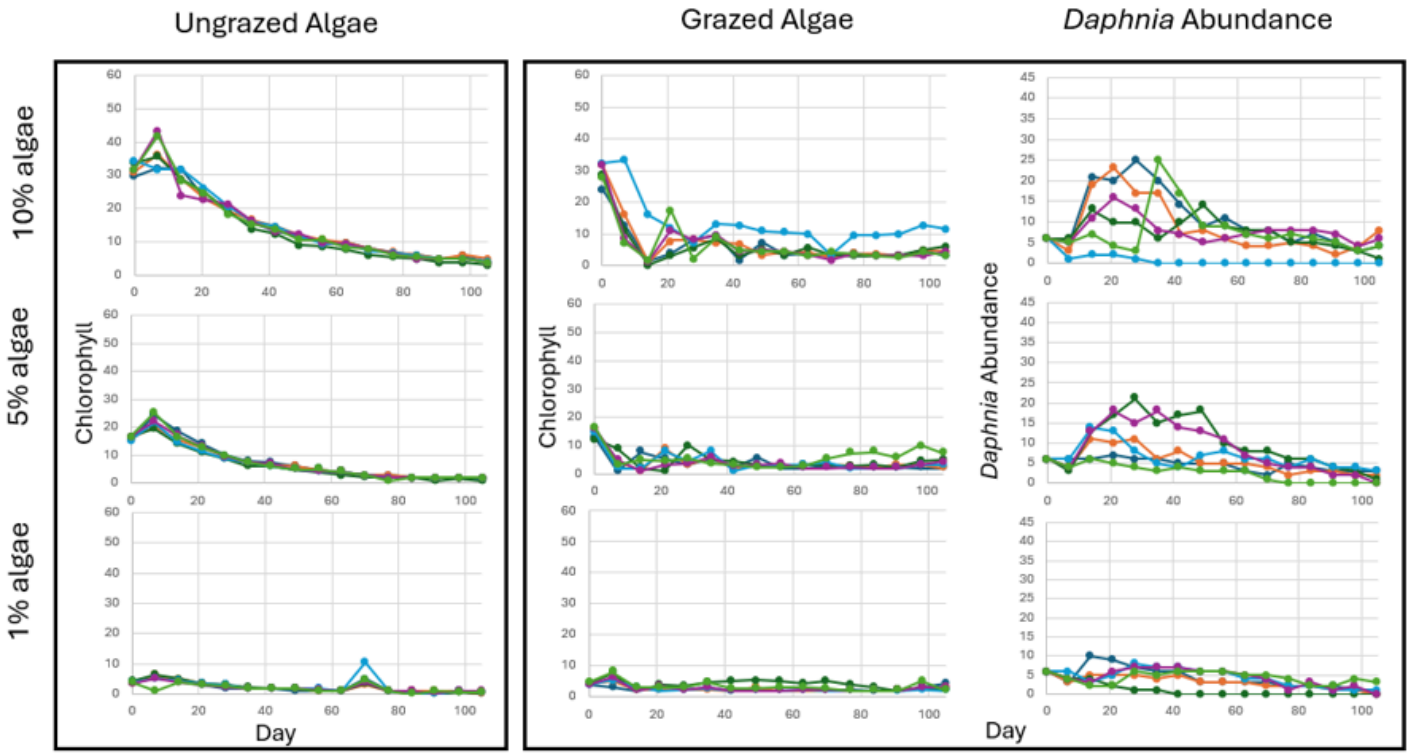

Figure 1

Chl a and *Daphnia* abundance of each treatment. Each line represents one replicate. This demonstrates that introducing less algae and algal nutrients resulted in lower Chl a and fewer *Daphnia*, but did not show a strong tendency for *Daphnia* populations to persist longer. Differences among replicates (e.g., some *Daphnia* populations not becoming established at the highest nutrient concentration) demonstrated an interaction in which high algal growth can overwhelm a *Daphnia* population, presumably through pH and O_2 concentration effects. Statistics S2.2.

