CMET
Center for Microbial Ecology and Technology

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# Space flight survival of nitrogen-cycle microorganisms

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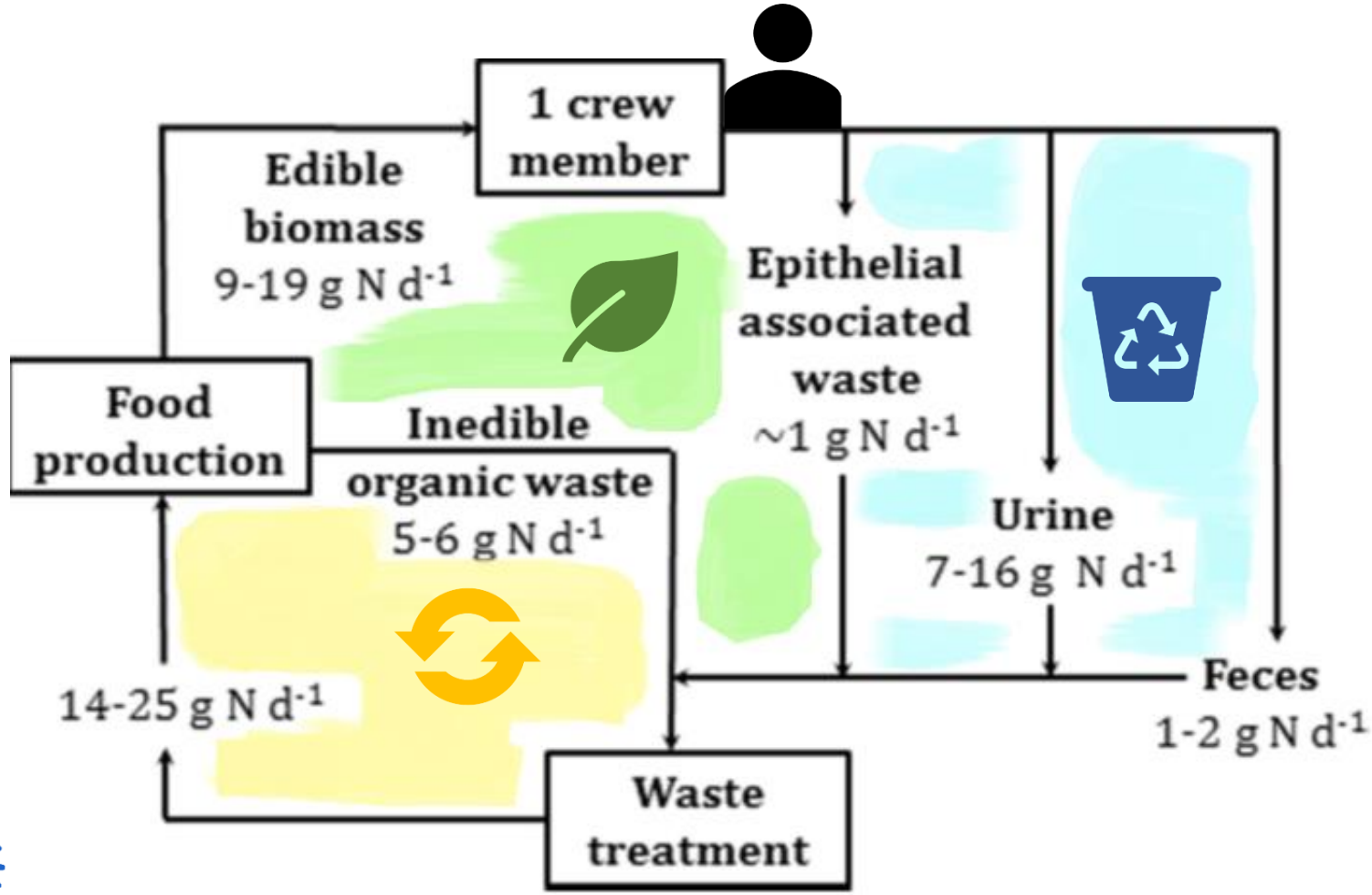
# NITROGEN'S IMPORTANCE TO LIFE

- Nitrogen is an essential building block for nucleic acids (DNA, RNA) and proteins.
- Crucial element in Life Support System to sustain human life away from Earth.

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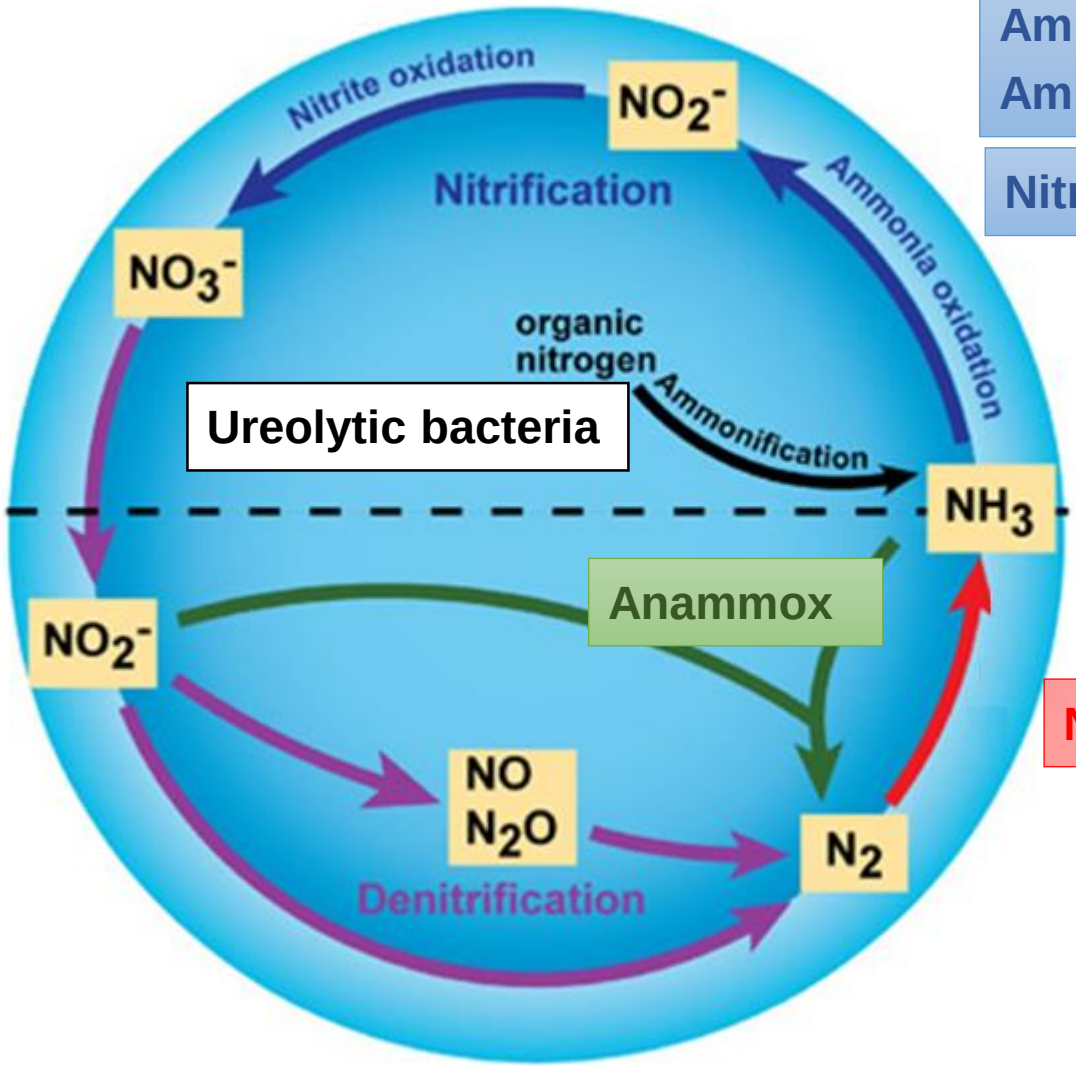
*Clauwaert et al. (2017) Progress in Aerospace Sciences (91)*

