Denitrifying bacterial abundance and diversity in wetland ecosystems

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Contents

Backgrounds

Denitrification process

- Reduction of NO_3^- to N_2O or N_2
- Terminal e^- acceptor for anaerobic respiration
- Key controlling variables
  - Anaerobic conditions
  - Carbon supply
  - NO_3^- availability

Importance

- Water quality amelioration
  - Key and permanent removal of NO_3^-
- Greenhouse gas
  - N_2O is *ca.* 250 times stronger than CO_2

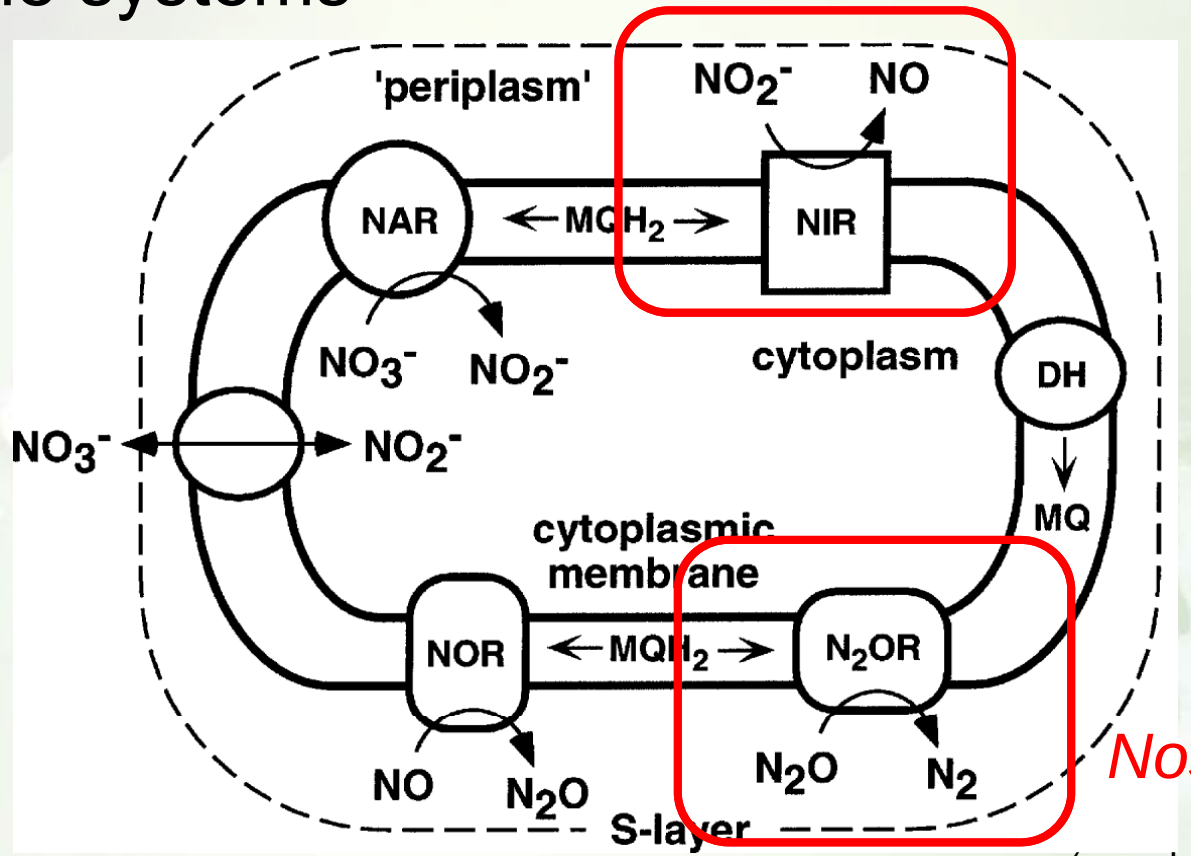
Measurements for the process rates

- Acetylene blocking method
- Disappearance or flux of NO_3^-
- Direct measurement of N_2
- ^{15}N NO_3^- dilution method
- ^{15}N isotope pairing method

