



# A microbe-based value chain: TREatment and valorisation of texTILE wastewater



## Caratterizzazione molecolare delle comunità responsabili dei processi biodegradativi

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Evento Finale \_Webinar 3 giugno 2021



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# Aims and objectives

## Aims

- Implementation of alternative bioremediation technologies based on Partial Nitritation (PN)-anaerobic ammonium oxidation (ANAMMOX)
- N removal with less sludge production and reduced operational costs

## General objectives

- Characterization of the key role bacterial and algal species in the treatment samples
- Quantification of the microbial communities involved in the biotransformation process by qPCR
- Monitor the microbial consortia related to PN-ANAMMOX processes
- Correlate the bacterial consortia dynamics with reactor performance

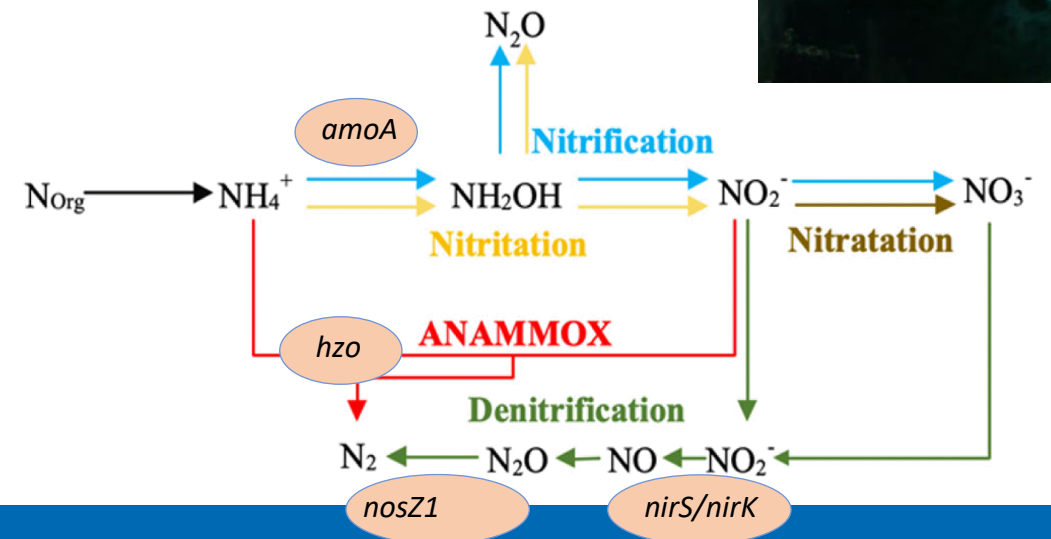
# SBR pilot plant

## Set up

- Same as previously explained by Roberto

## Molecular analyses:

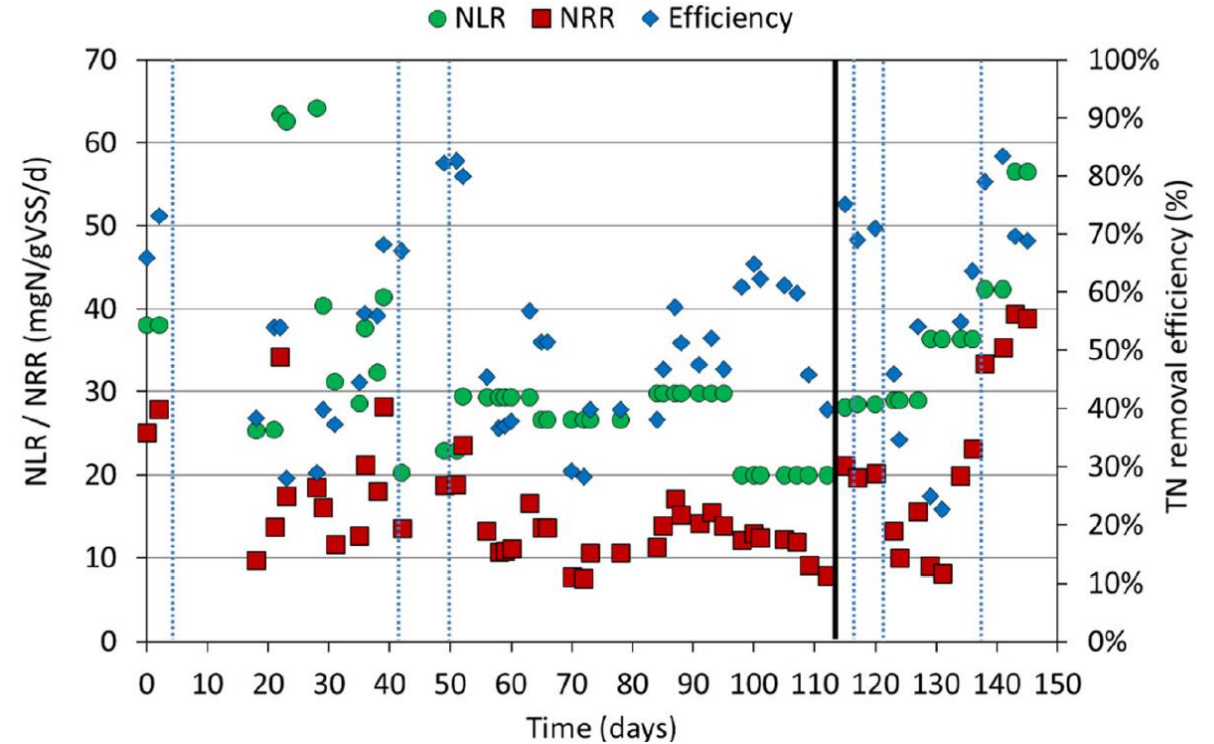
- Quantitative PCR on N cycle genes
  - Denitrification (*nirS*, *nirK*, *nosZ1*)
  - Bacterial nitrification (*amoA*)
  - Anammox (*hzo*)



