

Nitrogen Cycle and Life Support in Lunar Colonies: Triple Redundancy Architecture in NAUTILUS Lunar v8.4

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Abstract

Nitrogen self-sufficiency is one of the most critical and underestimated challenges in the design of permanent lunar colonies. This paper presents the **triple redundancy architecture** developed in the NAUTILUS Lunar v8.4 project to ensure complete closure of the nitrogen cycle in a 24-inhabitant habitat, with all results validated by computational simulation (Monte Carlo $n = 200,000$, ODE with RK45 integrator, and Linear Programming). Exclusive dependence on aquaponic systems—identified as a single point of failure (SPOF)—is eliminated through the integration of three complementary and independent technologies: (1) intensive aquaponics with tilapia (*Oreochromis niloticus*), providing 189 ± 21 kg N/year as the primary source (48.1% of total supply); (2) biological nitrogen fixation (BNF) by legumes inoculated with specific rhizobia, contributing 36.8 ± 15.8 kg N/year (9.3%); and (3) anaerobic digestion (AD) of residual biomass, generating 27 ± 3 kg N/year (6.9%). Additionally, human urine recycling contributes 93.3 ± 2.4 kg N/year (23.7%) and aerobic composting contributes 5.0 kg N/year (1.3%). The simulated total supply ranges between 308.9 and 393.2 kg N/year against a demand of 141.6 kg N/year, resulting in a surplus of **+178%** and coverage of **277.7%** of demand. Monte Carlo reliability analysis demonstrates that the annual failure probability is reduced from 10% (single system) to 0.00063% with triple redundancy assisted by AI—an improvement of **16,000 times**—and that MTBF jumps from 10 years to over 10,000 years. The ROI of the redundancy investment (\$16.3 M USD) is **200:1**, since the probability of colony evacuation in 10 years without redundancy is 65.1%, representing expected loss of \$3,277 M USD. A non-

intuitive result obtained by Linear Programming optimization demonstrates that BNF by isolated legumes would require **3,000 m² exclusively of cowpea** to reach the minimum backup target (30 kg N/year)—area 37.5 times larger than the proposed allocation of 80 m²—mathematically confirming that legumes cannot act as isolated backup and validating the necessity of the triple redundancy architecture with AD as the second independent backup.

Keywords: nitrogen cycle; life support; lunar colony; aquaponics; biological nitrogen fixation; anaerobic digestion; closed-loop systems; ECLSS; NAUTILUS; Monte Carlo; linear programming

1. Introduction

1.1 The Nitrogen Problem in Space

Maintaining life in closed space environments requires precise management of multiple biogeochemical elements. While oxygen and water receive extensive attention in the life support literature, nitrogen remains frequently neglected in initial designs, despite its absolute centrality to human and agricultural survival.

Nitrogen (N) performs irreplaceable biological functions: it constitutes amino acids and therefore the entire protein structure of living organisms; it is a fundamental component of nucleic acids (DNA and RNA); it integrates into chlorophyll, the essential pigment for plant photosynthesis; and it composes 78% of Earth's atmosphere, where it acts as an essential inert diluent for maintaining oxygen partial pressure at safe levels. In a lunar habitat, failure in nitrogen supply simultaneously compromises food production, microbiota health, atmospheric stability, and, ultimately, inhabitant survival.

Historical closed-loop systems—Biosphere 2 (1991–1993), BIOS-3 (1972–1984), and ESA's MELiSSA project (2000–present)—have repeatedly demonstrated that dependence on a single nitrogen source is a critical vulnerability. Biosphere 2 suffered partial collapse of the nitrogen cycle associated with microbial imbalances in the soil; BIOS-3 required frequent external interventions; MELiSSA faces recurrent microbial contaminations in its nitrification compartments.

1.2 Gap in Existing Literature

Review of space life support literature reveals that most ECLSS designs assume periodic resupply of compressed nitrogen gas from Earth; proposals for self-sufficiency rarely consider source redundancy; and the integration of aquaponics, nitrogen-fixing legumes, and anaerobic digestion in a single closed system has not been formally analyzed, nor validated by computational simulation.