Denitrifying bacteria

- Facultative anaerobes
- Enzyme systems

NirS gene



NosZ gene

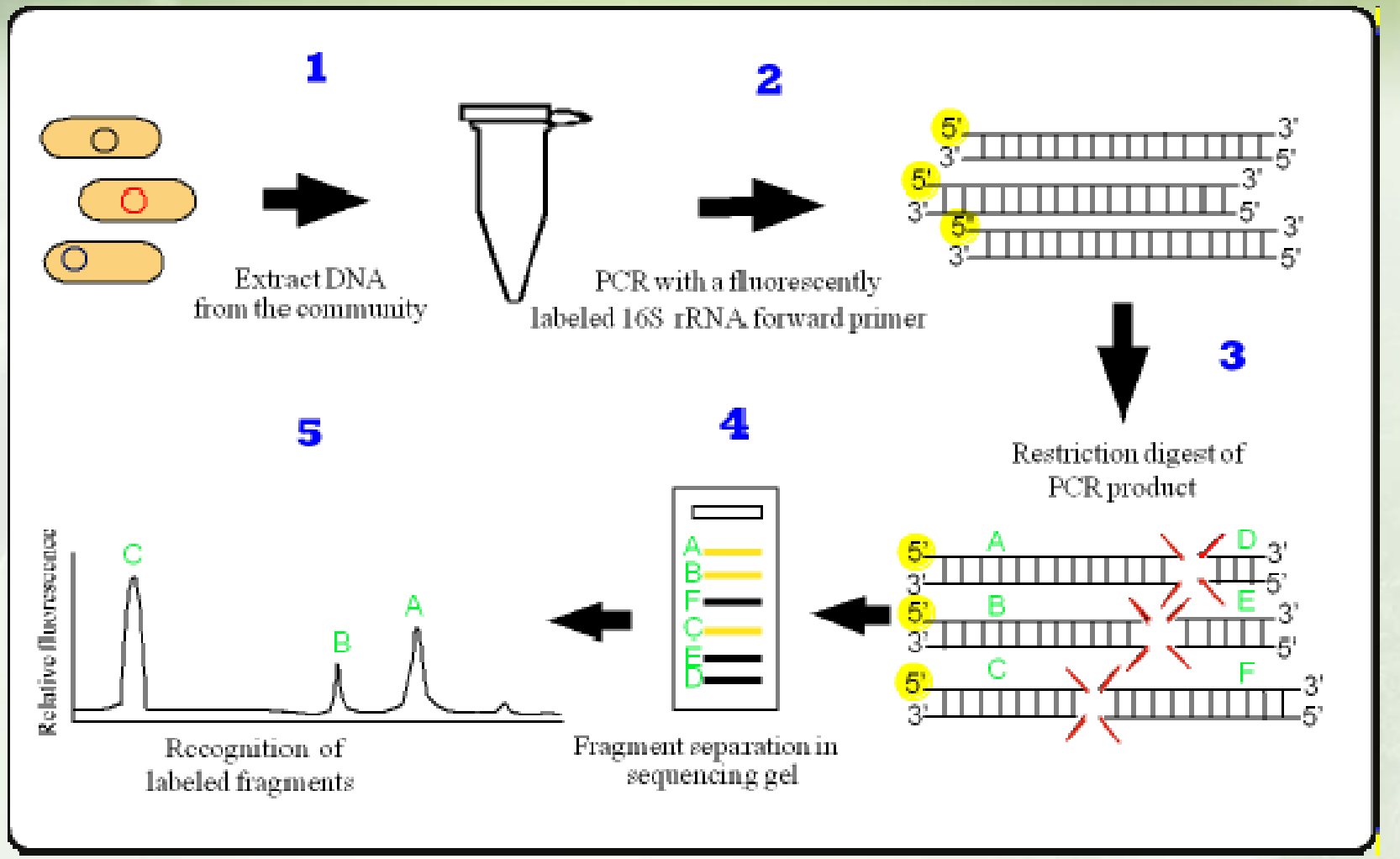
(www.biochemsoctrans.org)

Methods for denitrifiers

- Conventionally, culture based methods were employed → only 1-5 % are culturable
- Molecular approaches
  - Cloning & sequencing
  - Fingerprinting methods (T-RFLP)
  - Q-PCR