# N removal

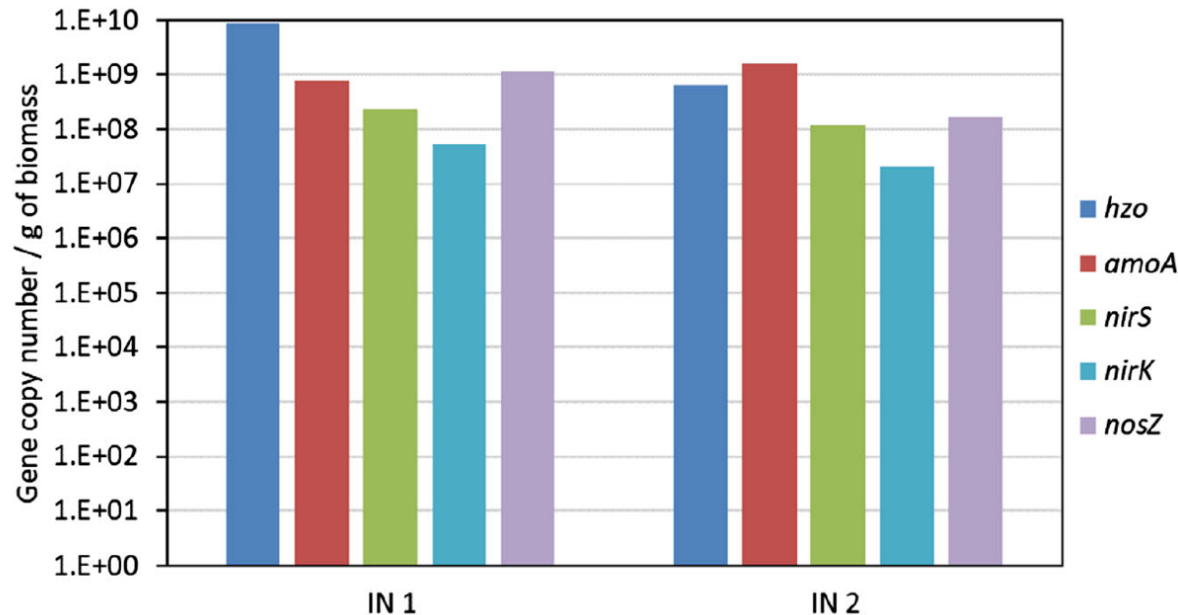
- Acclimation periods are important to reach steady-state conditions
- Bacteria adaptation to undiluted influent allows increased N removal efficiency (60–70%)
- Abrupt changes in the influent lead to a reversible shock for bacteria reducing their metabolic activity
- Quick stabilization leads to N removal efficiency between 70 and 80%
- Compliance with discharge in sewer system (100 mg N/L) was successfully demonstrated.
- N removal efficiency achieved maximum values of 40 mgN/gVSS/day

→ the PN/anammox process represents an important solution

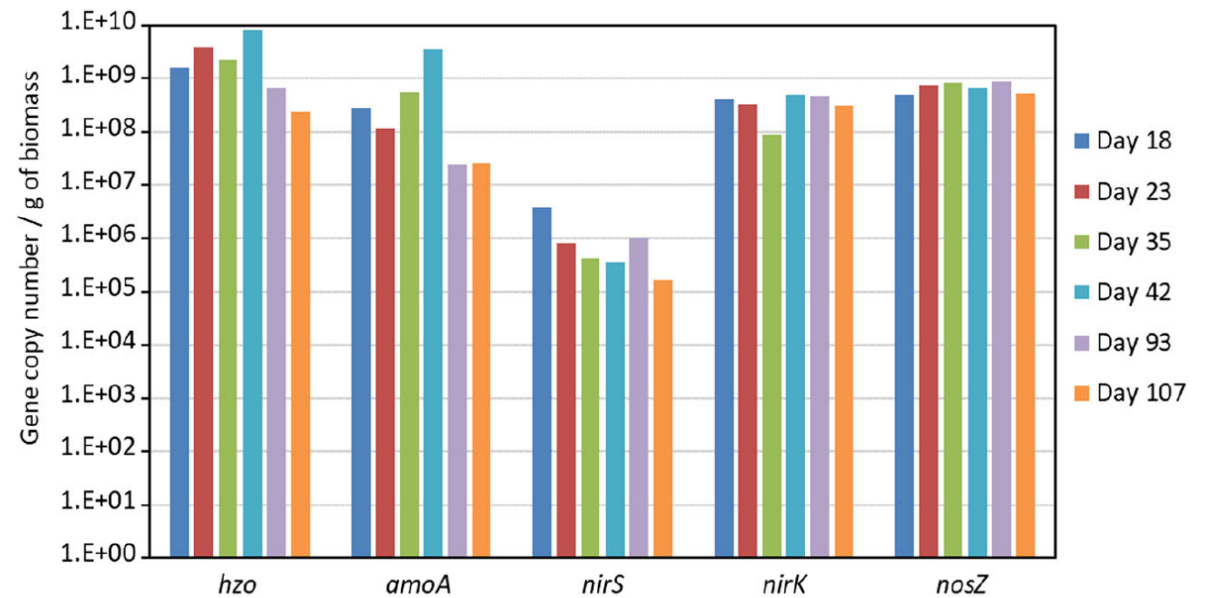


# Dynamic changes in microbial functional genes

- Anammox bacteria GCCs in the first batch (IN 1) was one order of magnitude higher than in the second (IN 2)
- Higher abundance of AOB was detected in IN 2
- A conspicuous number of denitrifiers were detected in both biomasses.



