Fiscal Year:	FY 2015	**Task Last Updated:**	FY 07/28/2014
PI Name:	Mancinelli, Rocco Ph.D.		
Project Title:	Elucidating The Nitrogen Cycle of Eu:CROPIS (Euglena: Combined Regenerative Organic-food Production In Space)		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	SPACE BIOLOGY--Cellular and molecular biology		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Microbiology		
Space Biology Cross-Element Discipline:	(1) Reproductive Biology		
Space Biology Special Category:	(1) Cell Culture (2) Translational (Countermeasure) Potential (3) Bioregenerative Life Support		
PI Email:	mancinelli@baeri.org	**Fax:**	FY
PI Organization Type:	NON-PROFIT	**Phone:**	(650) 604-6165
Organization Name:	Bay Area Environmental Research (BAER) Institute		
PI Address 1:	Mail Stop 239-4, NASA Ames Research Center		
PI Address 2:			
PI Web Page:			
City:	Moffett Field	**State:**	CA
Zip Code:	94035	**Congressional District:**	18
Comments:			
Project Type:	FLIGHT	**Solicitation:**	Space Biology Unsolicited
Start Date:	10/01/2013	**End Date:**	09/30/2017
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
Contact Monitor:	Smith, Jeffrey	**Contact Phone:**	650-604-0880
Contact Email:	jeffrey.d.smith2@nasa.gov		
Flight Program:	Small Satellites		
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Hauslage, Jens (DLR (German Aerospace Center))		
Grant/Contract No.:	Coop Agreement via NNX12AD05A		
Performance Goal No.:			
Performance Goal Text:			

Task Description:

Editor's Note (12/2/2013): Funding is for Dr. Mancinelli's participation as Co-I on the German Aerospace Center (DLR)'s Eu:CROPIS (Euglena with Combined Regenerative Organic-food Production In Space) mission and the PI of the nitrogen cycling portion of the mission, entitled "Elucidating The Nitrogen cycle of Eu:CROPIS (Euglena: Combined Regenerative Organic-food Production In Space)".
The objective of the proposed study is to determine the effect of different gravity levels on the nitrogen cycle leveraging experiments to be flown on DLR's Eu:CROPIS mission. This is of importance to NASA because The National Research Council's Plant and Microbial Biology Decadal Survey (2011) states that there is a need for understanding the role of gravity on microbe-microbe interactions and microbe-plant interactions. The research proposed here will do just that. Nitrogen is an essential element for life. It is present in all living systems, occurring in several important molecules including proteins and nucleic acids. Without nitrogen life as we know it could not exits. Thus, the nitrogen cycle is important to supporting life whether it is on Earth, in space, or on other planets or moons. Because only Earth has a 1 x g environment understanding how the nitrogen cycle operates as a function of gravity is key to sustaining life off of Earth. To change the gravity levels the spacecraft will be maneuvered (by spinning) to produce three different gravity regimes during the courser of the mission. The three gravity regimes will be 0.01 x g - 0.1-x g (essentially microgravity); 0.16 x g (Moon gravity) and 0.38 x g (Mars gravity). Each gravity regime will last for six months. Eu:CROPIS will be used in reducing organic waste and in the development of efficient life support systems. Its core element is a microbiological trickling filter of lava rock – the habitat of a multitude of microorganisms that purify and decontaminate water. The development aims at a wet composting system that may be used in closed life support systems such as waste water recovery. A key component of the system is the nitrogen cycle. So, modeling the nitrogen cycle of the system is essential to understanding how the system functions. It will be the first time nitrogen-transformation reactions will be measured as a function of gravity. NASA has an excellent opportunity to participate in the DLR's Eu:CROPIS mission that allows us to obtain data by leveraging their laboratory work and hardware at a fraction of what it would cost if NASA had to pay for it.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

The need for fundamental research to understand the role of gravity on microbe-microbe interactions microbe-plant interactions and microbe human interactions in space is recognized in the National Research Council's Plant and Microbial Biology Decadal Survey (2011) on Biological and Physical Sciences in Space (chapter 4). Through the proposed work, data from the Eu:CROPIS mission will address microbe-microbe and microbe plant interactions through cycling of key nutrients, specifically nitrogen, oxygen and carbon.
Eventually, space travel will require the ability for self-sufficiency. Once mission profiles extend beyond short trips to the lunar surface, the duration of each mission will mean it will no longer remain cost-effective - or indeed feasible - to dispose of all waste and resupply oxygen, water, and food to crew members from Earth. NASA has acknowledged this reality for more than two decades with programs exploring the development of both physicochemical and bioregenerative life support systems. The program on bioregenerative capabilities arose from observations that the only truly long-term, self-sustaining life support system that has a demonstrated stability and efficacy relies upon biological systems for its function; that system is the life support afforded by Earth. Since bioregenerative life support systems are not high on the NASA priority list at this time it was stated in the report: Because international collaborations will be essential to make rapid progress with these aims, NASA should support collaborations, where appropriate, with partners that are already pursuing these goals, such as European scientists….