# TERRESTRIAL MICROBIAL NITROGEN CYCLE

**Complete ammonia oxidizer (COMMAMOX)**

**Ammonia oxidizing bacteria (AOB)**
**Ammonia oxidizing archaea (AOA)**

**Nitrite oxidizing bacteria (NOB)**




**Denitrifying bacteria**

**Nitrogen fixing bacteria**

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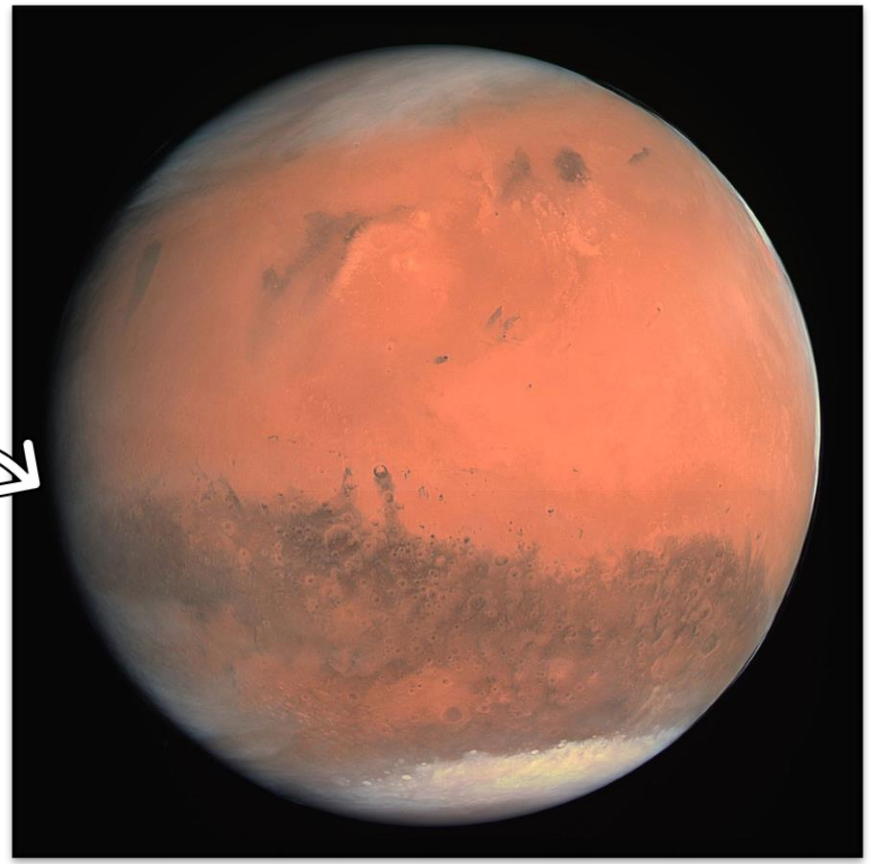
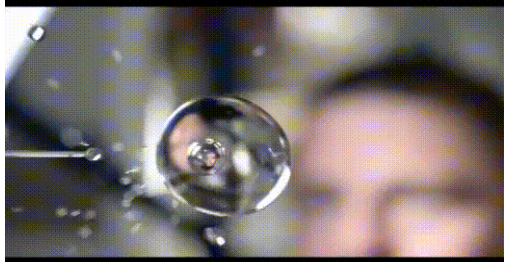
*Bernhard, A. (2010) Nature Education Knowledge*

# PROBLEM STATEMENT

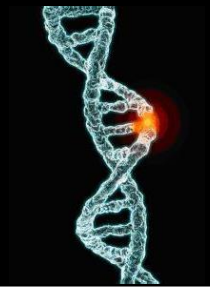


?

**LOW GRAVITY**



**HIGH RADIATION**



**LOGISTIC-STORAGE**

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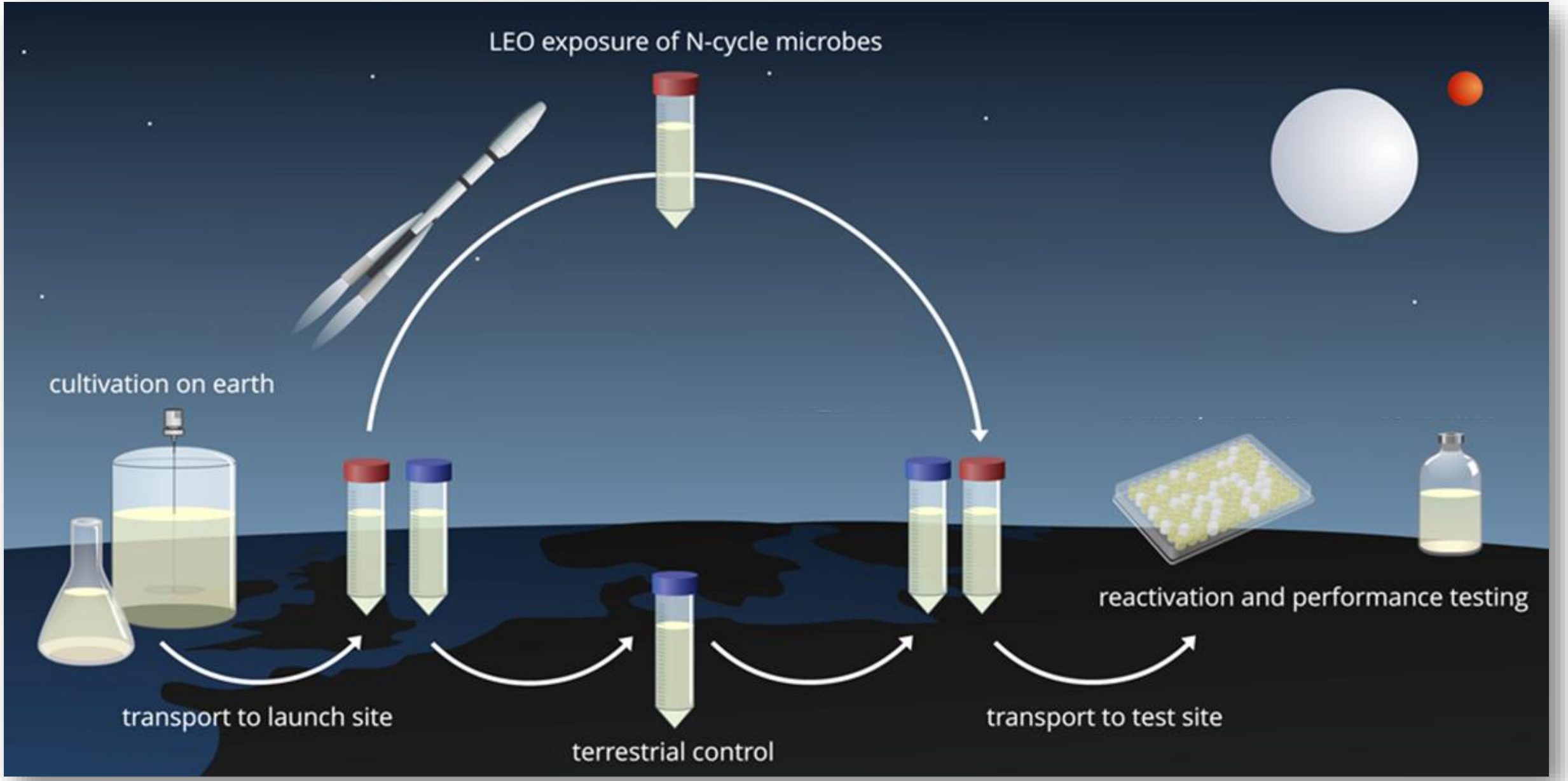
# Reactivation after Space flight



# LOW EARTH ORBIT EXPOSURE

| | ISS | FOTON-M4 |
|---|---|---|
| Duration | 7 days – whole storage 10 days | 44 days – whole storage 104 days |
| | **Short mission** | **Long mission** |