- Anammox and AOB bacteria increased when fed with a mixture of WW 1 (30%) and synthetic wastewater (70%). → pivotal role in the process → need for an acclimation phase
- Drastic decrease in anammox and AOB at the end of the period for undiluted wastewater due to toxic compounds affecting reactor performance.



# Conclusions

- A process stabilization strategy was reported, highlighting the importance of the initial acclimation period.
- An increase of anammox and AOB bacteria was observed in line with the nitrogen removal rate of the reactors
- Significant reversible reductions in bacterial activity were observed due to wastewater toxicity.
- A conspicuous number of denitrifiers were detected in both biomasses



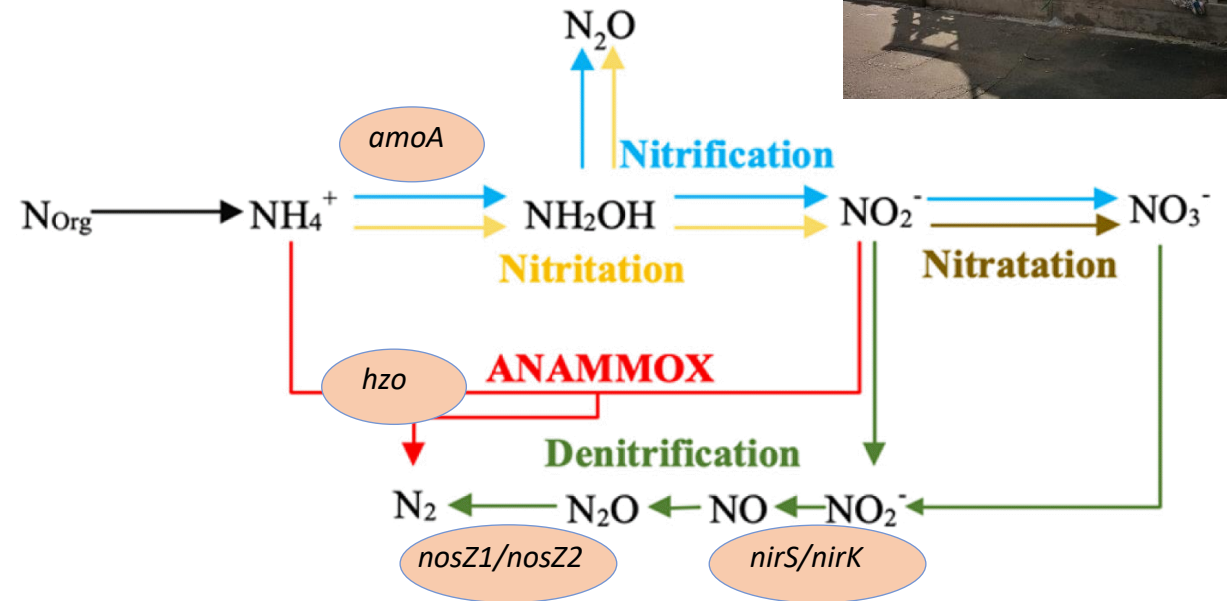
# SBR demonstrative plant

## Set up

- Same as previously explained by Roberto Canziani