T-RFLP

(Terminal Restriction Fragment Length Polymorphism)

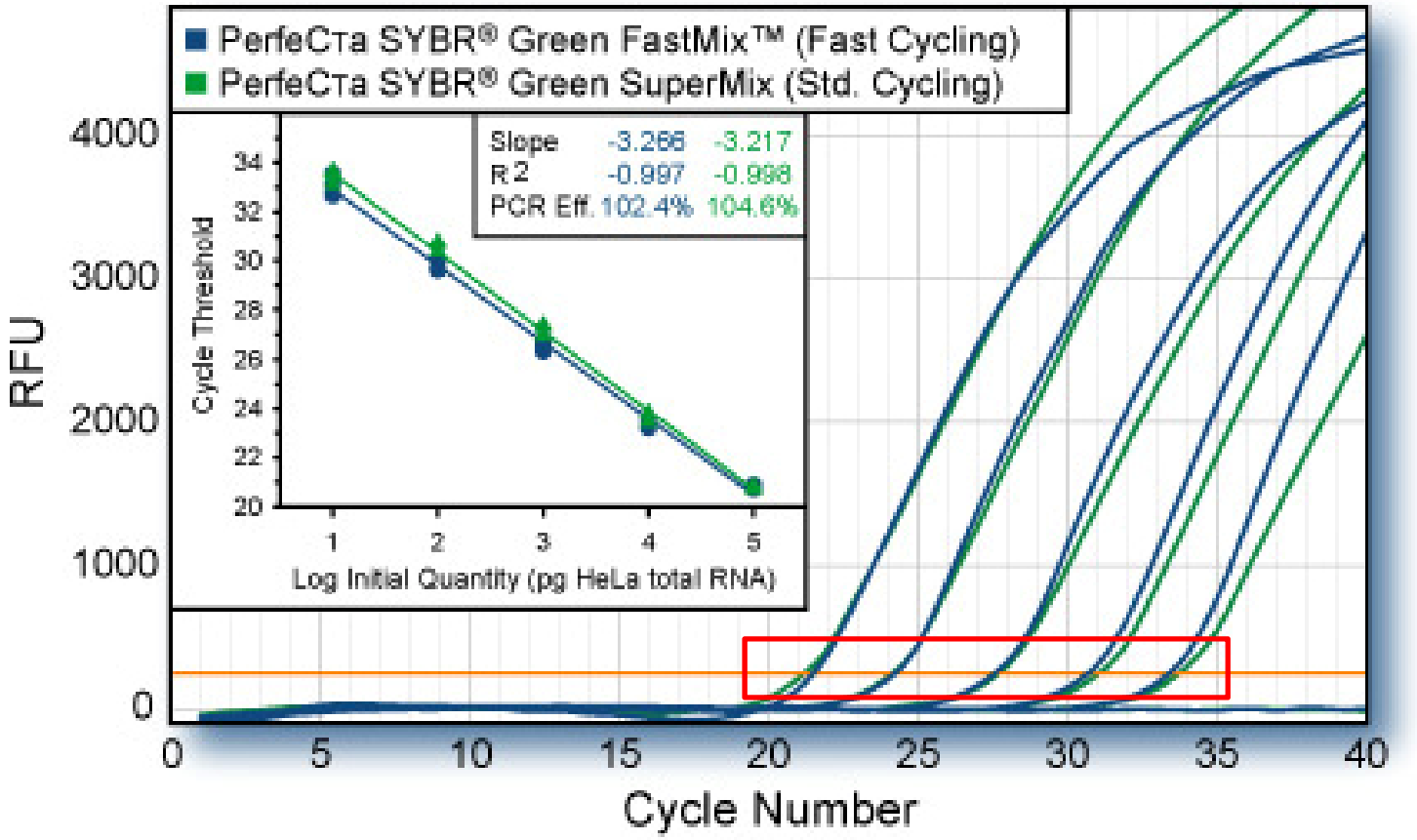


(rdp8.cme.msu.edu/html/t-rflp_jul02.html)

Real Time Q-PCR

1. **Reaction setup:** The SYBR® Green I Dye fluoresces when bound to double-stranded DNA.