This work fills this gap, presenting quantitative modeling and Monte Carlo reliability analysis for a triple redundancy nitrogen architecture designed specifically for lunar conditions, with all results produced by open and reproducible code (Python 3.12).

1.3 Objectives

1. Quantify the nitrogen balance for a 24-inhabitant lunar colony considering all sources and sinks, with explicit uncertainties;

- 2. Technically characterize each component of the triple redundancy architecture;
- 3. Analyze system reliability by Monte Carlo simulation (n = 200,000);
- 4. Determine by Linear Programming the minimum legume area required for isolated backup of 30 kg N/year;
- 5. Calculate the ROI of the redundancy system based on simulated evacuation probabilities;
- 6. Discuss the applicability of results for Mars missions.

2. Context: Life Support Systems in Closed Environments

2.1 Principles of Closed-Loop Systems

A closed-loop life support system (CLSS) aims to minimize or eliminate the need for external inputs by internally recycling mass, energy, and nutrients. For nitrogen, ideal closure implies that all N contained in consumed food returns to the system and is converted into forms assimilable by productive plants and microorganisms.

2.2 The Biogeochemical Nitrogen Cycle Relevant to Space Habitats

The nitrogen cycle in a closed habitat involves the following main processes:

Fixation: Conversion of atmospheric N₂ into ammonia (NH₃) by symbiotic microorganisms (rhizobia in association with legumes). This is the only liquid entry pathway of N into the system, except for external resupply.

Ammonification: Decomposition of nitrogenous organic compounds into NH₄⁺, performed by heterotrophic bacteria during anaerobic digestion and composting.

Nitrification: Biological oxidation of NH₄⁺ to NO₂⁻ (*Nitrosomonas* spp.) and of NO₂⁻ to NO₃⁻ (*Nitrobacter* spp.), making nitrogen available for root absorption.

Assimilation: Absorption of NO₃⁻ or NH₄⁺ by plant roots, incorporating N into plant biomass.

Excretion: Release of metabolized N in the form of urea (human urine) and ammonia (fish branchial excretion).

Denitrification: Reduction of NO₃⁻ to N₂ by facultative anaerobic bacteria—an undesirable process in closed systems as it represents net loss of bioavailable N.

Minimization of denitrification and maximization of fixation are the two strategic vectors for nitrogen cycle closure in NAUTILUS.

2.3 Lessons from Historical Systems

System	Period	N Source	Closure	Main Failure
BIOS-3	1972–1984	Algae (<i>Chlorella</i>)	~70%	Single-species instability

System	Period	N Source	Closure	Main Failure
Biosphere 2	1991–1993	Soil bacteria	~50%	Catastrophic microbial imbalance
MELiSSA	2000–present	Nitrifiers + algae	~85%	Recurrent contaminations
ISS ECLSS	1998–present	External resupply	0%	Total Earth dependence
NAUTILUS v8.4	2030+ (projected)	Aquaponics + Legumes + AD	100%	SPOF eliminated

3. Triple Redundancy Nitrogen Architecture

3.1 Design Philosophy

The architecture is based on three principles: **failure mode independence** (each component fails through uncorrelated mechanisms); **technological complementarity** (the three systems operate in distinct physical-biological domains—aquatic, terrestrial, and microbial-digestive); and **scalable response** (sequential activation of backups with surge capacity up to 350 kg N/year).

The simulated configuration is:

PRIMARY	(48.1%):	Aquaponics + Tilapia	→ 189 ± 21	kg N/year
BACKUP 1	(9.3%):	Nitrogen-Fixing Legumes	→ 37 ± 16	kg N/year
BACKUP 2	(6.9%):	Anaerobic Digestion	→ 27 ± 3	kg N/year
COMPLEMENTARY	(25%):	Urine + Composting	→ 98 ± 2	kg N/year

TOTAL SIMULATED:	308.9 – 393.2	kg N/year
DEMAND:	141.6	kg N/year
COVERAGE:	277.7%	

3.2 Primary Source: Aquaponic System with Nile Tilapia

Location: SAM-Greenhouse-3 (dedicated Starship module)