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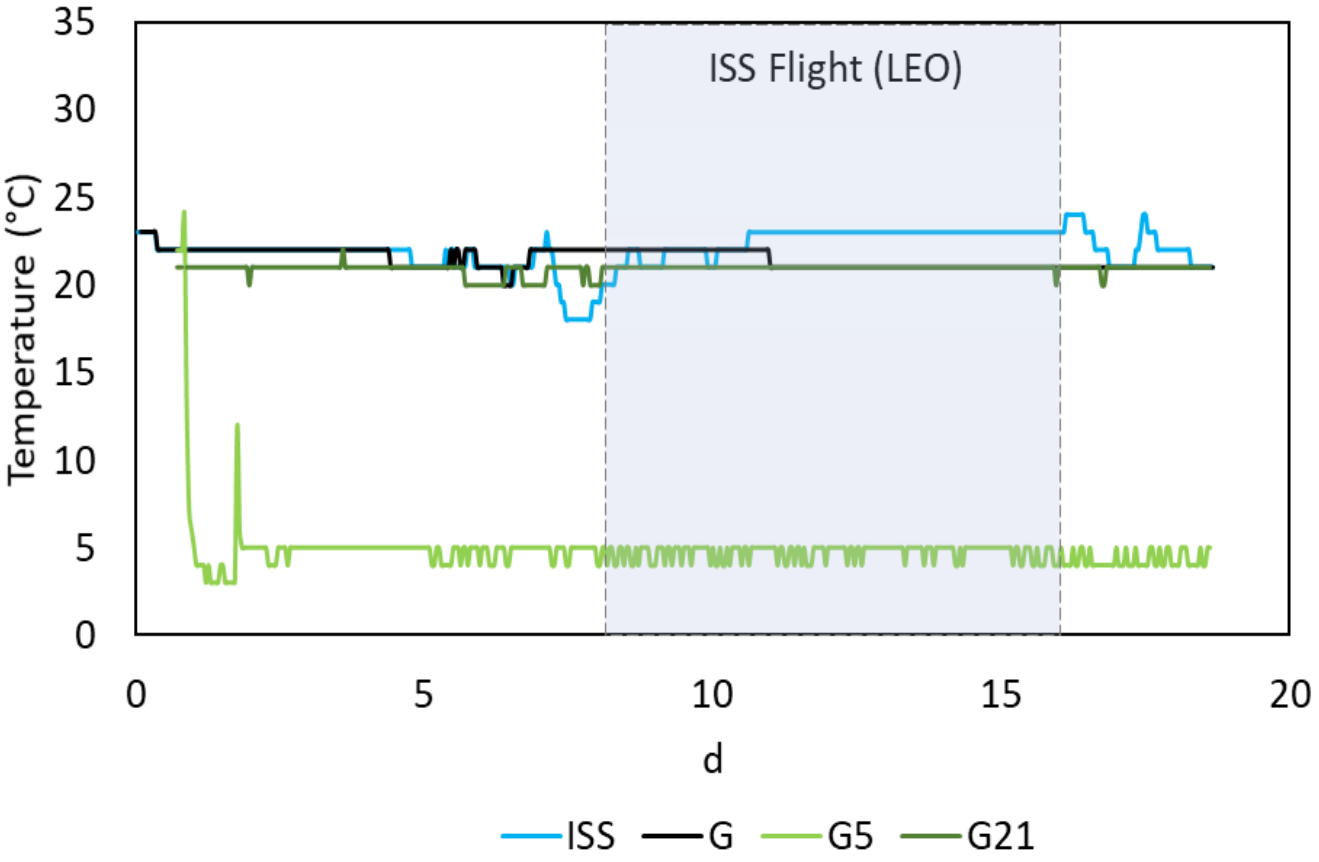
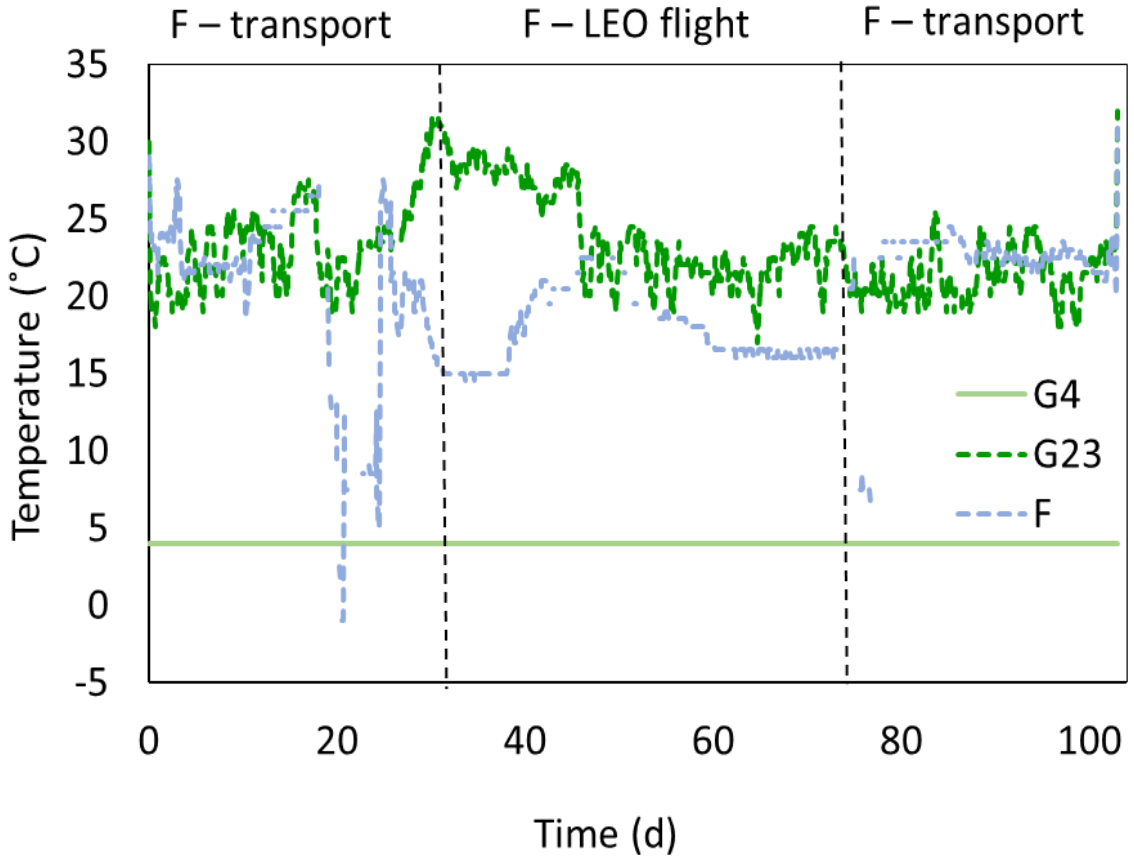
# STORAGE CONDITIONS

| | SHORT MISSION | LONG MISSION |
|---|---|---|
| **Radiation dose of flight samples:** | **140 times higher than on Earth** | **250 – 400 times higher than on Earth** |



# CULTURES SELECTION

| | PURE STRAINS | | | SYNTHETIC COMMUNITIES | |
|---|---|---|---|---|---|
| | *Cupriavidus pinatubonensis* | *Nitrosomonas europaea* | *Nitrobacter winogradskyi* | *N.europaea* + *Nb.winogradskyi* | *C.pinatubonensis* + *N.europaea* + *Nb.winogradskyi* |
| | **C** | **Ns** | **Nb** | **NsNb** | **CNsNb** |
| **Ureolysis** | X | | | | X |
| **Ammonia oxidation** | | X | | X | X |
| **Nitrite oxidation** | | | X | X | X |

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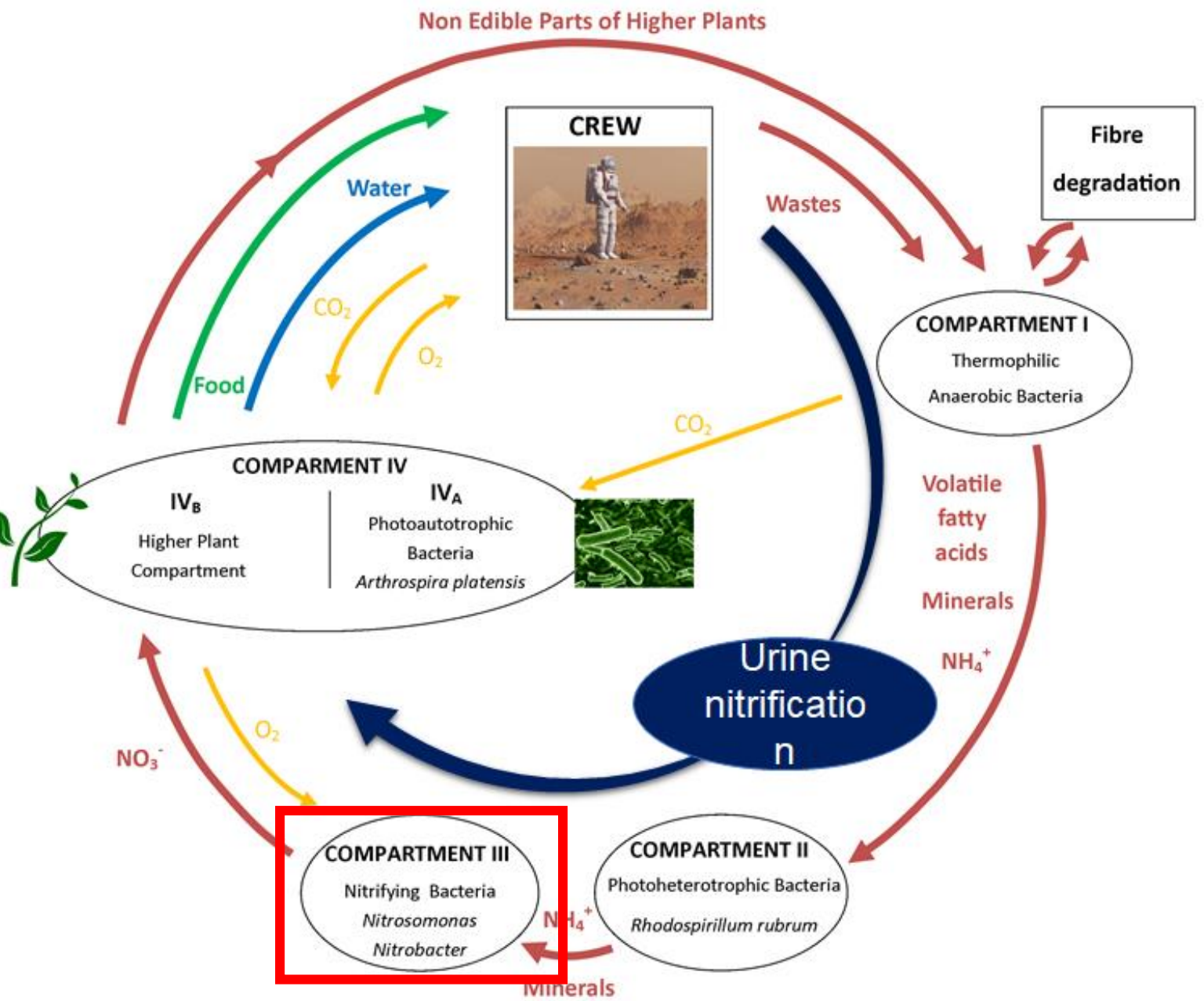
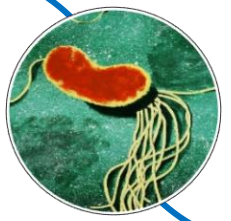
# STRAINS PRESENT IN MELISSA




