



CREATING  
A CIRCULAR  
**FUTURE**

# Lettuce cultivation in a urine recycling scenario: Effects of different $\text{NH}_4^+:\text{NO}_3^-$ ratios

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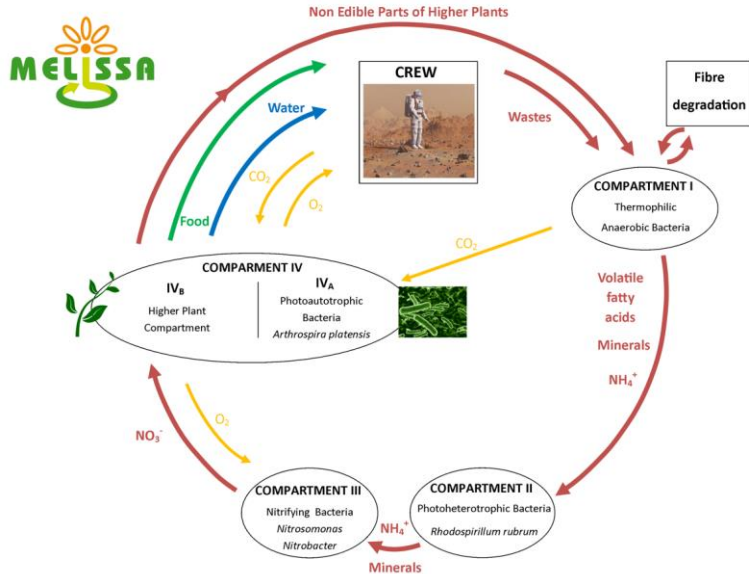
<sup>3</sup> EnginSoft S.p.A, Italy





# INTRODUCTION

# Urine-based fertilizer for cultivation of higher plants



Hypothesis:

the outcome of the upstream process for urine nitrification may result in a varying ammonium:nitrate ratio, which can affect plant growth and development

Test campaign:

Two tests with different  $\text{NH}_4:\text{N}$  ratio  
(N provided as a mix of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ )

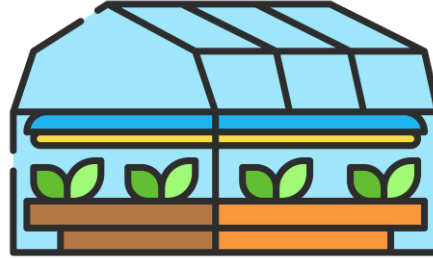
# Urine-based fertilizer for cultivation of higher plants



Urea conversion  
 $\text{NH}_4^+$  nitrification



$\text{NH}_4:\text{N}?$   $\updownarrow$





# Nutrient solution strategy

Reference crop: control and starting point

- Lettuce test performed by the University of Naples in standard nutrient solution

NaCl addition to mimic urine

- Based on natural NaCl:N ratio in urine (5 mM Na in final nutrient solution, not accounting for possible accumulation)

Nutrient solution strength reduced to allow EC comparable to Reference

	Reference	LoNH4:N	HiNH4:N
NH4:N	0.22	0.11	0.50
Na:N	0.01	0.42	0.42
Cl:N	0.02	0.42	0.42
EC /pH	1.9 / 5.9	1.9 / 5.9	2.2 / 5.9

# Nitrate control

- Feeding of nutrient stock solutions based on nitrate level
  - Optical nitrate sensor in the hydroponic loop
- Same total N concentration in both tests 146 mg/l N, 10.4 mM
- Test 1 (LoNH<sub>4</sub>:N): NH<sub>4</sub>:N = 0.11, 130 mg/l NO<sub>3</sub>-N (9.3 mM)
- Test 2 (HiNH<sub>4</sub>:N): NH<sub>4</sub>:N = 0.50, 73 mg/l NO<sub>3</sub>-N (5.2 mM)

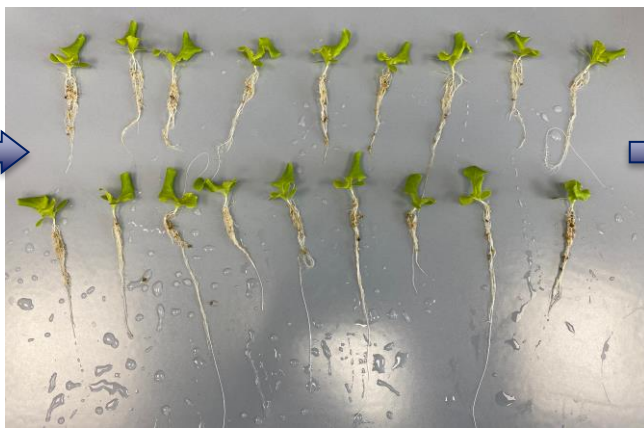




# PCU – Plant Characterization Unit

- Developed in the MELISSA PaCMan1 project (2018-2020)
- Located at the Federico II University of Naples
- Closed atmospheric and hydroponic loop
- 18 plant positions
- Advanced gas and liquid monitoring
- Color and thermal camera systems