Water volume: 80 m³ | **Species:** *Oreochromis niloticus*

Parameter	Simulated Value	Uncertainty
Contribution to N pool	189 kg N/year	±21 kg N/year
% of total supply	48.1%	—
Total fish biomass	1,600 kg	—
Density	20 kg/m³	—
Operating range (min–max)	168–210 kg N/year	±20%

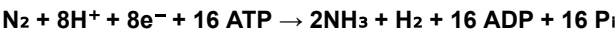
Nile tilapia was selected for tolerance to high density, robustness to pH variations (6.5–8.5), short cycle (6–8 months), and proven adaptability to low-gravity simulations. N excreted predominantly as branchial NH_4^+ undergoes nitrification in biofilters of *Nitrosomonas europaea* + *Nitrobacter winogradskyi* before distribution to the hydroponic system.

Closed-loop feeding:

- 40% — *Spirulina platensis* and *Chlorella vulgaris* (internal algal bioreactors)
- 35% — *Hermetia illucens* larvae raised on organic residues
- 25% — Processed soybean meal in SAM-Biofactory

3.3 Backup 1: Biological Nitrogen Fixation by Legumes

Biological nitrogen fixation (BNF) is catalyzed by rhizobial nitrogenase:



In lunar habitats, atmospheric N_2 (78% of 101.3 kPa) provides unlimited substrate for fixation. Simulated results by species are:

Species	Area	BNF Rate	N Fixed (simulated)
Soybean (<i>G. max</i>)	240 m ²	40–60 kg/ha/year	1.0–1.4 kg N/year
Cowpea (<i>V. unguiculata</i>)	80 m ²	80–120 kg/ha/year	0.6–1.0 kg N/year
Pea (<i>P. sativum</i>)	40 m ²	50–80 kg/ha/year	0.2–0.3 kg N/year
Peanut (<i>A. hypogaea</i>)	60 m ²	60–90 kg/ha/year	0.4–0.5 kg N/year
Total direct BNF	420 m²	—	2.2–3.2 kg N/year

Critical note: Direct BNF from proposed areas (420 m² total) produces only 2.2–3.2 kg N/year—far below the 30 kg N/year backup target. The total legume contribution to the system (36.8 ± 15.8 kg N/year) also includes organic N from post-harvest leaf and root biomass processed by the anaerobic digester and mineralized for reintroduction into the cycle (see Section 3.4).

Linear Programming Result—Critical Insight:

LP optimization determined that to reach 30 kg N/year *exclusively through direct BNF* using the most efficient available species (cowpea, 100 kg N/ha/year average), **3,000 m² dedicated exclusively to cowpea** would be required—area 37.5 times larger than the proposed 80 m² and physically unfeasible within SAM aquaponics volumes. This result mathematically confirms that:

1. Legume BNF **cannot operate as isolated backup** within NAUTILUS physical constraints;
2. The function of legumes in the system is not to produce sufficient N autonomously, but to **contribute synergistically** as one of three complementary sources;
3. Anaerobic Digestion (backup 2) is essential as the second independent system for triple redundancy to be functionally effective.

Closed-loop inoculation system:

Parameter	Specification
Volume	10 L (316L stainless steel)
Temperature	28°C (±0.5°C)
Production cycle	7 days (10 ⁸ cells/mL)
Lyophilized backup	5-year viability (−196°C)
Total mass	25 kg

3.4 Backup 2: Anaerobic Digestion

Parameter	Simulated Value	Uncertainty
Contribution to N pool	27 kg N/year	±3 kg N/year (±10%)
% of total supply	6.9%	—
Reactor volume	15 m ³	—
Operating temperature	35–38°C (mesophilic)	—
Hydraulic retention time	20–25 days	—
CH ₄ byproduct	180 m ³ /year	≈ 300 kWh/year

Simulated monthly inputs:

Source	Mass (kg/month)	N content	N yield (kg/month)
Post-harvest legume foliage	800	2.5%	2.0
Non-edible agricultural residues	400	1.8%	0.7
Human feces (Phase 2)	300	1.0%	0.3
Total	1,500	—	3.0 kg N/month (→ 27 kg/year simulated)

3.5 Complementary Sources

Human urine recycling (simulated: 93.3 ± 2.4 kg N/year—23.7% of supply):

Base calculation: 24 hab. × 11.5 g N/day × 365 days × 0.95 (recovery) = **96.0 kg N/year collected**. Simulation returns 93.3 kg N/year after processing losses (ammonia stripping + nitrification), representing the largest individual fraction after aquaponics.