UREA

**Ureolysis**

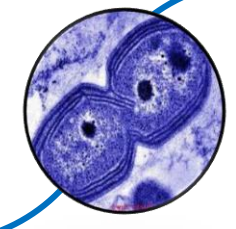
*C. pinatubonensis*



$NH_4^+$

**Ammonia oxidation**

*Ns. europaea*



$NO_2^-$

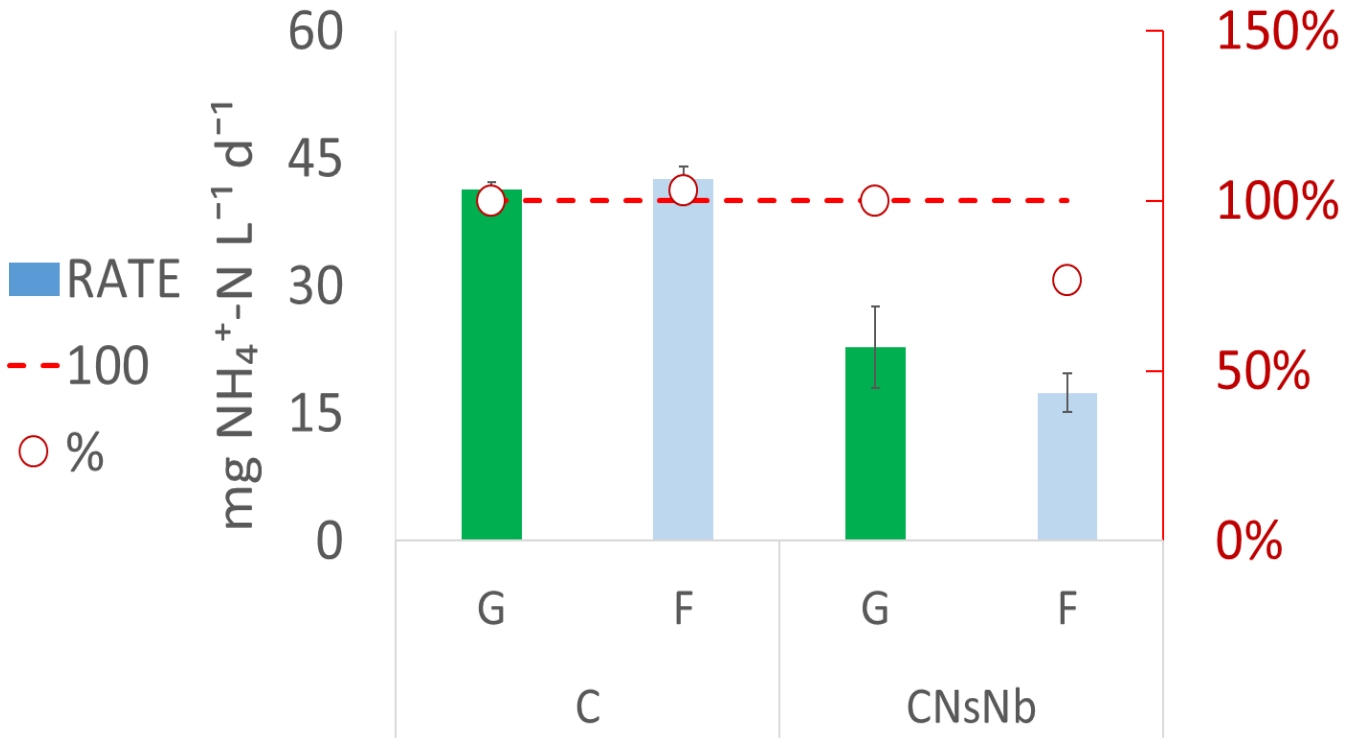
**Nitrite oxidation**

*Nb. winogradskyi*

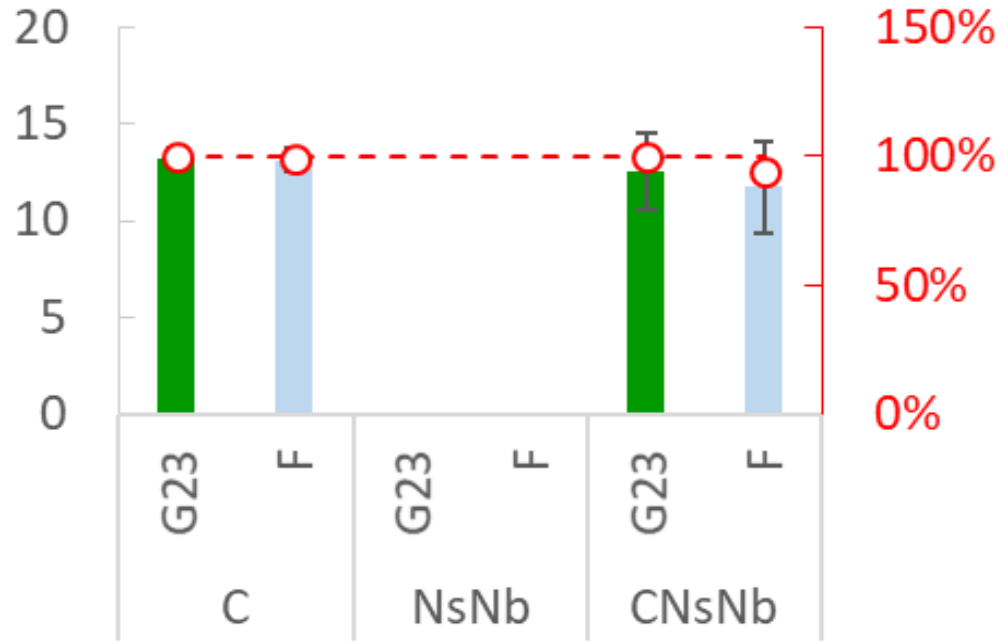
$NO_3^-$

# UREOLYSIS BY *C. PINATUBONENSIS*

SHORT MISSION




LONG MISSION

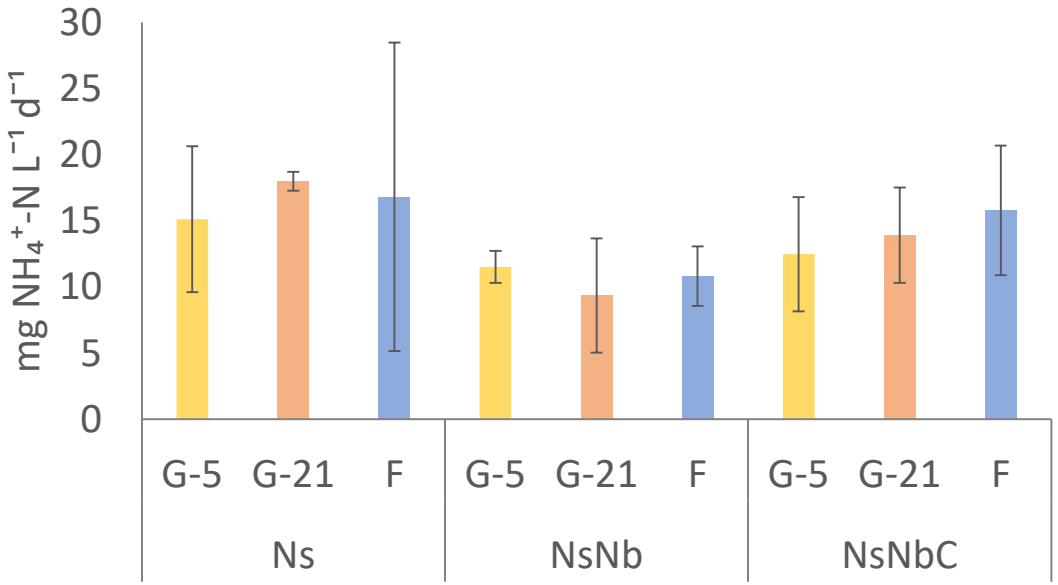



**Take home**

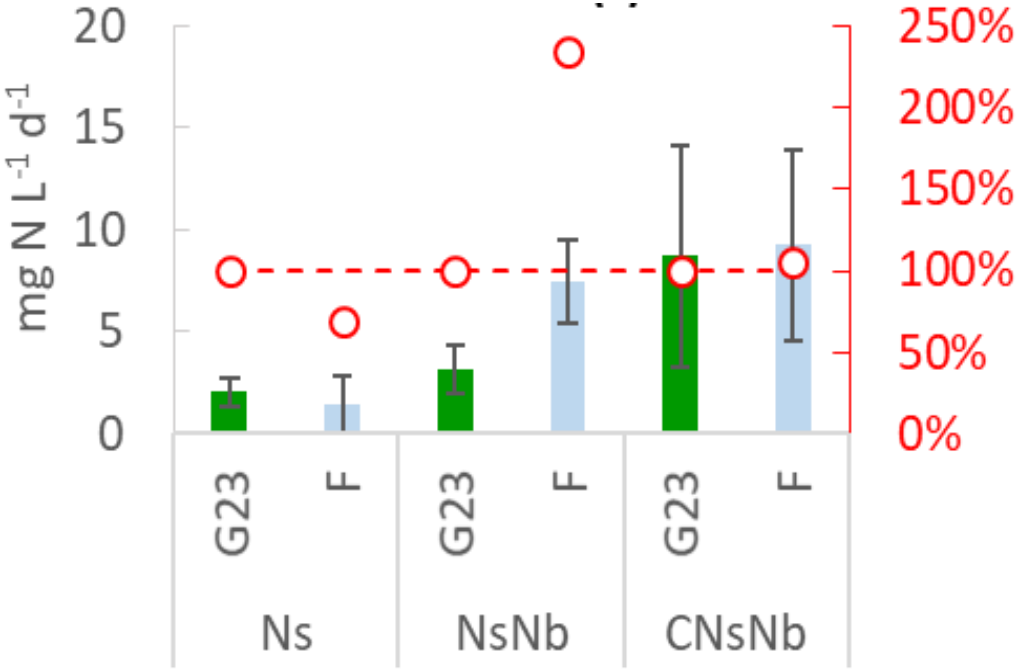
- No effect of flight on *C. pinatubonensis* (both for long and short mission)