## Seedling selection



Lettuce *Lactuca sativa* L.  
cultivar 'Grand Rapids'







# RESULTS



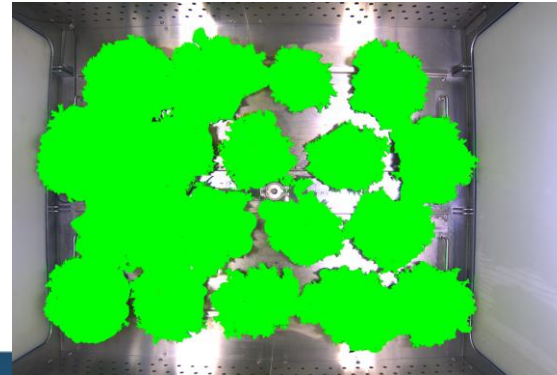
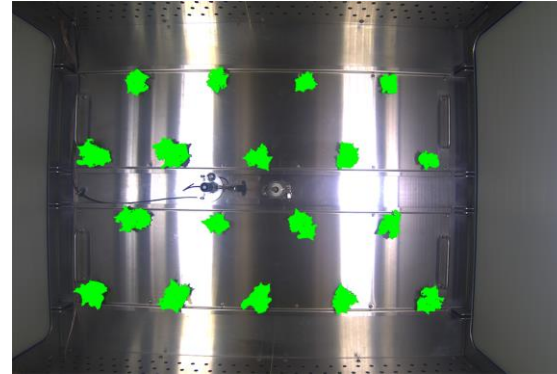
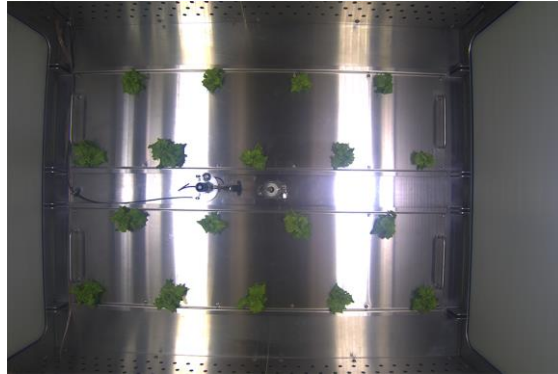
Harvest after  
28 days in PCU  
38-day old plants

Parameter	LoNH <sub>4</sub> :N (0.11)	HiNH <sub>4</sub> :N (0.50)	Significance
Shoot FW (g)	290	120	P < 0.01
Plant DW (g)	21	13	P < 0.01
No. of leaves	40	26	P < 0.05
Leaf area (cm <sup>2</sup> )	3907	1961	P < 0.01