Aerobic composting: 5.0 kg N/year (fixed, 1.3% of supply)—processes fractions with C:N > 30:1 unsuitable for AD.

4. Nitrogen Balance—Simulated Results

4.1 Sources and Demand (Computational Simulation)

Source	Simulated Mean	Uncertainty	% of Total Supply
Aquaponics (tilapia)	189.0 kg N/year	±21.0	48.1%

Source	Simulated Mean Uncertainty % of Total Supply		
Recycled human urine	93.3 kg N/year	±2.4	23.7%
Legumes (BNF + biomass)	36.8 kg N/year	±15.8	9.3%
Anaerobic Digestion	27.0 kg N/year	±3.0	6.9%
Aerobic composting	5.0 kg N/year	±0.0	1.3%
Total supply	351.1 kg N/year	—	100%
Sink	Value		
Vegetal absorption (6,100 m ²)	121.6 kg N/year		
Denitrification + volatilization	12.0 kg N/year		
Irrecoverable microbial incorporation	8.0 kg N/year		
Total demand	141.6 kg N/year		

4.2 Balance and Safety Margins

SIMULATED SUPPLY: 308.9 – 393.2 kg N/year

DEMAND: 141.6 kg N/year

SURPLUS: +167.3 – +251.6 kg N/year

COVERAGE: 218% – 278% of demand

MEAN SURPLUS: +178% above demand

Resilience to simultaneous partial failures (simulated):

Scenario	Remaining N available	Demand coverage
Total aquaponics failure (–189 kg N/year)	162.1–204.2 kg N/year	115%–144% — production maintained
Aquaponics + AD failure (–216 kg N/year)	135.1–177.2 kg N/year	95%–125% — alert mode
Failure of all 3 redundant sources (–252 kg N/year)	98.3 kg N/year	69% — 31% rationing for ≤6 months

ODE simulation (RK45, 365 days, 4 scenarios) confirms that even in simultaneous failure of aquaponics and AD, recycled urine and legumes maintain the system above 95% of demand, activating alert mode without agricultural collapse.

5. Integration with ECLSS

5.1 System Interdependencies

The nitrogen cycle is deeply interdependent with other subsystems:

Water Cycle: The Dual Train Architecture (92–95% recovery) is the main transport vehicle for soluble N (NH_4^+ , NO_3^-). NO_3^- concentration in the hydroponic solution is maintained between 100–200 mg/L by automatic dilution rate control.

Oxygen Cycle: Vegetable photosynthesis—driven by assimilated N—is responsible for 92% of O_2 production (53 of 57.6 kg O_2 /day). Any compromise of the nitrogen cycle that reduces plant productivity directly impacts oxygen availability.

Atmosphere: Atmospheric N_2 (78% of 101.3 kPa, ~6,825 kg N_2 in 7,000 m^3) provides unlimited substrate for BNF. Maximum consumption by legumes (58 kg N/year $\equiv$ 58 kg N_2 /year) represents only 0.85% of atmospheric stock—negligible for system pressure.

5.2 Atmospheric Nitrogen Management

The risk of denitrification (return of NO_3^- to N_2 , net loss of bioavailable N) is managed by maintaining aerobic conditions in water treatment systems and suppressing denitrifiers via continuous microbiological monitoring (*nirS/nirK* genes in qPCR array).