# AMMONIA OXIDATION

## SHORT MISSION




## LONG MISSION

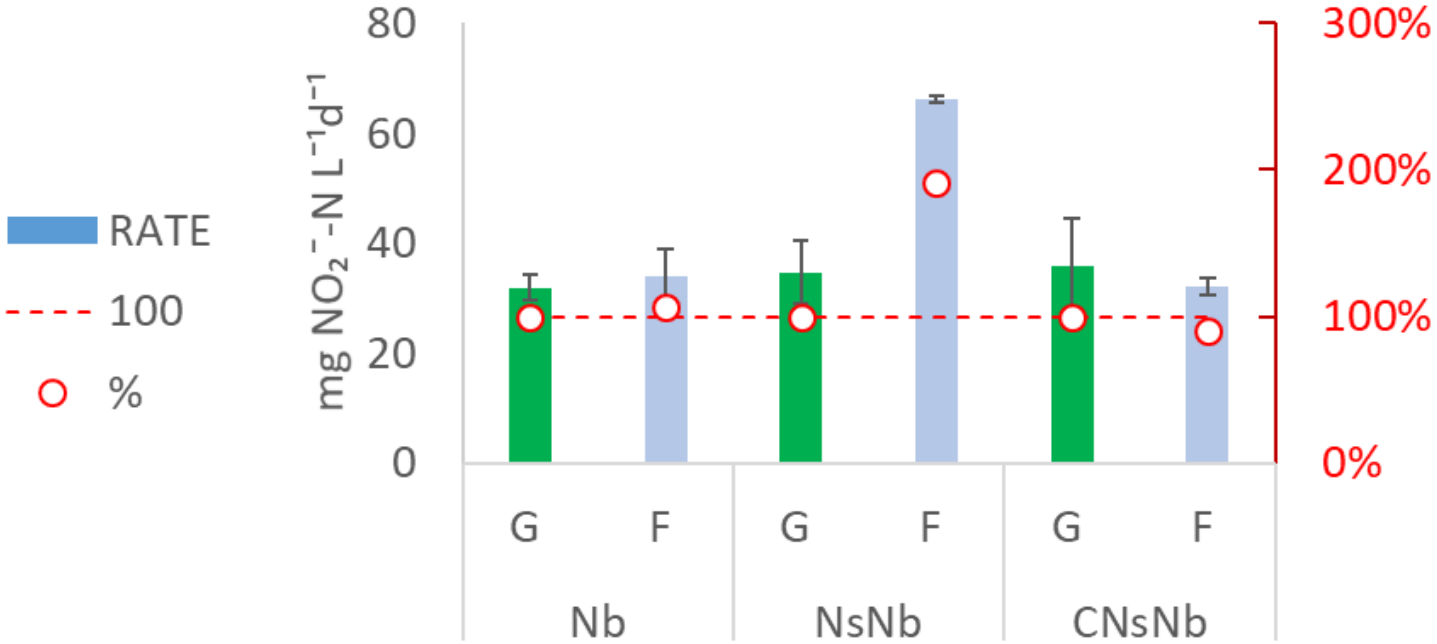



**Take home**

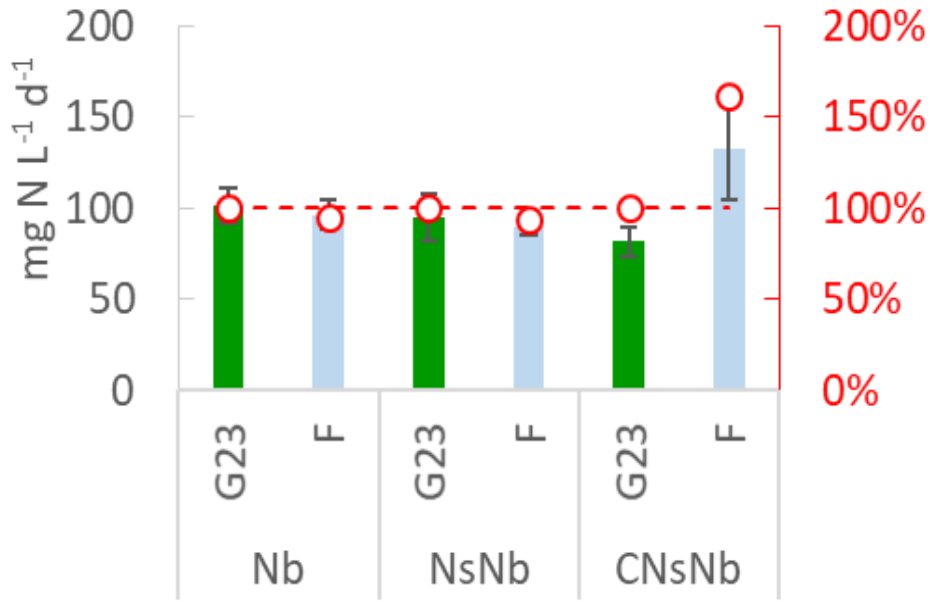
- No effect during short term mission
- Negative effect of long term storage of *Nitrosomonas europaea*
- Positive effect of co-culturing with *Cupriavidus* / *Nitrobacter*

# NITRITE OXIDATION

## SHORT MISSION




## LONG MISSION

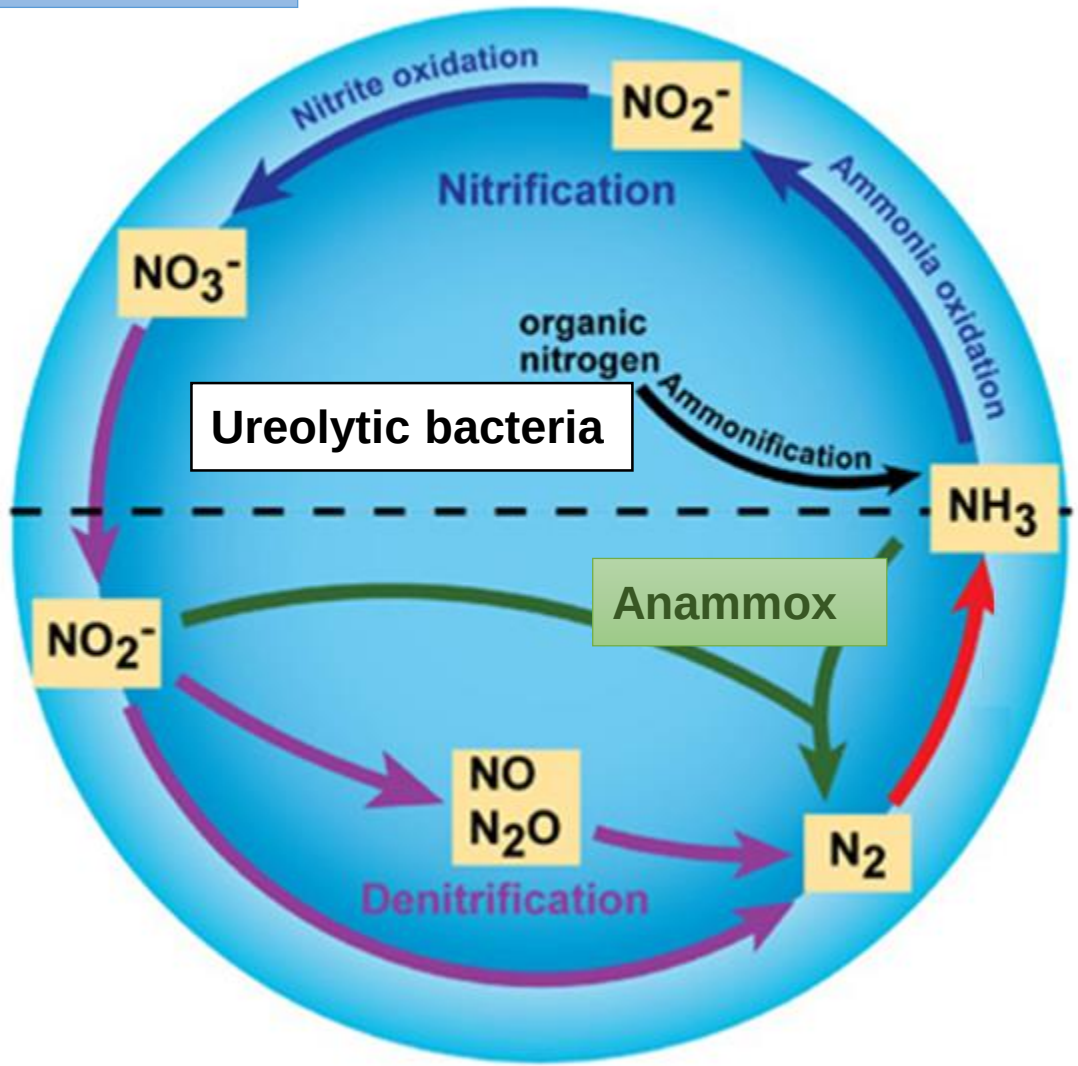



**Take home**

- No effect of flight on Nb -> No difference between long and short mission

# TERRESTRIAL MICROBIAL NITROGEN CYCLE

**Ammonia oxidizing archaea (AOA)**




**Denitrifying bacteria**

**SAFETY CONCERNS**

Possible presence of pathogen in reactor cultures. Not allowed in crewed space vehicle