## Molecular analyses:

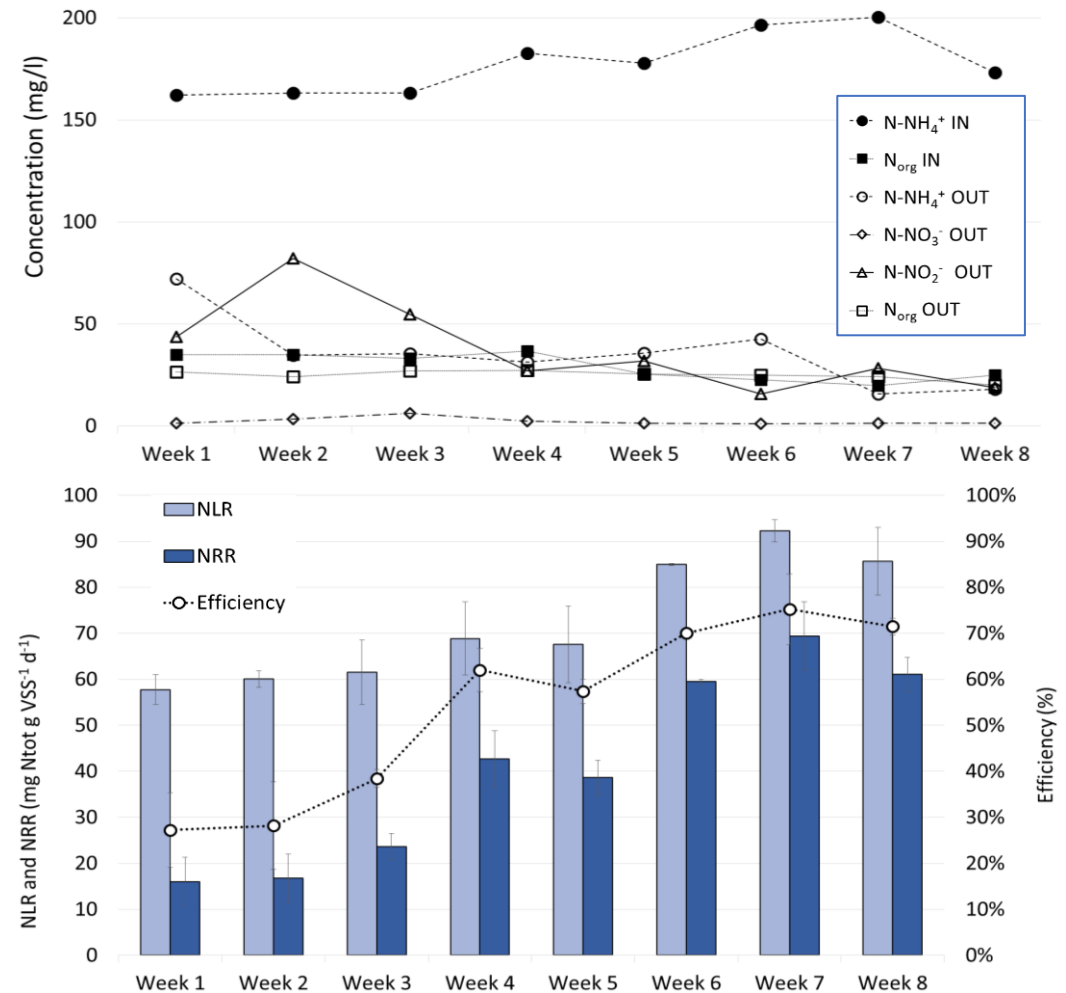
- 16S rRNA NGS
- Quantitative PCR on N cycle genes
  - Total bacterial and anammox (*16S rRNA*)
  - Denitrification (*nirS*, *nirK*, *nosZ1* and *nosZ2*)
  - Bacterial and archaeal nitrification (*amoA*)
  - Anammox (*hzo*)





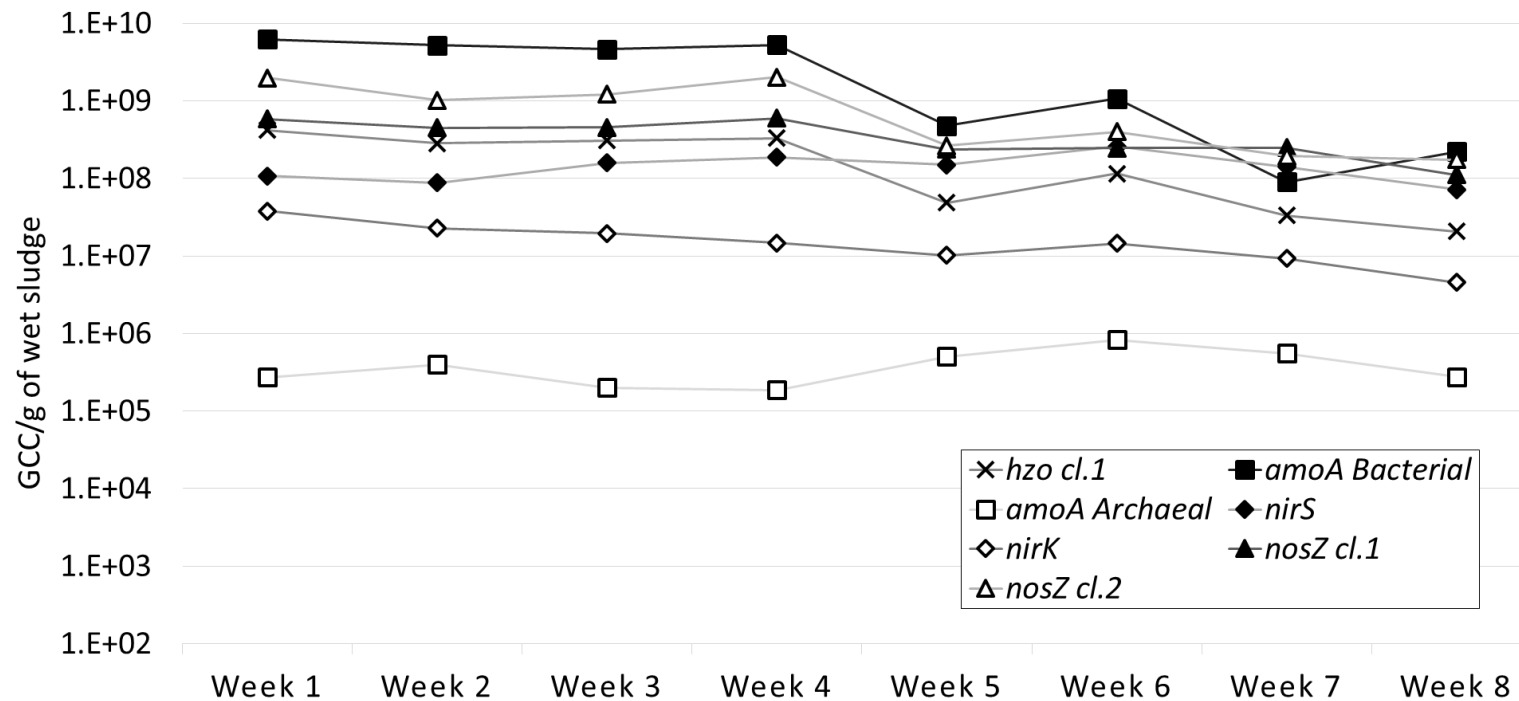
# N removal

- **N** residue of  $\text{N-NH}_4^+$  and  $\text{N-NO}_2^-$ , could be always detected in the effluent → the **PN/ANAMMOX** process was inhibited
- A constant level of **organic N** was detected probably derived from the recalcitrant **azo dyes**
- The  $\text{N-NO}_3^-$  level was **negligible**
- The **N removal rate** increased over time reaching a removal efficiency up to **70%** during the last three weeks