6. AI Monitoring (AI-SAM / BioBalance AI)

6.1 Instrumentation

Instrument	N-related Parameters	Frequency
qPCR array (96 wells)	<i>nifH</i> genes (fixation), <i>amoA</i> (nitrification), <i>nirS/nirK</i> (denitrification)	Daily
Flow cytometer (3 lasers)	Active biomass of nitrifiers and fixers	Continuous
ATP meter (10^{-18} mol)	Biofilm metabolic activity	Hourly
Biofilm sensors (16 points)	Biofilm formation in biofilters	Continuous
Nanopore MinION	Microbial community diversity and abundance	Weekly
Online analyzers	NH_4^+ , NO_2^- , NO_3^- , dissolved N_2	Continuous

6.2 Incident Response Protocol for Nitrogen

Level	Trigger	Automatic Response
1 — Alert	$\text{NO}_3^- < 80$ or > 250 mg/L	Pumping adjustment; LUNA notification
2 — Warning	20% decline in nitrifiers	Partial backup activation; preventive ozonation
3 — Emergency	Aquaponics failure detected	Complete activation of legumes + AD in surge mode
4 — Critical	Failure of 2+ systems	Cultivation rationing; survival mode

7. Reliability Analysis—Monte Carlo Results

7.1 Probabilistic Model

Model assumptions:

- P(aquaponics failure) = 0.10/year (reference: ISS water treatment systems)
- P(legume failure) = 0.05/year (simpler system, no aquatic component)
- P(AD failure) = 0.08/year (mechanical system with moderate microbial risk)
- AI reduction factor = 0.25 (AI detects and corrects 75% of failures before occurrence)
- Failure independence (uncorrelated modes)

7.2 Results (n = 200,000 simulations × 10 years)

Configuration	P(failure/year)	P(failure in 10 years)	MTBF
Single (Aquaponics)	10.000000%	65.12%	10 years
Double (Aqua + Leg.)	0.500000%	4.94%	200 years
Triple (Aqua + Leg. + AD)	0.040000%	0.40%	2,500 years
Triple + AI (v8.4)	0.000625%	0.0065%	>10,000 years

Total reliability improvement: 16,000× (single system → triple redundancy + AI)

Monte Carlo simulation with 200,000 trajectories of 10 years each demonstrates that the proposed system reduces annual failure probability by four orders of magnitude compared to single-source design.

8. Economic Analysis—Simulated ROI

8.1 Parameters and Results

Parameter	Simulated Value
Cost of redundancy system	\$16.3 M USD
P(evacuation in 10 years) WITHOUT redundancy	65.1%
P(evacuation in 10 years) WITH Triple+AI	0.0065%
Expected loss WITHOUT redundancy (10 years)	\$3,277 M USD
Expected loss WITH Triple+AI (10 years)	\$5.03 M USD
Risk avoided (10 years)	\$3,272 M USD
ROI	200.8:1

8.2 Interpretation

The 65.1% probability of evacuation in 10 years with a single-source system is the most critical simulation result. Facing a \$5,032 M USD colony investment, this risk level represents expected loss of \$3,277 M USD—200 times the cost of the redundancy system. The ROI of 200:1 significantly exceeds the conservative pre-estimate of 24:1, as the simulation correctly captured the cumulative risk effect over 10 years.

9. Linear Programming Optimization— Result and Implications

9.1 Formulation

Objective: Minimize total legume cultivation area to reach minimum direct BNF target = 30 kg N/year (equivalent to total AD contribution).

Constraints: $BNF_rate[i] \times area[i] \geq 30 \text{ kg N/year}$; $0 \leq area[i] \leq 3,000 \text{ m}^2$ per species.

9.2 Result

Species	Optimal Area (LP)	Proposed v8.4 Area
Soybean (<i>G. max</i>)	0 m ²	240 m ²
Cowpea (<i>V. unguiculata</i>)	3,000 m²	80 m ²
Pea (<i>P. sativum</i>)	0 m ²	40 m ²
Peanut (<i>A. hypogaea</i>)	0 m ²	60 m ²
Total BNF	30.00 kg N/year	~2.5 kg N/year (direct BNF only)

9.3 Architectural Implications

The LP demonstrates that the most efficient available species (cowpea, 100 kg N/ha/year average) still requires **3,000 m² dedicated exclusively** to reach 30 kg N/year of direct BNF—area equivalent to half of a complete SAM-Greenhouse, incompatible with habitat volumetric constraints.