Tested only on long missions (FOTON flight)

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# CULTURES SELECTION

| | REACTOR COMMUNITIES | | |
|---|---|---|---|
| | **Nitr-ur** | **Nitr-au** | **OLAND** |
| | Partially nitrified real urine ($NO_3^-$-N/$NH_4^+$-N ≈ 1) | Synthetic – Urea & nitrite | Synthetic – Ammonium |
| **Ammonia oxidation** | *Nitrosomonas* | *Thaumarchaeota* | *Nitrosomonas* |
| **Denitrification** | Present | Present | Present |
| **Anammox** | | | *Ca. Kuenenia* |

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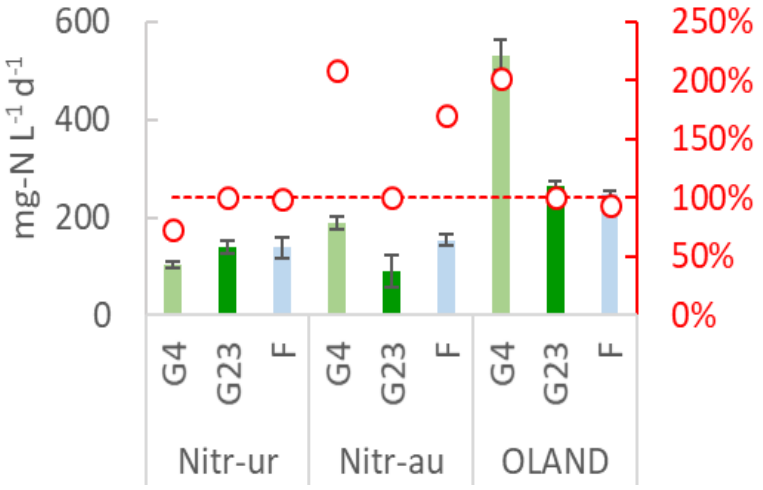
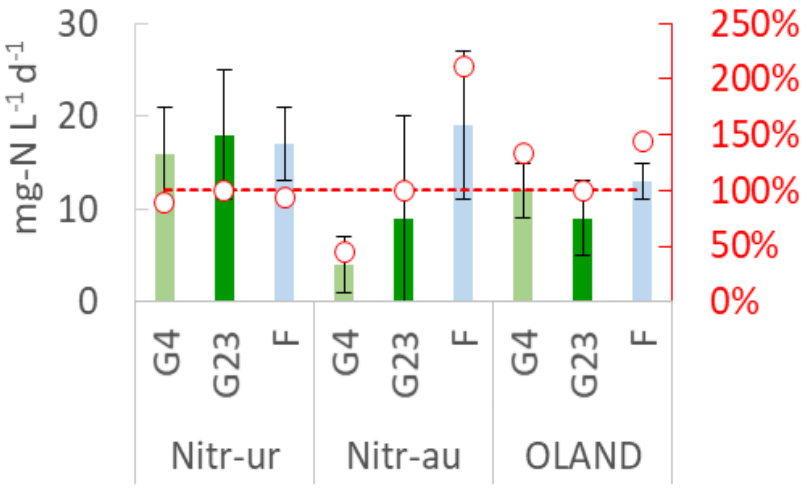
# REACTIVATION – DENITRIFICATION AND ANAMMOX

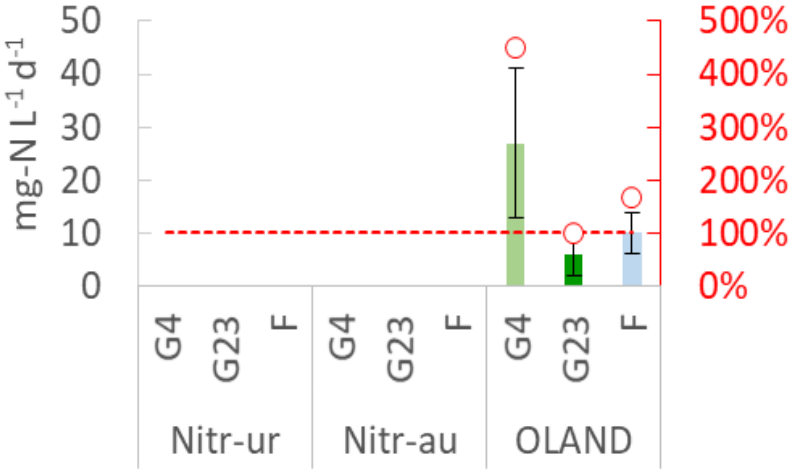
Ammonia oxidation




Denitrification




Anammox




**Take home**

- AOA/AOB, denitrification and anammox could be reactivated
- No effect of long term storage & flight

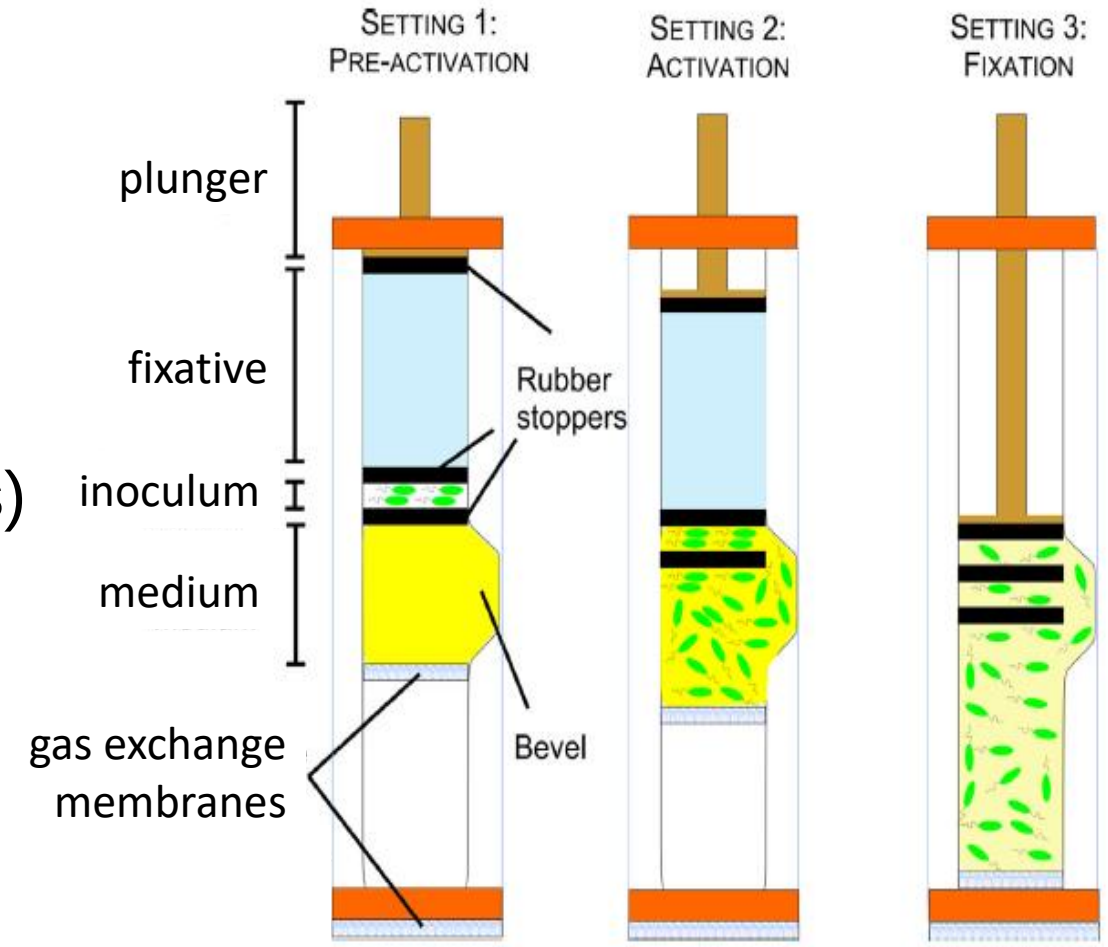
# Nitrification activity tests (ISS): URINIS A

~~Earth~~ → **Space**

**Synthetic urine** → ~~Real urine~~

~~Open community~~ → **Synthetic community**

- Gravity independent aeration
- Effect of microgravity on:
  - biofilm structure/formation
  - nitrification rate
  - metabolism (transcriptomics/proteomics)
- ISS (<2020?)