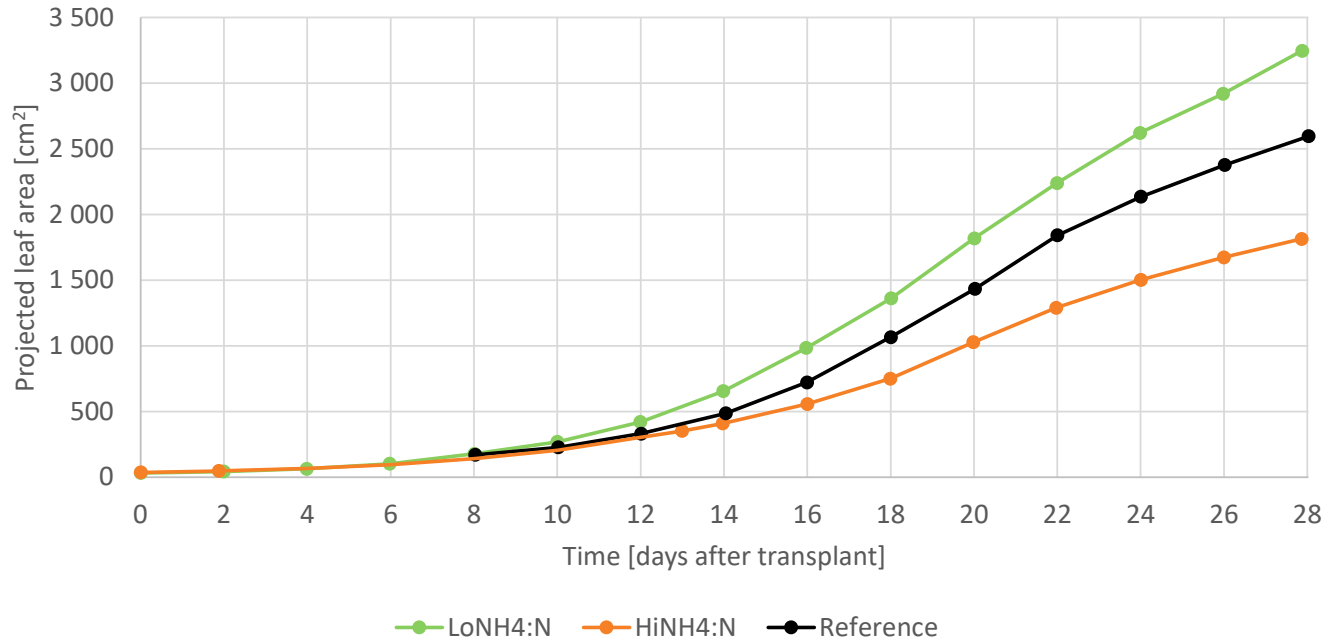
# Projected leaf area

Image segmentation of top view images

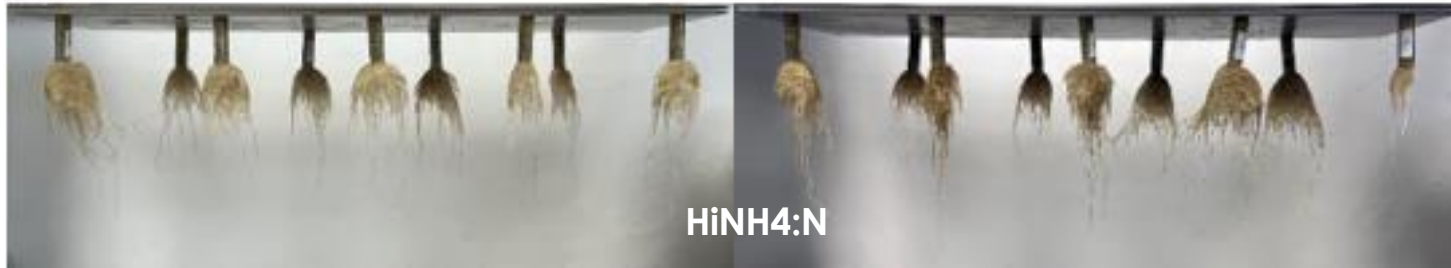


# Shoot growth

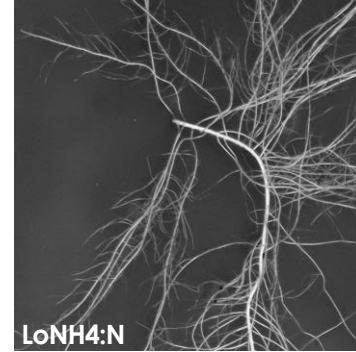
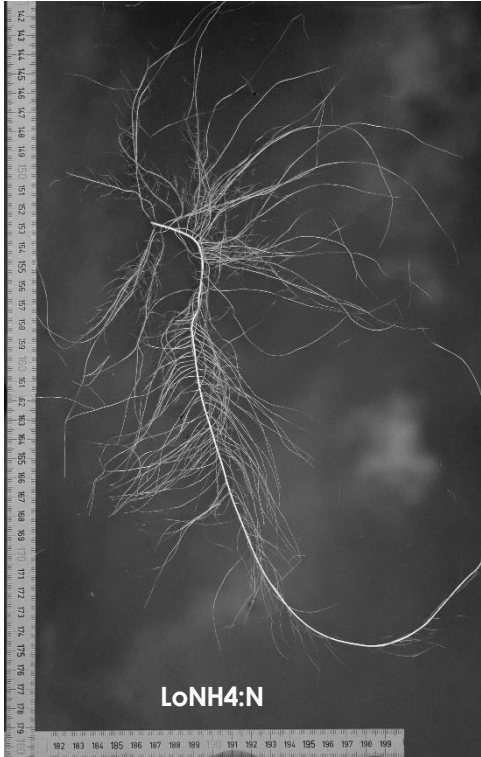
as projected leaf area