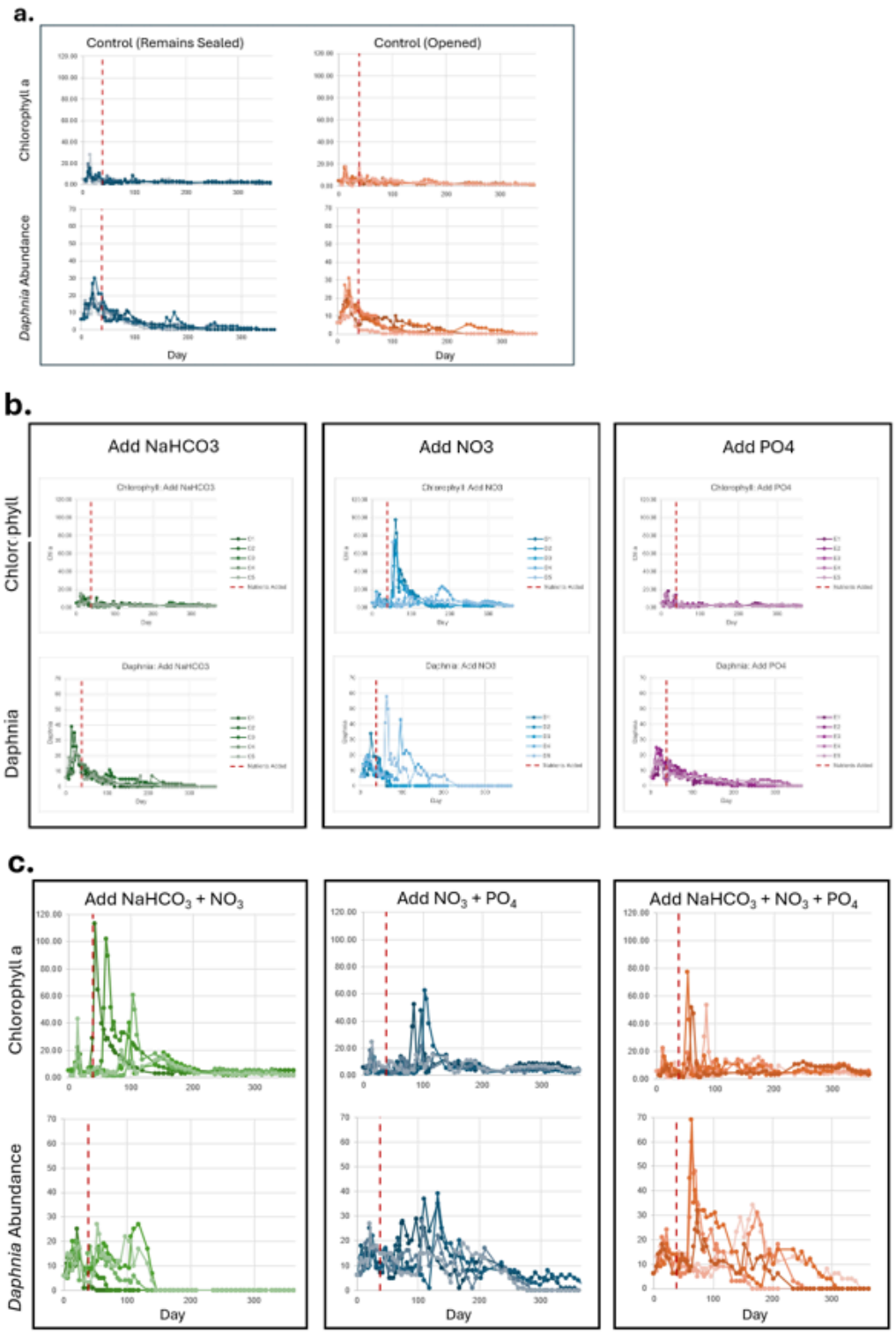

Figure 2

All CESs started with the same nutrient concentrations; they differed only by various nutrient concentrations added on day 38 (vertical line). Each line represents one replicate. 2.a. represents the Chl a and *Daphnia*responses of the control (unopened) and the opened system (< 10 minutes); 2.b. represents the CESs that received one additional chemical, as labeled; and 2.c. represents CESs that received multiple chemical additions. Opening had no effect. Adding C alone or P alone had no effect. Adding N alone had complex effects; if *Daphnia* grazing was low, the Chl a increased greatly (3 replicates); if the *Daphnia* grazing was high (2 replicates), no Chl a increase was seen. The addition of multiple chemicals significantly increased Chl a and *Daphnia*. Statistics are in S3.5.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx
- SupplementaryData.xlsx