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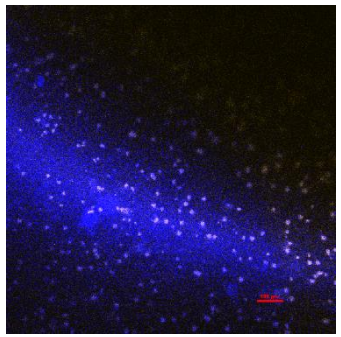
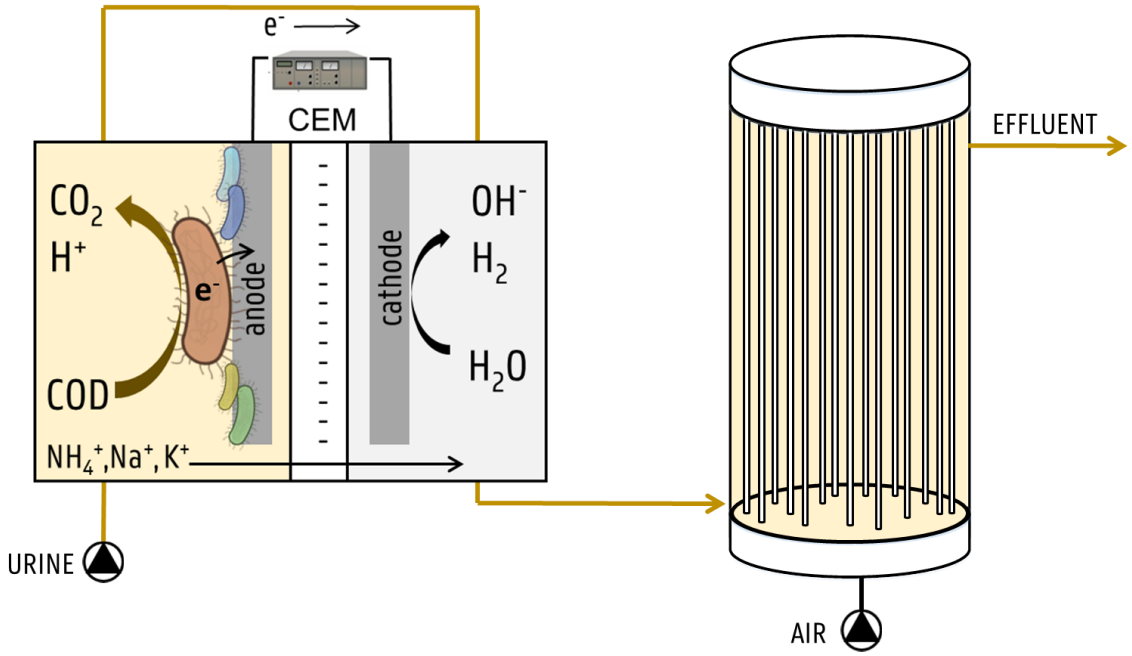


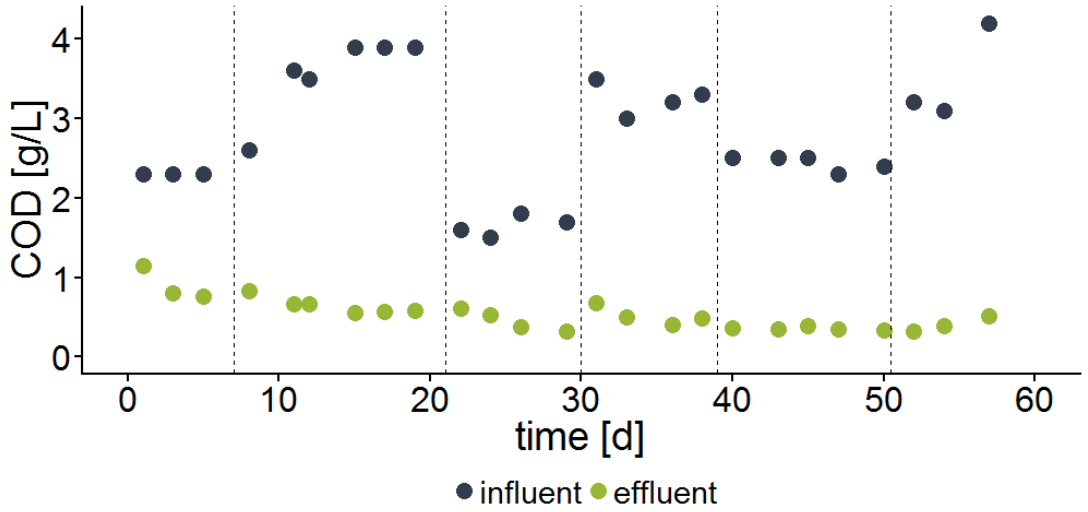
# Bioanodic oxidation as a pretreatment for membrane aerated nitrification

**Earth → (Space)**

**~~Synthetic urine~~ → Real urine**

**Open community → Synthetic community**




- Avoid denitrification in MABR
- In MFC-mode → power production of aeration nitrification?

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*De Paepe et al. (in preparation)*

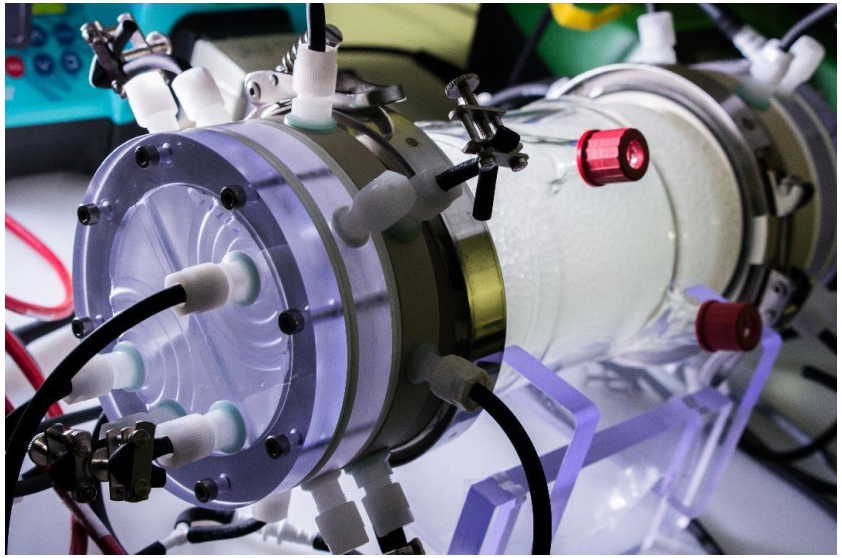
# Urine nitrification in a bioreactor (ISS?): URINIS B