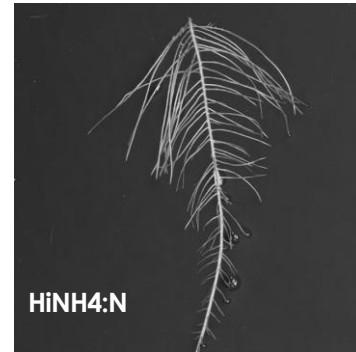
Parameter	LoNH <sub>4</sub> :N (0.11)	HiNH <sub>4</sub> :N (0.50)	Significance
Root DW (g/plant)	2.4	1.9	--
Root:Shoot ratio	0.14	0.19	P < 0.01
Harvest index	0.88	0.85	P < 0.01



# Root morphology



10 x 10 cm



# Leaf chlorophyll and color

- SPAD: LoNH<sub>4</sub>:N significantly lower than HiNH<sub>4</sub>:N
- Color index: LoNH<sub>4</sub>:N had color index significantly more towards yellow than HiNH<sub>4</sub>:N.



LoNH<sub>4</sub>:N

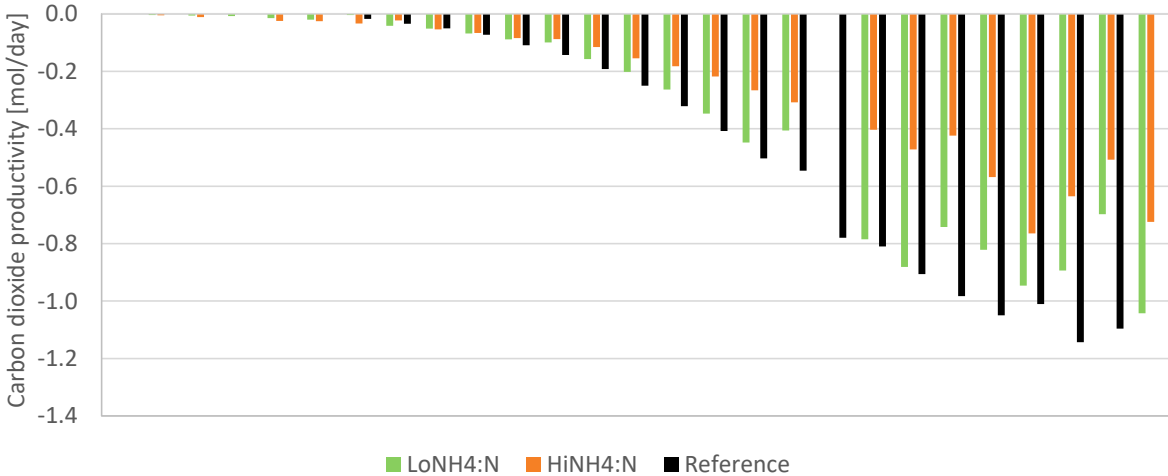
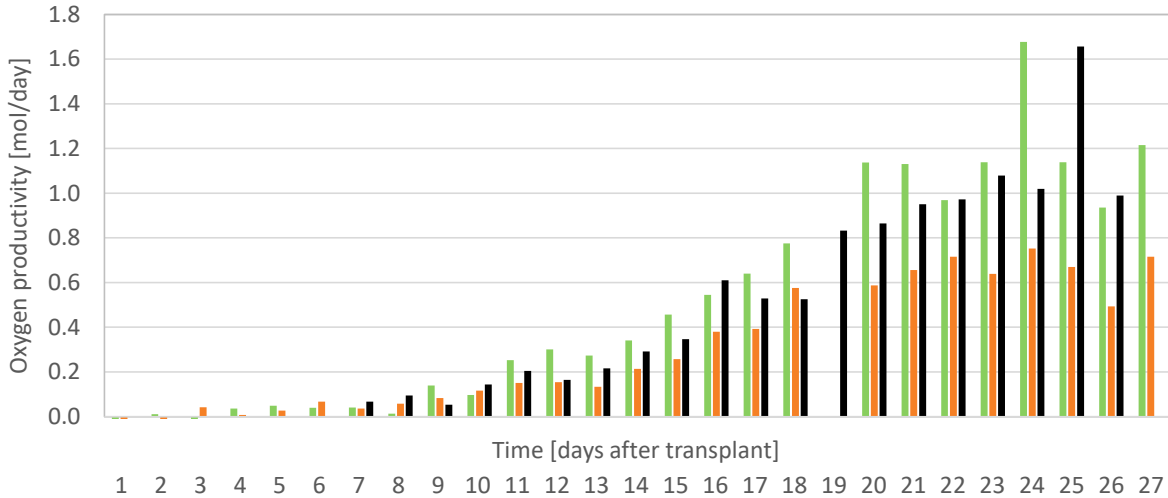