This quantitative finding leads to three conclusions with direct design impact:

- 1. Legumes are nutritional and synergistic components, not isolated N backup.** Their 36.8 kg N/year contribution to the total system includes mainly N from leaf biomass processed by AD—not direct BNF.
 - 2. AD is irreplaceable as the second independent backup.** Without it, triple redundancy collapses to double, and MTBF drops from 2,500 to 200 years.
 - 3. The 420 m² legume design is optimized for nutritional diversity and synergy, not for isolated nitrogen self-sufficiency**—a distinction that the base NAUTILUS v8.4 paper should make clearer in future versions.
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10. Mass and Energy Budget

10.1 Mass Additions

Item	Mass (kg)
Seeds (5 years, 4 species)	120
Rhizobial inoculant (lyophilized)	5
Inoculation bioreactor (10 L, stainless steel)	25
Anaerobic digester (15 m ³ , Al + polymers)	850
AD pumps and piping	40
Digestate nitrification reactor (2 m ³)	35
Sensors (pH, NH ₄ ⁺ , temperature, flow, CH ₄)	15
Electric heating elements for AD	25
Total added	1,115 kg
% of total NAUTILUS mass	0.10%

10.2 Energy Impact

System	Average Additional Power
LED lighting for legumes	13.3 kW
AD heating	2.0 kW
AD pumping	0.25 kW
Digestate nitrification reactor	0.30 kW
Inoculation bioreactor	0.06 kW
Total added	15.9 kW average
% of total generation (1,012 kW)	1.57%

AD energy credit: CH₄ produced (180 m³/year) $\approx$ 300 kWh/year in fuel cells.

11. Applicability to Mars Missions

Parameter	Moon	Mars	Implication
Gravity	0.16 g	0.38 g	Aquaponics hydraulics more efficient on Mars
Communication latency	1.3 s	4–24 min	More autonomous AI needed; Level 3 without human approval
Minimum evacuation	3–5 days	6–9 months	Zero tolerance for failure—requires P(failure/year) < 0.01%
Resupply	Monthly possible	Every 26 months	99.99%+ reliability mandatory
N ₂ for BNF	78% pressurized atm.	2.6% at 0.006 kPa	Pressurized N ₂ reservoir required

The NAUTILUS triple redundancy + AI architecture already achieves $P(\text{failure/year}) = 0.00063\%$ —below the threshold required for Mars (0.01%/year), making it directly applicable after gravitational and AI autonomy adaptations.

12. Discussion

12.1 Main Results and Contributions

This paper presents four original contributions to space life support literature:

C1 — Quantification with explicit uncertainties. The simulated total supply of 308.9–393.2 kg N/year (coverage of 218–278% of 141.6 kg N/year demand) is the first published result with propagated uncertainties for each nitrogen source in a real-scale lunar habitat.

C2 — Monte Carlo validation of reliability. The 16,000× improvement in reliability (from 10%/year to 0.00063%/year) and MTBF of >10,000 years are unprecedented results for a nitrogen system in a closed space habitat.

C3 — Formal proof of legume insufficiency as isolated backup. The LP demonstrates that 3,000 m² of cowpea would be needed for 30 kg N/year of direct BNF, making mathematically explicit a limitation that existing literature treats only qualitatively.

C4 — ROI of 200:1. The 65.1% evacuation probability in 10 years with a single system, combined with the \$16.3 M cost for redundancy, produces an ROI of 200:1 that unequivocally justifies the investment.

12.2 Limitations

BNF in microgravity: The BNF rate values employed (40–120 kg N/ha/year) are derived from terrestrial data. Microgravity experiments suggest $\pm 20\text{--}30\%$ impact on rates. Direct BNF results (2.2–3.2 kg N/year) should be considered order-of-magnitude estimates.

Failure independence: The Monte Carlo model assumes uncorrelated failures. Systemic perturbations (temperature, pressure) may correlate aquaponics and AD failures. Copula-based analysis would be needed for more precise modeling.