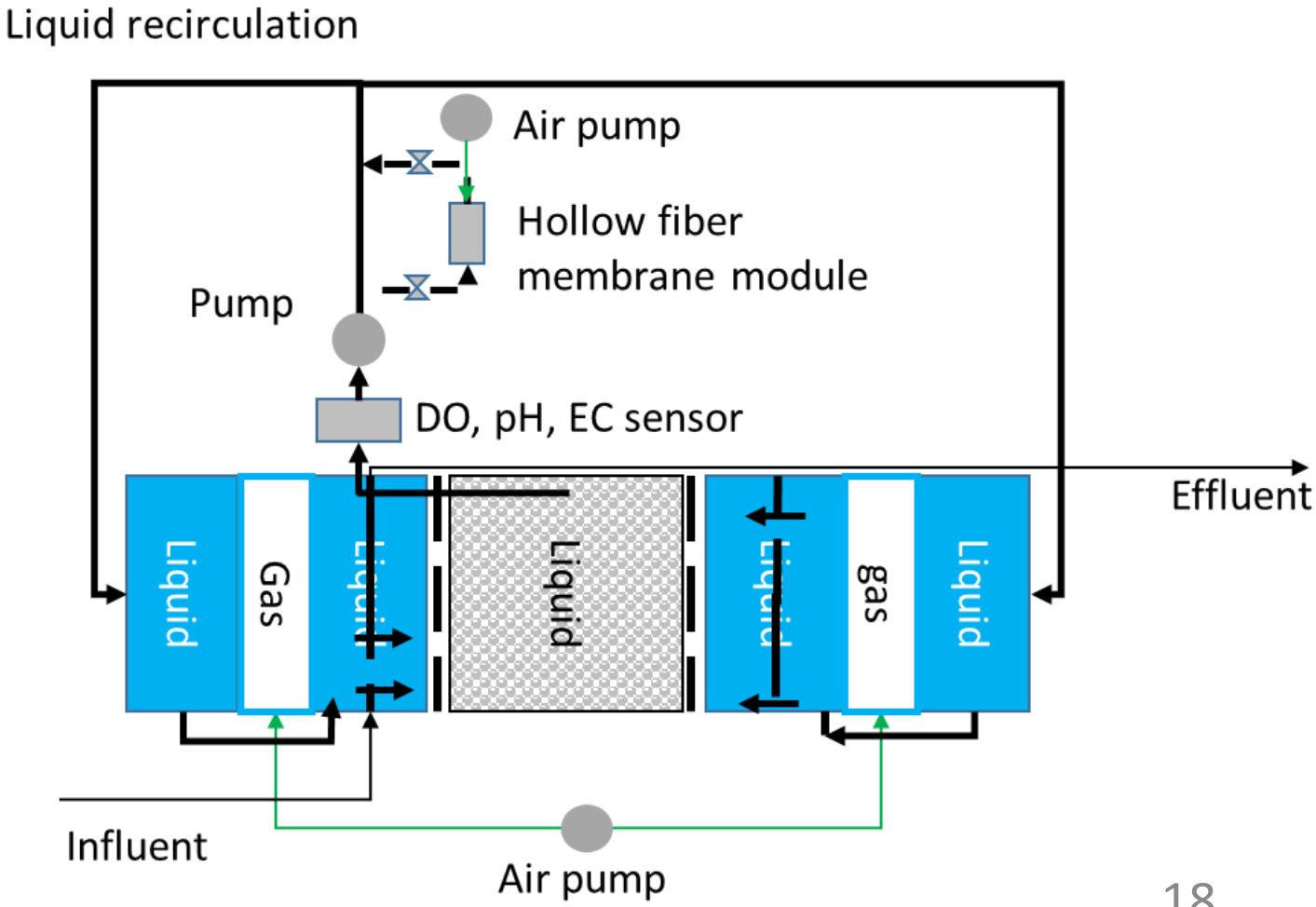
~~Earth~~ → **Space**

**Synthetic urine → Real urine**

~~Open community~~ → **Synthetic community**

Membrane aeration with flat sheet or hollow fiber membranes for gravity independent aeration





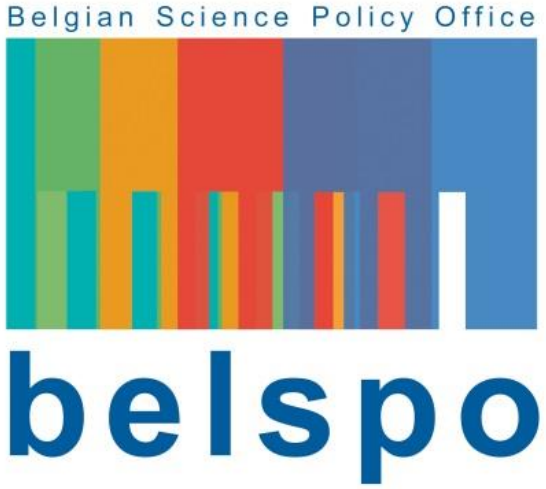
# CONCLUSIONS

- LEO exposed samples present similar (or higher) rates than terrestrial ones
- Long mission affected ammonia oxidation of Ns
- During long mission, the presence of Nb and C had a positive effect on ammonia oxidation

**Successful reactivation of five key nitrogen conversions, that can be safely applied for resource recovery in human Space exploration.**

# Acknowledgements





European Space Agency


# Contact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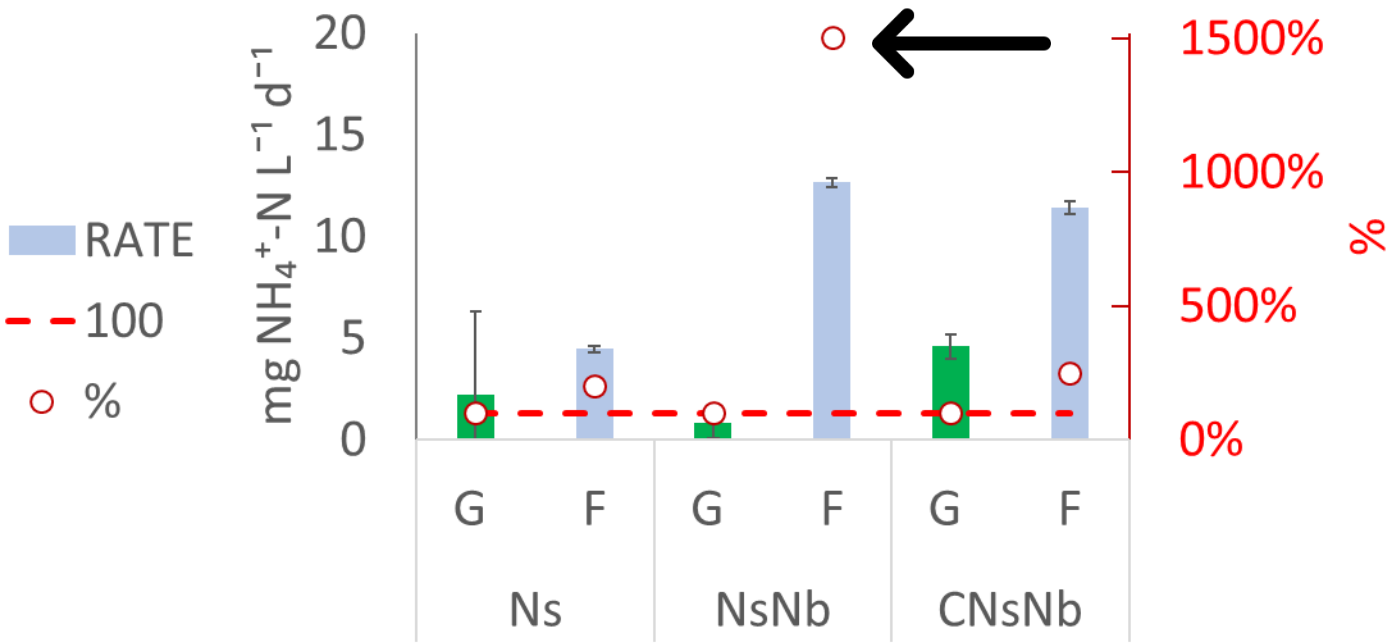
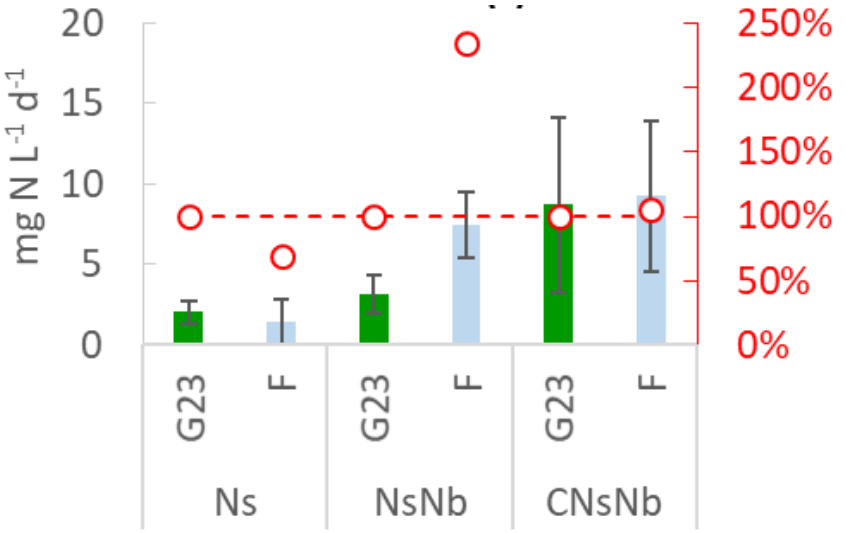
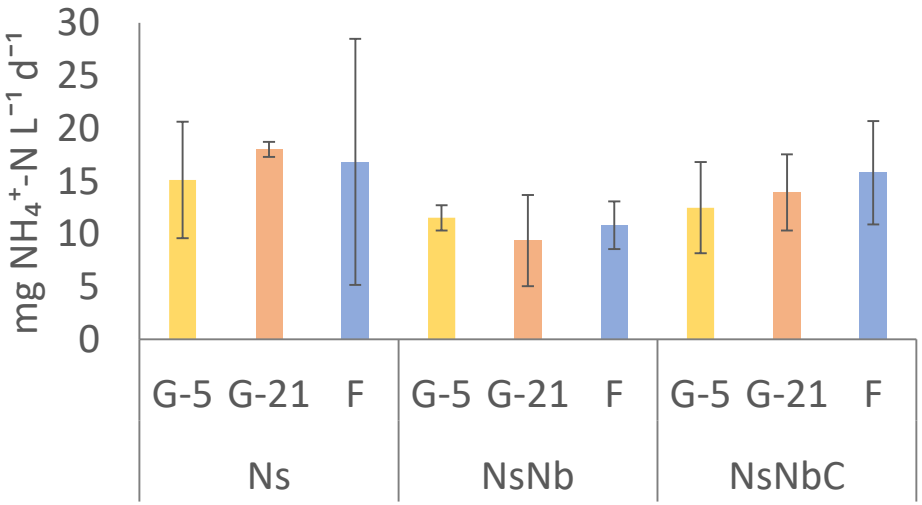
# AMMONIA OXIDATION

## SHORT MISSION




## LONG MISSION




**Take home**

- No effect of short term mission
- Negative effect of long mission on Ns, but positive effect of Cupriavidus and Nitrobacter