HiNH<sub>4</sub>:N





# O<sub>2</sub> and CO<sub>2</sub> productivity

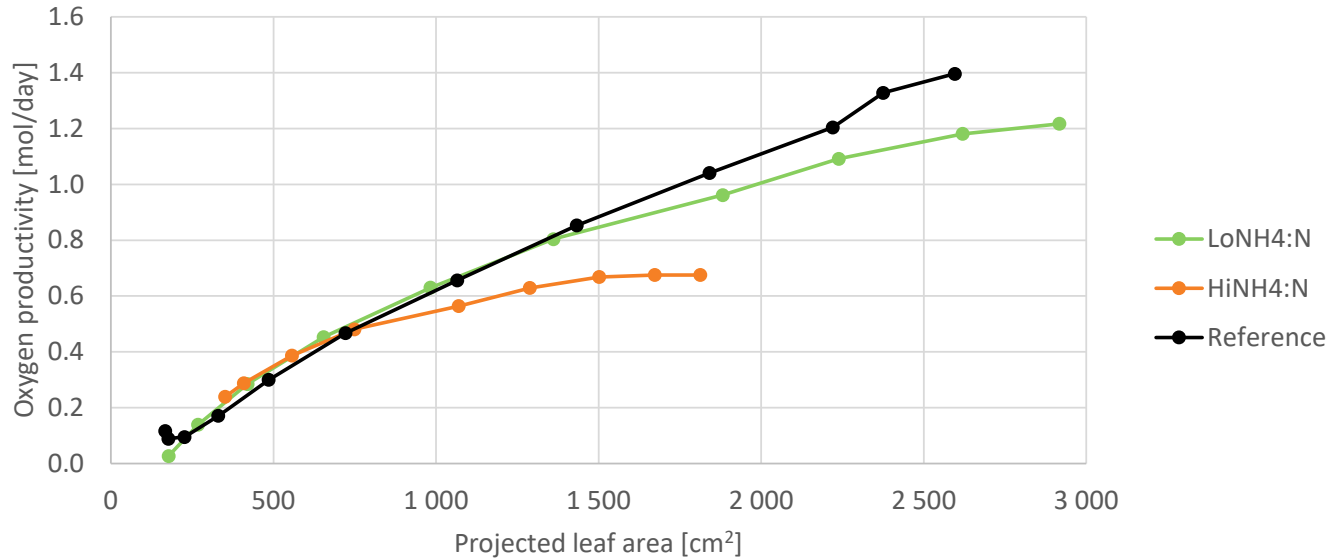


Estimated O <sub>2</sub> production (moles), day 7 – 28		
Ref	Lo	Hi
12.9	14.2	8.4