2. **Denaturation:** When the DNA is denatured, the SYBR® Green I Dye is released and the fluorescence is drastically reduced.



(Source: Quantabio.com)

Study sites

Olentangy constructed wetland

9 stream sediment & riparian

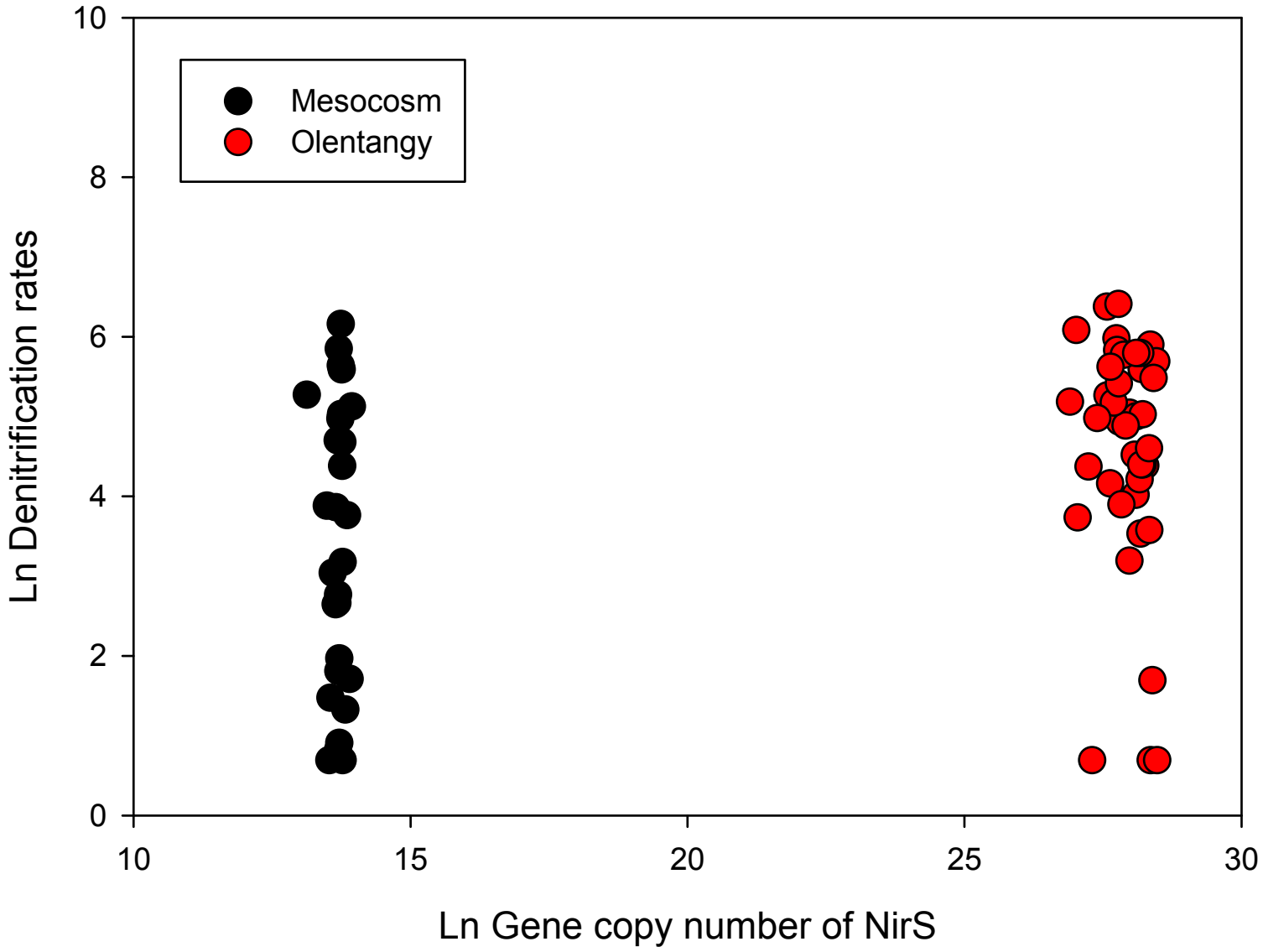