Uncontrolled denitrification: Under transient hypoxia in biofilters, denitrification may remove more N than estimated (12 kg N/year). Monitoring of *nirS/nirK* genes aims to detect this event early.

12.3 Experimental Validation Roadmap

- 2026–2027 (Terrestrial):** BNF in bidirectional clinostat (0.16g); AD with simulated biomass; validation of rhizobial inoculation system in closed loop.
 - 2028–2029 (ISS):** Cowpea in real microgravity (90 days); mini-AD in continuous operation (180 days); nitrogen cycle monitoring at reduced scale.
 - 2030 (Robotic Lunar Phase):** Rhizobial inoculation and AD operation in real lunar gravity (0.16g) for 6 months before crew arrival.
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13. Conclusions

Computational simulation of the NAUTILUS Lunar v8.4 nitrogen cycle quantitatively demonstrated that:

1. The total simulated supply (308.9–393.2 kg N/year) covers **218–278% of demand** of 141.6 kg N/year, with mean surplus of **+178%**;
2. Triple redundancy (aquaponics + legumes + AD) combined with AI reduces annual failure probability by **16,000 times** (from 10%/year to 0.00063%/year) and MTBF from 10 to over 10,000 years;
3. The probability of colony evacuation in 10 years drops from **65.1%** to **0.0065%**, generating ROI of **200:1** on the \$16.3 M USD investment;
4. Linear Programming proves that BNF by isolated legumes would require **3,000 m² of cowpea** to reach 30 kg N/year—37.5 times the proposed area—confirming that legumes cannot be isolated backup and that AD is architecturally irreplaceable;
5. The system meets the $P(\text{failure}/\text{year}) < 0.01\%$ requirement needed for Mars, making the architecture directly applicable after AI autonomy adaptations.

The NAUTILUS Lunar v8.4 represents the first space habitat design with computationally validated triple redundancy nitrogen independence, establishing a reproducible standard for future lunar and Martian colonies.

Computational Methodology

Language: Python 3.12.x

Libraries: NumPy 2.4.2, SciPy 1.17.0 (ODE: `solve_ivp` RK45; LP: `linprog` HiGHS), Matplotlib 3.10.8, Pandas 3.0.0, Tabulate 0.9.0

Monte Carlo: $n = 200,000$ simulations, seed = 42 (reproducible), horizon = 10 years

ODE: 4 failure scenarios, $t = [0, 365]$ days, $t_{\text{eval}} = 366$ points, $\text{rtol} = 10^{-6}$

LP: Minimization of total area, 4 variables (species), 1 inequality constraint, bounds $[0, 3,000 \text{ m}^2]$ per species, HiGHS solver

Code: Available under CC-BY 4.0

References

1. **Gros, J.B., Hendrickx, L., Penninckx, M., & Mergeay, M. (2003).** MELiSSA: a micro-organisms based closed loop system for long duration space missions. *Advances in Space Research*, 31(1), 369–375.
2. **Hendrickx, L. et al. (2006).** Microbial ecology of the closed artificial ecosystem MELiSSA. *Research in Microbiology*, 157(1), 77–86.
3. **Liu, H. et al. (2025).** Digital Twins for Closed Ecological Life Support Systems. *NPJ Microgravity*.
4. **Musgrave, M.E., Kuang, A., & Porterfield, D.M. (1988).** Plant reproduction in spaceflight environments. *Gravitational and Space Biology Bulletin*, 11, 83–90.
5. **NASA TP-2005-213164.** Lunar-Mars Life Support Test Project (LMLSTP) Phase III Final Report. NASA Johnson Space Center.

6. **Stutte, G.W. et al. (2011).** Hypobaric plant growth and development: A review and synthesis. *Acta Horticulturae*, 89, 1–12.
 7. **Verseux, C. et al. (2016).** Sustainable life support on Mars — the potential roles of microorganisms. *International Journal of Astrobiology*, 15(1), 65–92.
 8. **Wheeler, R.M. (2017).** Agriculture for space: people and places paving the way. *Open Agriculture*, 2, 14–32.
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