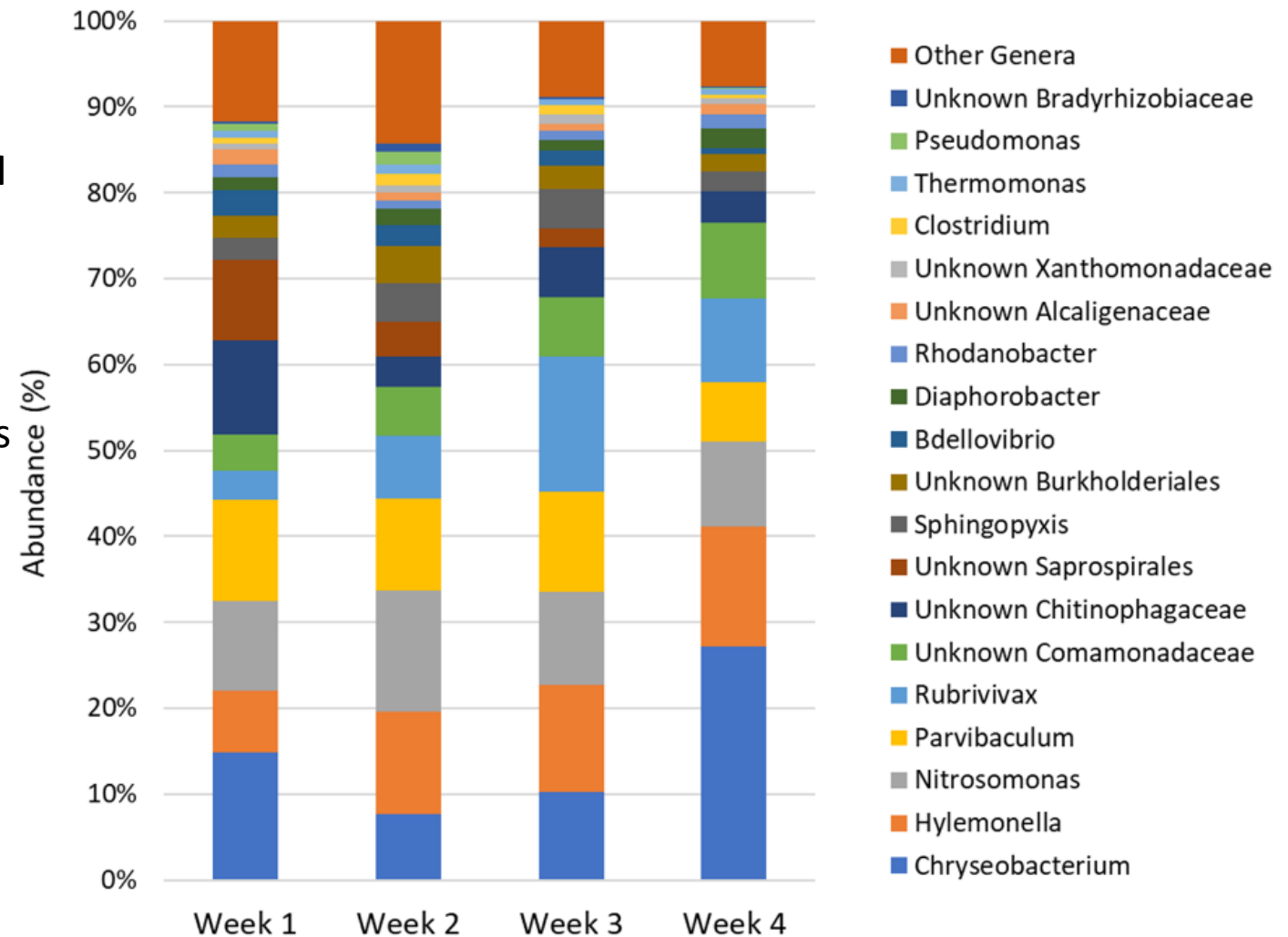
# Dynamic changes in microbial functional genes

- **High concentration** of gene copies associated to the function of **AOB, Anammox and Denitrifiers**
- **Decreasing trend** over time of gene copies associated to **Anammox** and **AOB**
- Possible **suboptimal performance** of the system across time