Mountain wetlands

Objectives

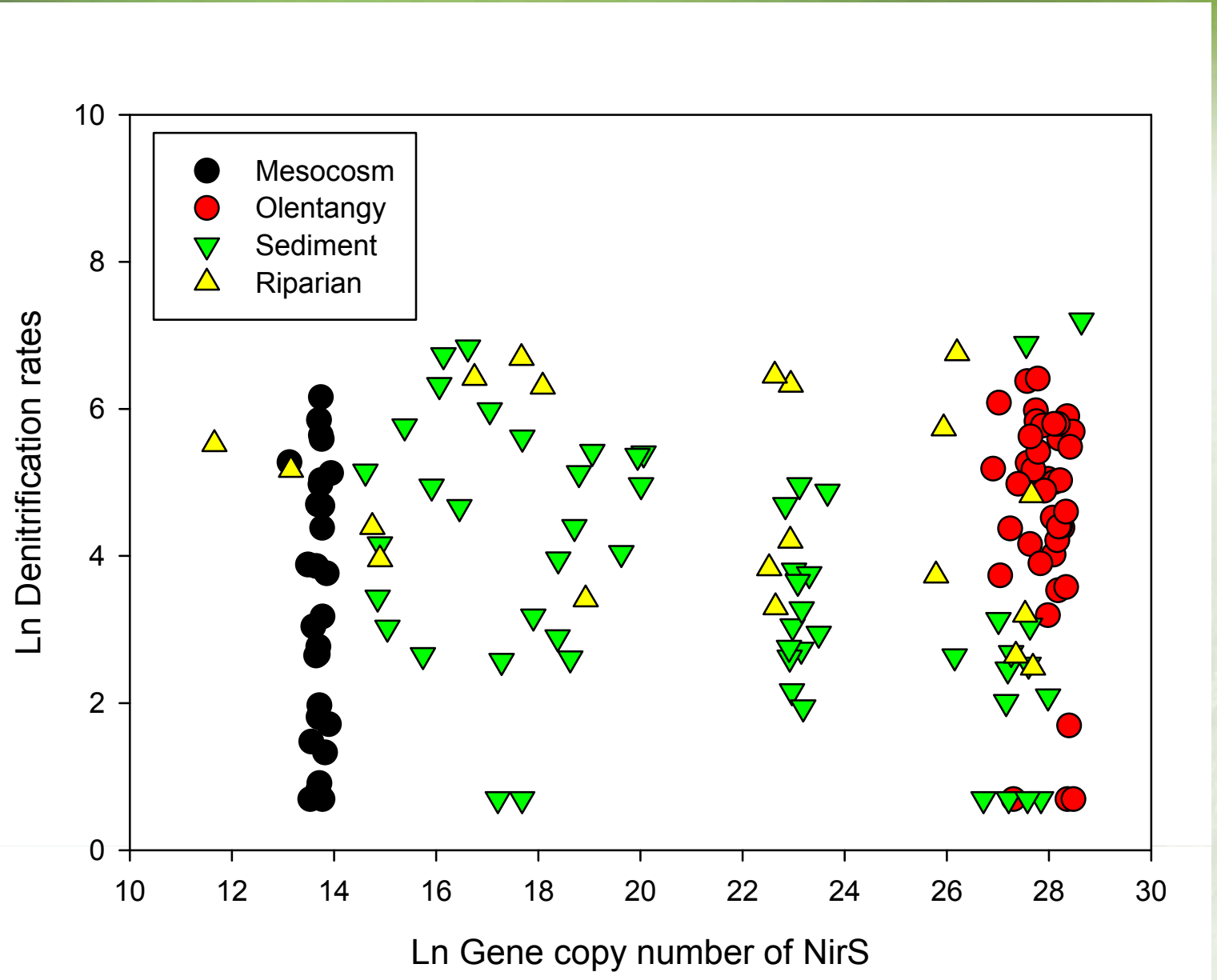
- To determine abundance (Q-PCR) & diversity (T-RFLP) of denitrifying bacteria in various types of wetlands
- To determine relationship between microbial information and denitrification process rate