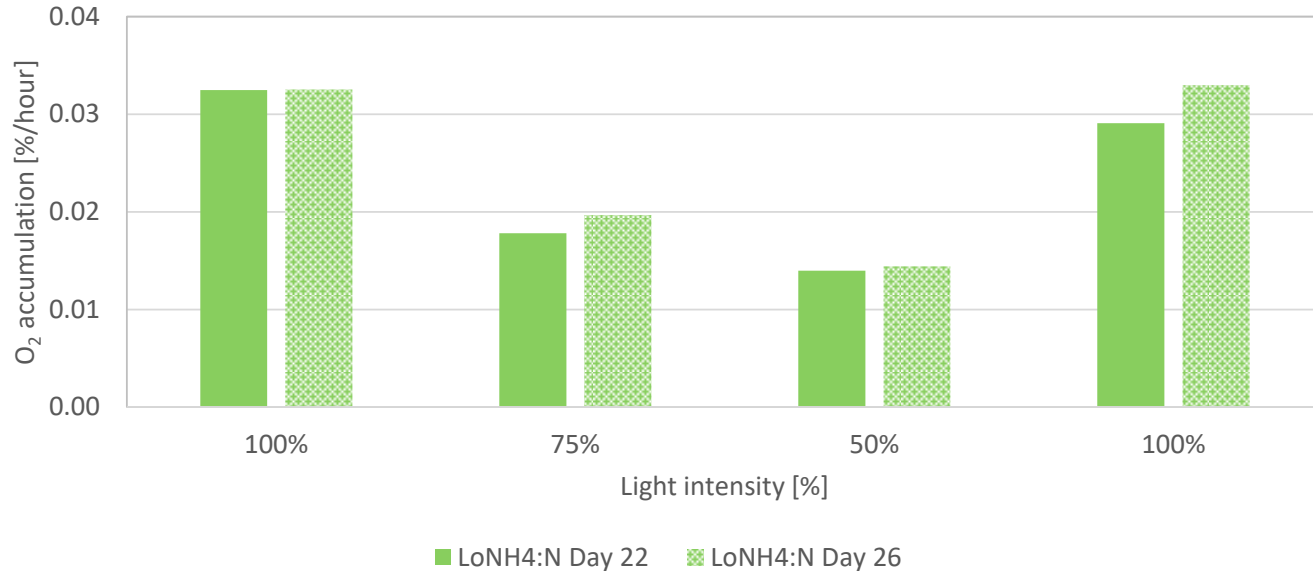
# Specific oxygen productivity

relative to projected leaf area



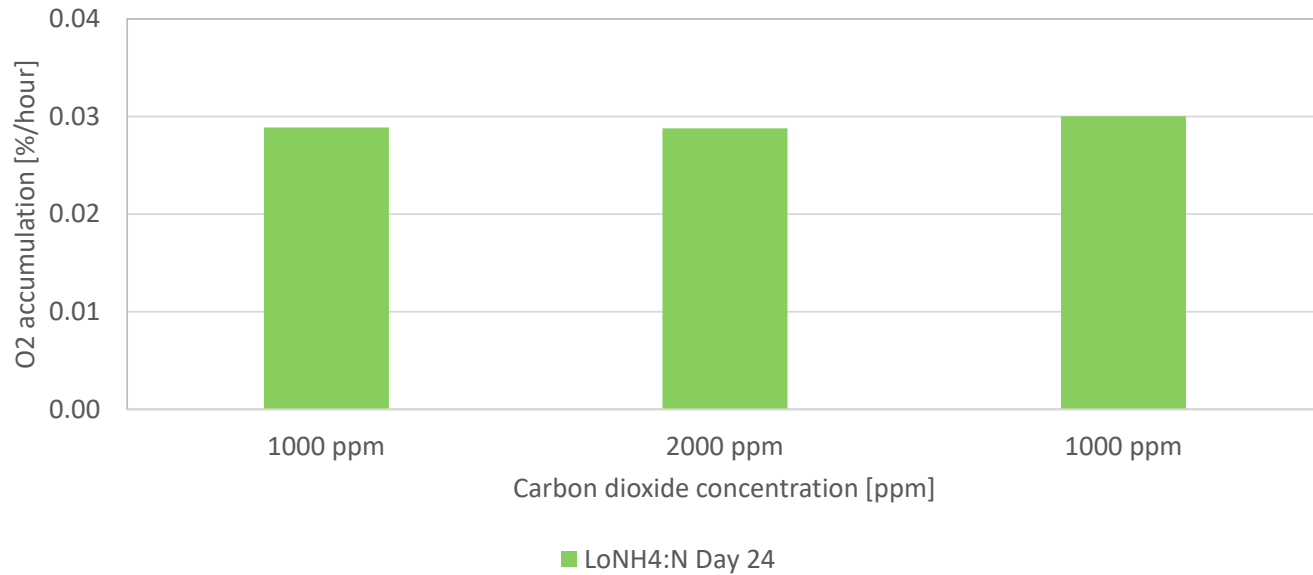
# Light test

## 2-hour stabilization



# CO<sub>2</sub> test

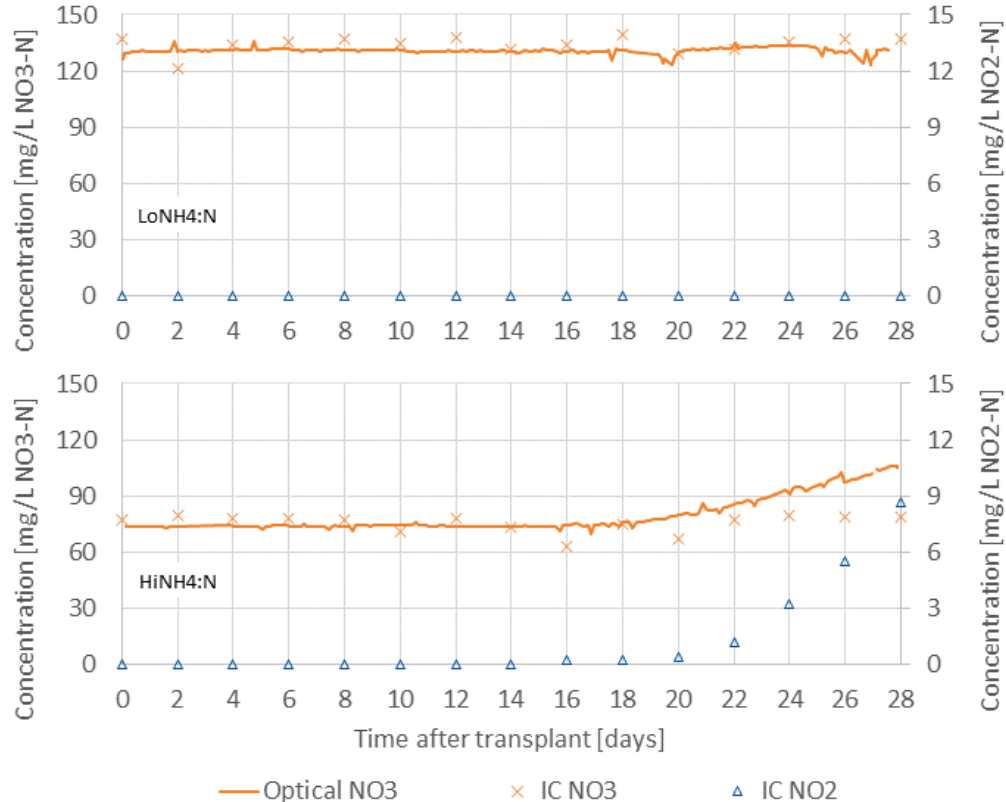
## 2-hour interval



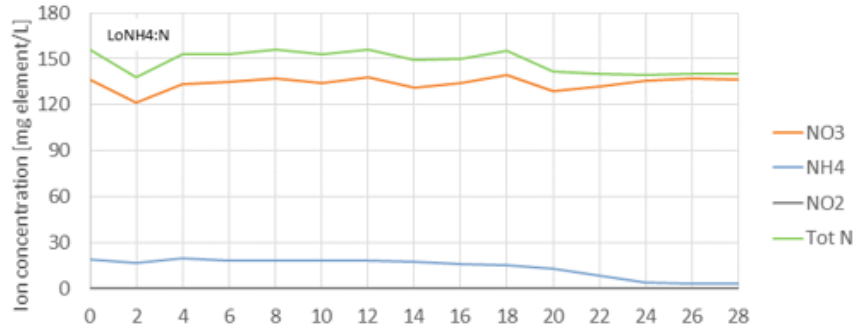


# Nutrient solution development

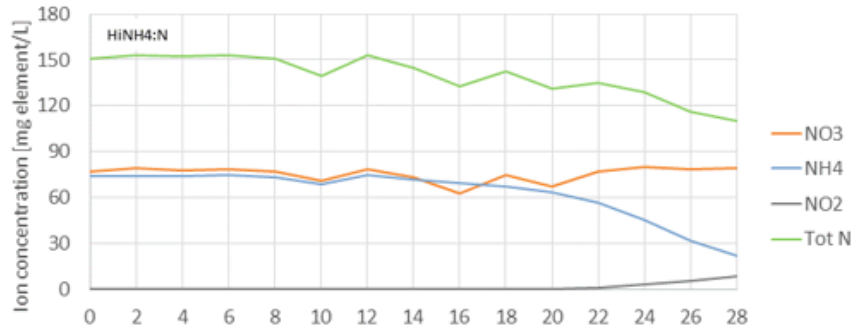
$\text{NO}_3^-$  control - Sensor measurements vs offline Ion chromatography



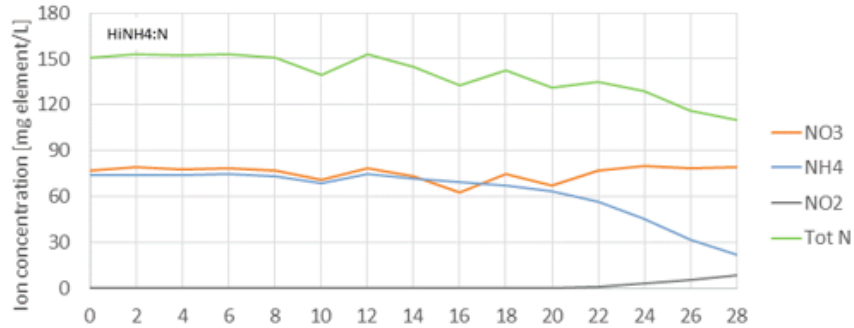
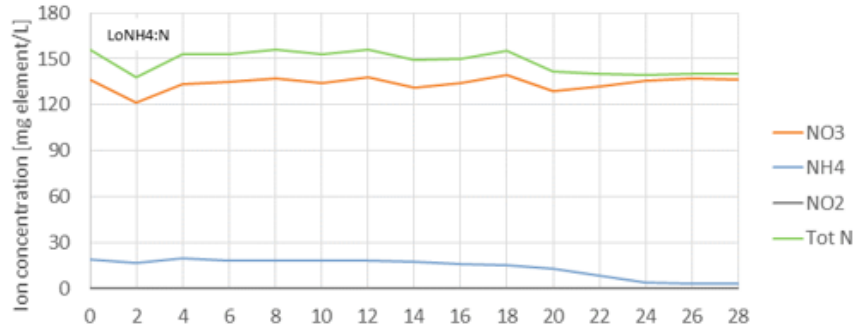
# Nutrient solution development