# Dynamic changes in microbial diversity and compositions

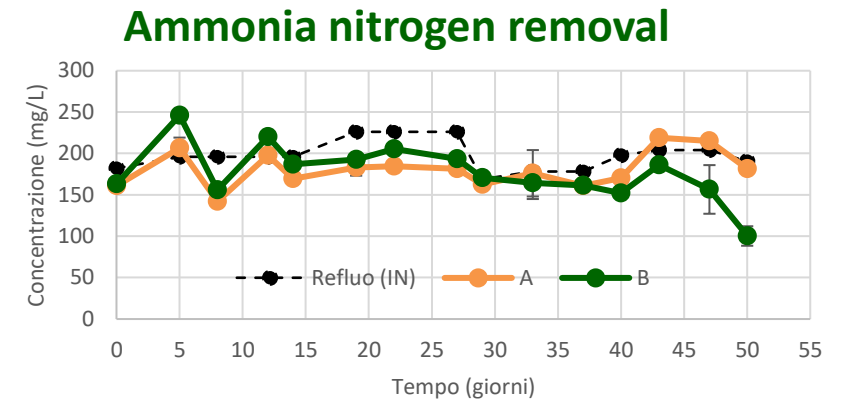
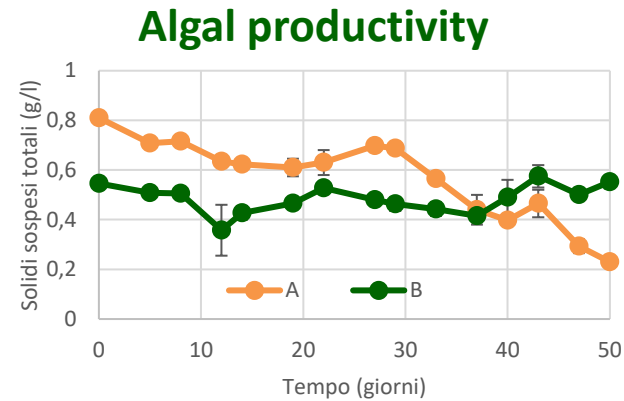
- Confirmation that the granules community is **dominated by nitrifying (*Nitrosomonas*: 10-14%) and denitrifying (*Hylemonella*: 7-14%) genera**
- **Anammox** bacteria (*Planctomyces*) accounted only for **0.0-0.1%** of the total population. More understanding on the reason why chemical evidences are contrasting with microbial species composition are in progress
- *Chryseobacterium* and *Rubrivivax* seemed to increase their abundance across the weeks



# Conclusions

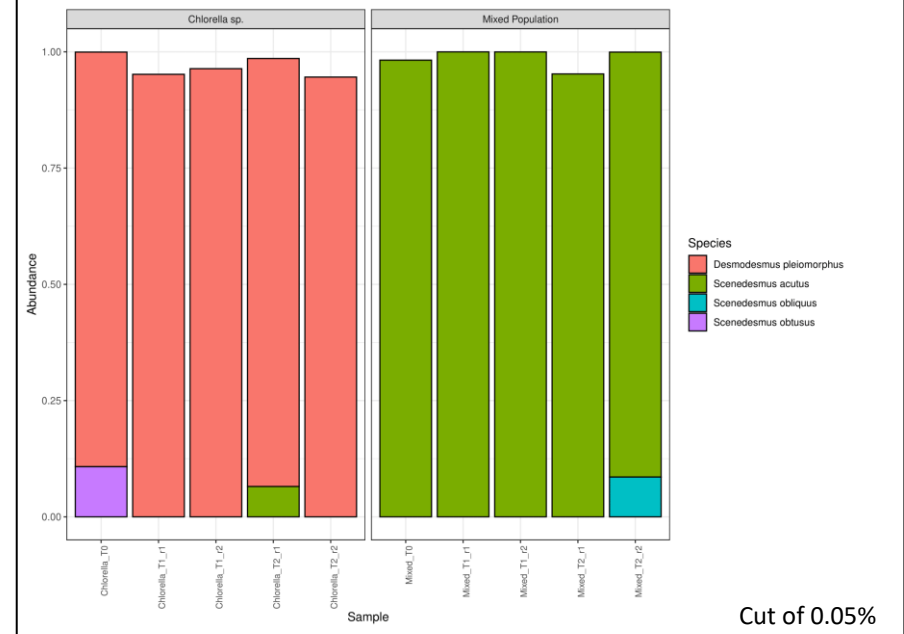
- A high N removal efficiency by anammox
- Sporadic denitrification and nitrite oxidation
- A community shifted towards nitrification with a small portion of anammox bacteria
- A suboptimal performance of the plant reflected in the community characterization.