Abundance

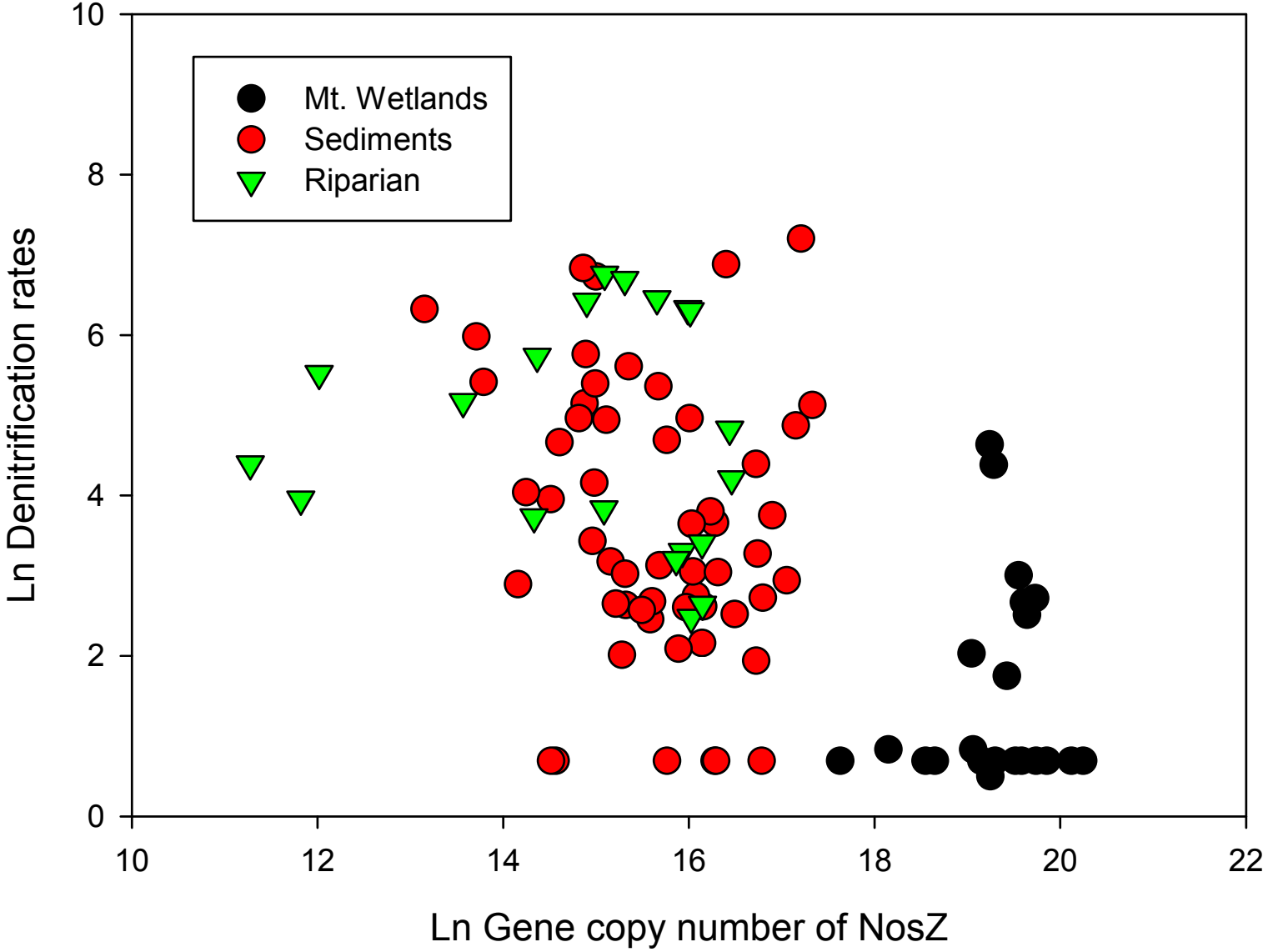
Abundance vs. Process rates (*NirS*)



Abundance vs. Process rates (*NirS*)

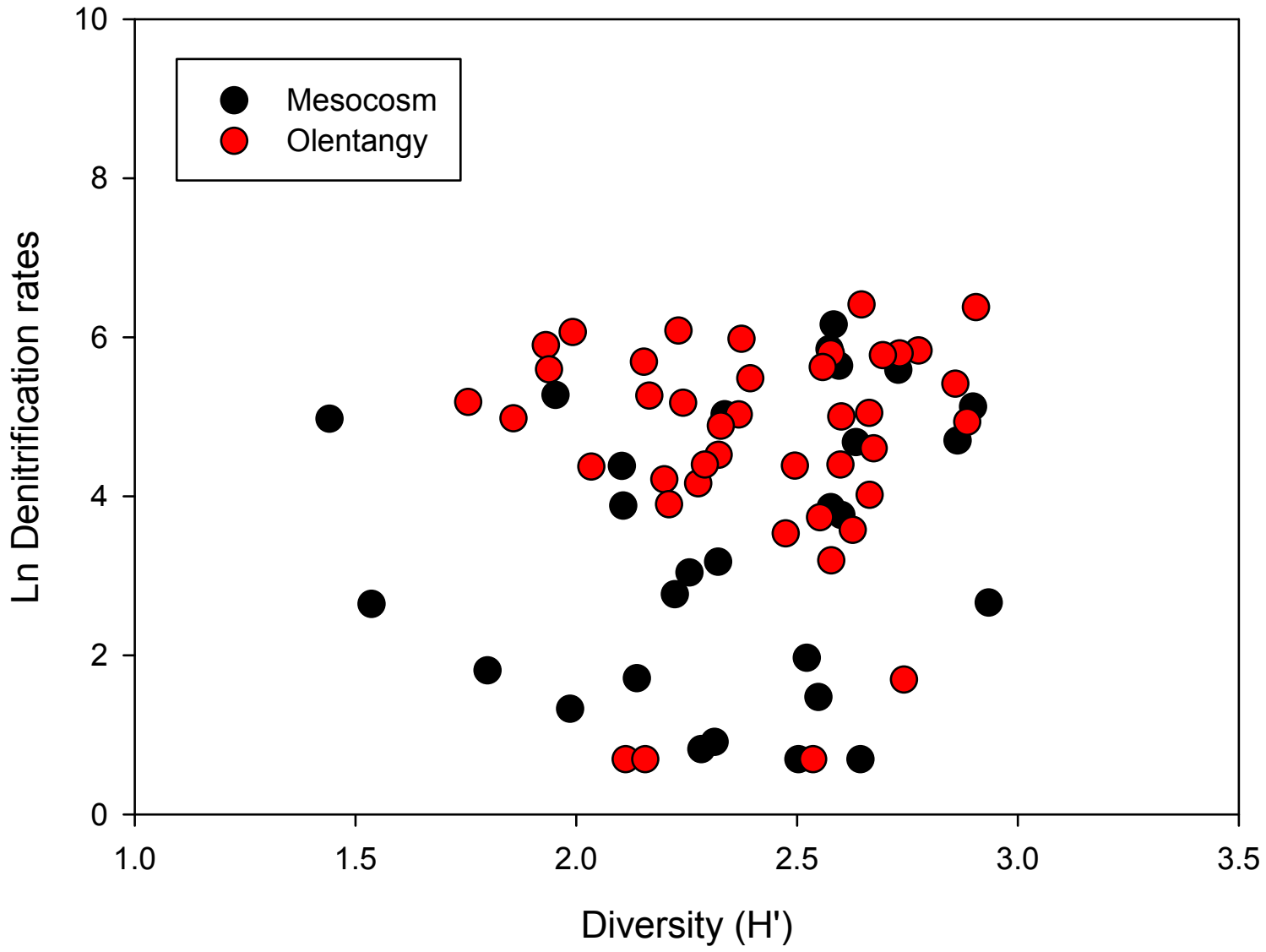


Abundance vs. Process rates (*NosZ*)

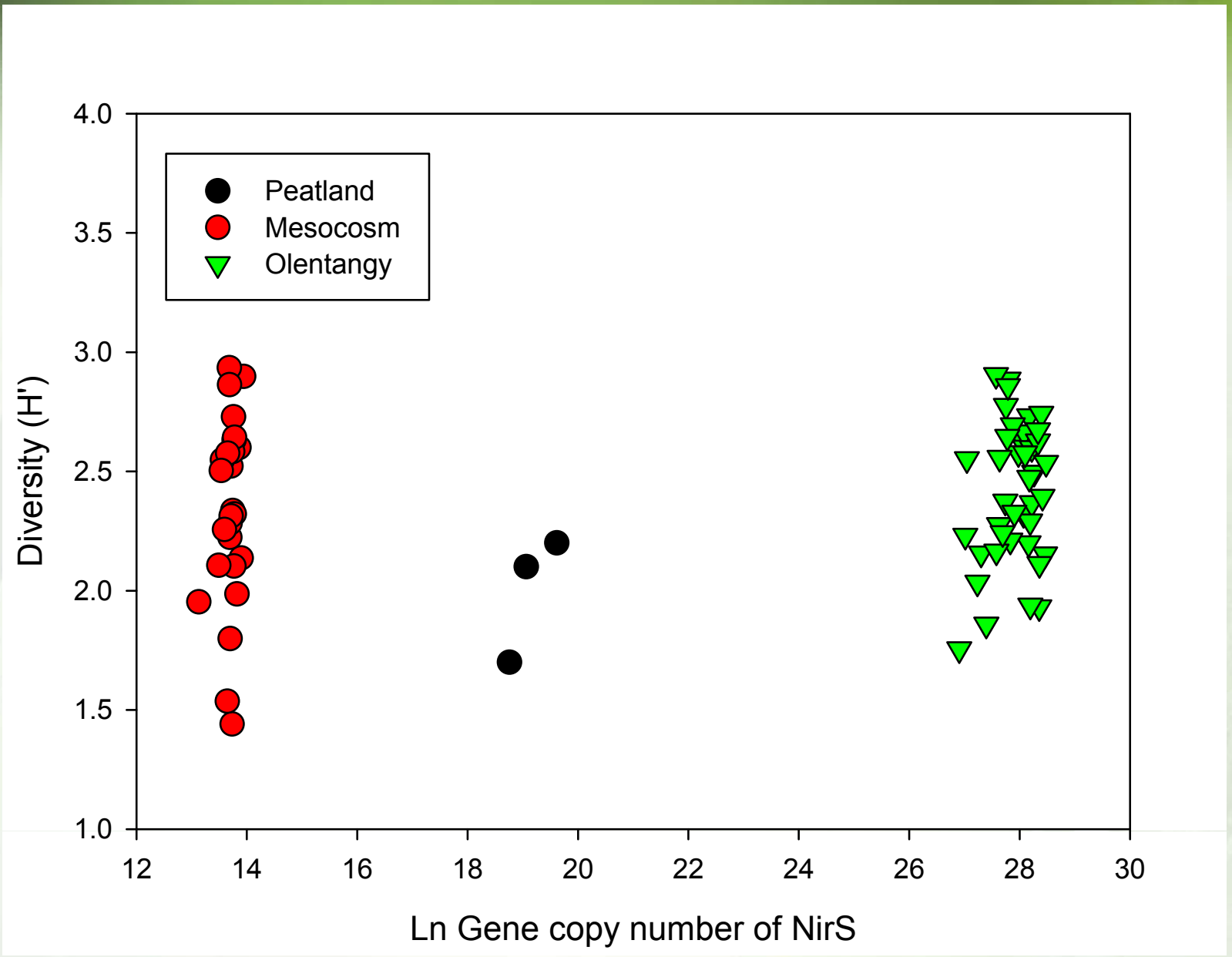


Diversity

Diversity vs. Process rates (*NirS*)



Abundance vs. Diversity (*NirS*)



Absence of correlation between microbes and process rates

- Most denitrifiers are facultative anaerobes
- Gene copy number in each bacterium is highly variable
- Shortcomings of acetylene blocking method

Summary

- Abundances (Q-PCR) are relatively stable in each system
- Process rates varies substantially

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