Eu:CROPIS is a clear example that fits in with this statement. It allows NASA to obtain this data at little cost by using the laboratories, the hardware, and the spacecraft paid for by the DLR. The Eu:CROPIS (Euglena: Combined Regenerative Organic-food Production In Space) experiment will test the feasibility and technology in the areas of life support systems and gravitational biological research. The mission offers for the first time the opportunity of analyzing coupled biological life support systems under different levels of gravity (space, moon, Mars) utilizing state-of-the-art methods for image and molecular analysis. It combines the C.R.O.P. system plant growth water purification system developed at the DLR in Cologne, Germany with the well studied Euglena gracilis space flight system. Euglena gracilis is a motile, photosynthetic, unicellular flagellate living in ponds and lakes. It uses gravity and light as hints to reach and stay in regions of the water column optimal for photosynthesis and growth. At low light irradiances, Euglena swims toward and at higher irradiances cells swim away from a light source (positive and negative phototaxis). In addition, Euglena typically orients away from the center of acceleration (negative gravitaxis). Euglena is considered a 'professional gravi-sensing organism', a term that was coined by ESA. In the past 15 years, Euglena has been established as a model organism for studying gravity perception of single cells. A model for gravitaxis was created by the combination of physiological, biochemical and molecular biological methods. In this context substantial contributions came from microgravity experiments in space.

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Progress to date:

• Demonstrated Euglena growth on NO_3^- as well as on NH_4^+. Significance: Complicates the interpretation of the

Task Progress:

N-transformation reactions and their rates in the primary payload.

• Demonstrated Euglena growth on NH4+ produced by cyanobacteria in co-culture in 2 types of media. Significance: Euglena able to grow on NH4+ produced by other organisms (ground control data for potential contaminants in system – the current prototype CROP system is full of cyanobacteria).

• Colorimetric assays for the various nitrogen species produced variable results leading to the decision to use ion-chromatography for the ground controls and flight experiment. Significance: Changes the hardware, its configuration, as well as mass, power, and volume in the payload.

• Decision finalized to use gas sensors to measure atmospheric gases in the primary payload instead of a gas chromatograph. Significance: Gas sensors are simpler and less prone to error and failure. It also results impacts the hardware configuration as well as resulting in a reduction in the mass and power requirements.

• Using the ion chromatograph we are monitoring the concentration of the ammonium, nitrate, and nitrite in the system as well as net rate of the reactions from a batch of 20% synthetic urine run through the CROP system. Thus far these data show an initial increase in ammonium (presumably from ureases acting on the synthetic urine to produce NH4+) and nitrite (most likely from the first step of nitrification, i.e., the conversion of NH4+ to NO2-). As the concentration of nitrite decreases there is a rise in the concentration of nitrate (from the second step of nitrification, i.e., the conversion of NO2- to NO3). At day 60 the nitrate begins to decrease (presumably due to denitrification; the gas sensors were not yet installed when these data were collected). Reaction rates can be empirically derived from the slope of the line. At day 62 the system is drained and a new batch of synthetic urine is added. Here we see the production of nitrate occurring immediately. One potential explanation is that the system is primed with all of the organisms at a higher population density in the trickling filter to readily begin metabolism, so as soon as ammonium is produced it is transformed to nitrate which is immediately oxidized to nitrate.

• The first phase of a computer model to simulate the microbial and nitrogen species changes in the Eu:CROPIS system was developed. In a test of the computer simulated system is was shown that when there is no light (primary energy source) entering the system oxygen production ceases, CO2 is no longer reduced to organic compounds, accumulates, and the system becomes anaerobic. The anaerobic microbial population increases as they consume the available carbon, but because this is a closed system the microbes run out of nutrients and die and the system ceases to function due to lack of nutrients and energy. In the presence of a light dark cycle the simulated system functions optimally. Unlike when it is in the dark the organic carbon rises ahead of the rise in CO2. The organic carbon then falls as it is consumed by heterotrophs. Slightly delayed from the rise and fall in CO2 is the increase in O2 from photosynthesis which is accompanied by an increase in the Euglena (phototroph) population. These results show that the computer simulation tests were successful and it is reflecting what should happen in the laboratory Eu:CROPIS system.

Bibliography Type: Description: (Last Updated: 02/21/2020)