# Implementation of N-removal with microalgae: Lab-scale PBR

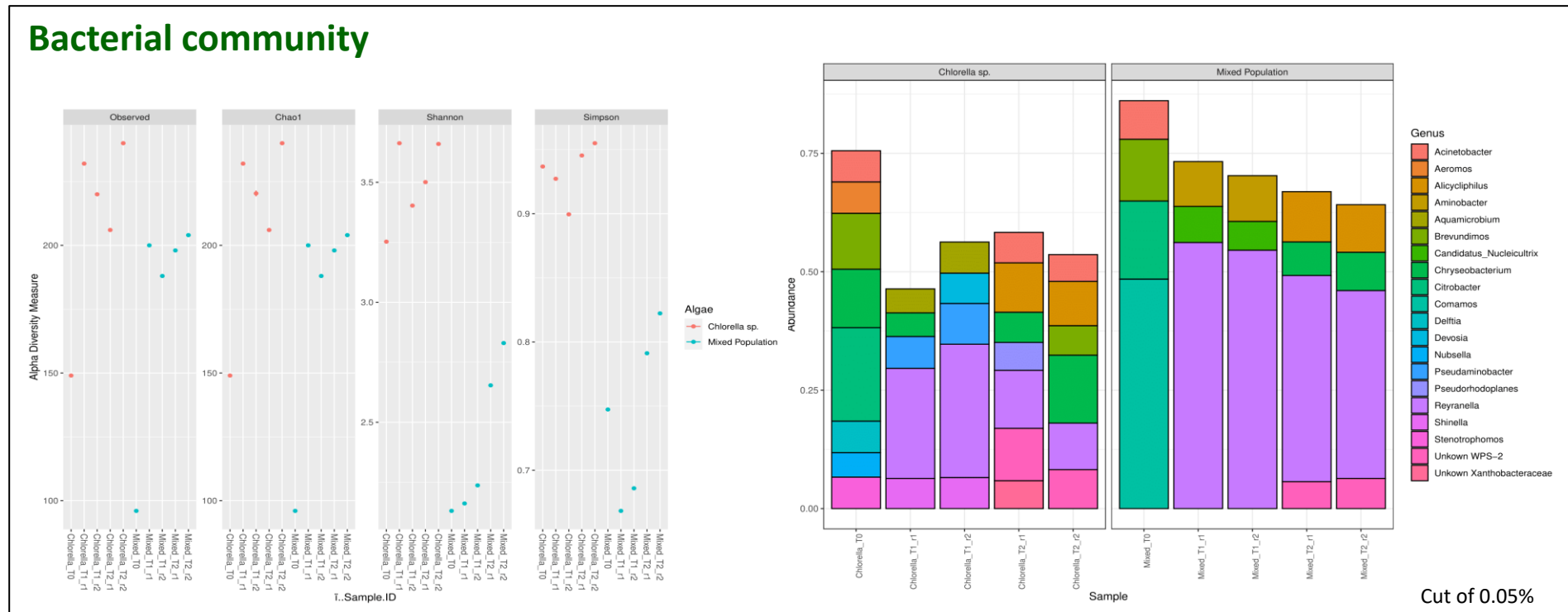


- The laboratory-scale photobioreactor showed a positive removal of  $\text{NH}_4^+$  after 50 days
- Main algal species were *Desmodesmus pleiomorphus* (synonym of *Scenedesmus pleiomorphus*) and *Scenedesmus acutus* in the two replica respectively

## Algal community



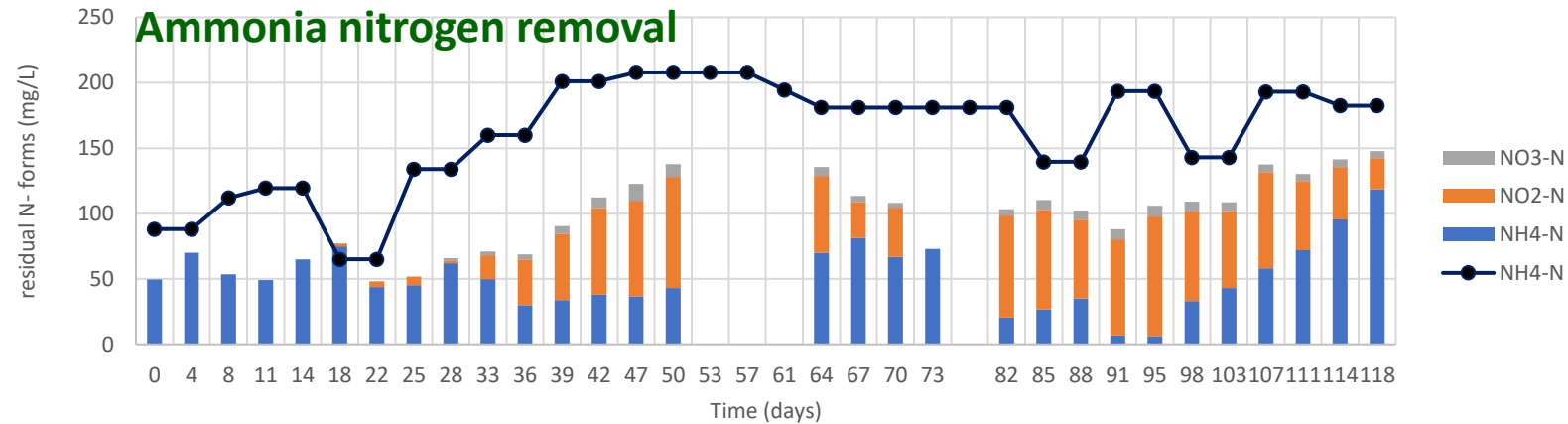
# Implementation of N-removal with microalgae: Lab-scale PBR



- The two reactors displayed different bacterial community assemblages with and increased importance of the genus *Reyranella*, a genus usually found in freshwater, and in forest or agricultural soils