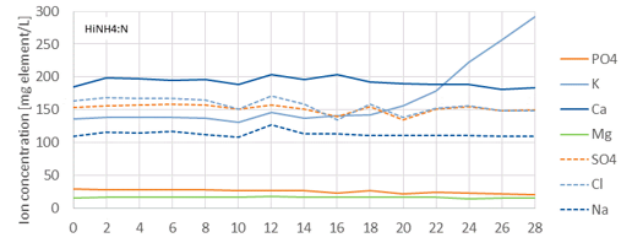
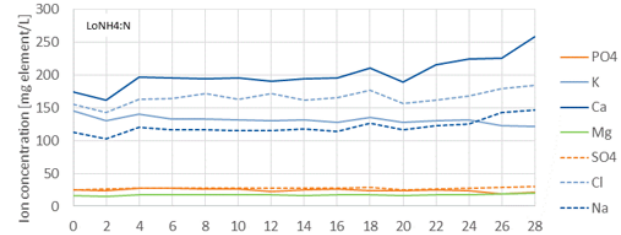
$\text{NO}_3^-$  constant due to control,  $\text{NH}_4^+$  “free”  
 $\text{NH}_4:\text{NO}_3$  ratio as function of time: HiNH4:N moves towards LoNH4:N with respect to  $\text{NH}_4:\text{N}$  ratio, as  $\text{NH}_4^+$  is depleted over time



# Nutrient solution development



NO<sub>3</sub><sup>-</sup> constant due to control, NH<sub>4</sub><sup>+</sup> “free”  
 NH<sub>4</sub>:NO<sub>3</sub> ratio as function of time: HiNH<sub>4</sub>:N moves towards LoNH<sub>4</sub>:N with respect to NH<sub>4</sub>:N ratio, as NH<sub>4</sub><sup>+</sup> is depleted over time



# Element content in plants (g/kg DW)

	Shoot				Root		
	Reference	LoNH4:N	HiNH4:N		Reference	LoNH4:N	HiNH4:N
NO <sub>3</sub> <sup>-</sup>	17	27	4	47	79	12	
NH <sub>4</sub> <sup>+</sup>	0.5	1.3	1.4	0.5	1.6	4.5	
Na	1.6	4.6	3.1	1.4	4.7	3.7	
Cl	17	20	17	3.9	6.7	1.9	
Ca	16	4	2	4.1	1.0	0.9	
K	58	70	34	97	97	62	
Mg	3	2	1	1.7	1.1	1.2	
PO <sub>4</sub>	14	15	8	45	43	47	

 Highest

 Middle

 Lowest

Different color  
= statistically different



# CONCLUDING REMARKS



# Concluding remarks

- The upstream process makes a difference
  - Lower growth rate with high  $\text{NH}_4^+$
  - Differences in root size and morphology between high and low  $\text{NH}_4^+$
  - Lower specific  $\text{O}_2$  production with high  $\text{NH}_4^+$
  - $\text{NH}_4:\text{NO}_3$  and  $\text{NaCl}$  influences element composition of the plant
- Plant uptake affects the  $\text{NH}_4:\text{N}$  ratio
  - The  $\text{NH}_4:\text{N}$  ratio decreases over time due to higher uptake rate of  $\text{NH}_4^+$
- Photosynthetic rate at increased  $\text{CO}_2$ 
  - Plants not capable of utilizing the additional  $\text{CO}_2$  in the 2-hours test (biomass not adapted)
- Photosynthetic rate at decreased light
  - Immediate photosynthetic response to reduced light intensity
- The PCU allows for measurements of  $\text{O}_2$  and  $\text{CO}_2$  produced/used by the canopy
  - Offers evaluation of  $\text{O}_2$  production as a function of projected leaf area - with interesting potentials
- The automatic nitrate control worked well
  - Opens new possibilities together with multiple nutrient stock solutions
- Accumulation of nitrite
  - Nitrifying bacteria in the system (buildup over time or increase due to high  $\text{NH}_4^+$ )
  - To consider in recirculating systems

# MELISSA



MICRO-ECOLOGICAL  
LIFE SUPPORT SYSTEM  
ALTERNATIVE



## THANK YOU.

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