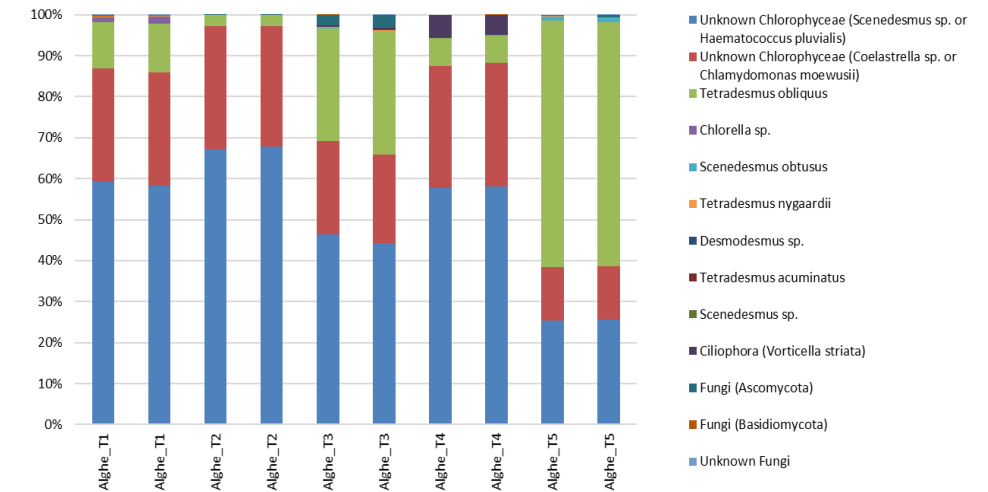


# Implementation of N-removal with microalgae: Pilot-scale PBR

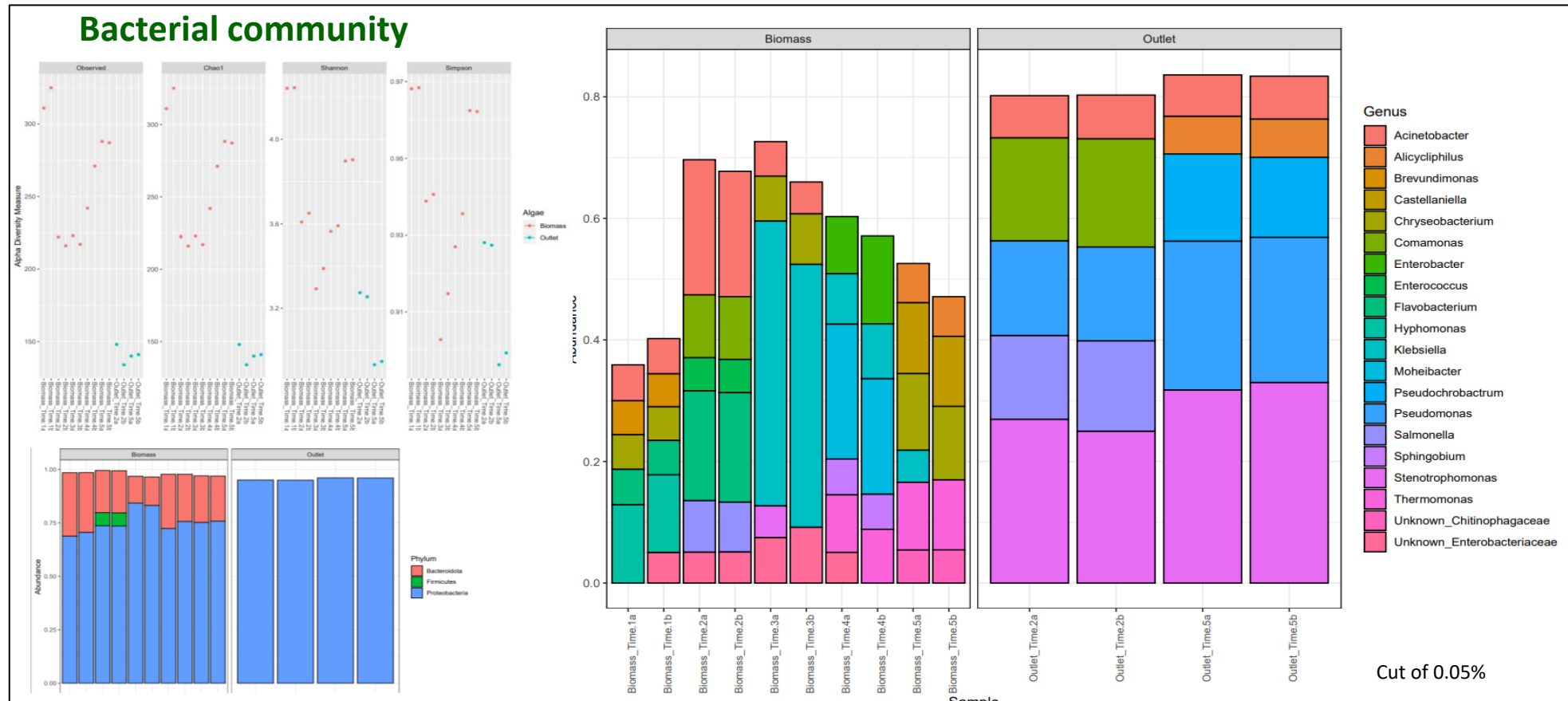


- The pilot-scale photobioreactor showed a positive removal of  $\text{NH}_4^+$  after 50 days, persisting until 118 days
- A more complex algal community assemblages have been found, with the final dominance of *Tetradesmus obliquus*

## Algal community



# Implementation of N-removal with microalgae: Pilot-scale PBR



- A complex dynamics of bacteria could be seen across the time, with bacteria associated to wastewaters and to high C loads
- Bacteria involved in pollutant degradation can be observed in the outlet (*Pseudomonas*, *Stenotrophomonas*)

# Implementation of N-removal with microalgae: conclusions

- The pilot-scale photobioreactor showed a very diverse algal and bacterial communities with respect to the lab-scale one
- Algal diversity was minor than bacterial diversity, but in both cases a complex dynamic was visible
- Interesting bacterial genera has been found in the pilot scale PBR and in the outlet: most of these genera are associated to pollutant degradation and in C-recycling, but some of them are also associated to human-impacted environments
- Their role and contribution in the richness and functionality of the algal consortium deserve to be further investigated

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Progetto finanziato da:

