

BENEATH THE OXIA:

Insight into the prokaryotes of marine meromictic Rogoznica lake (Croatia) and their potential in elemental cycling

IRB Ruđer Bošković Institute

M. Čanković¹, G. Collins², E. Porca², I. Petrić¹, I. Ciglencčki¹
¹Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia
²School of Natural Sciences - NUI Galway, Microbiology, Galway, Ireland

NUI Galway
OĒ Gaillimh

Microbial Ecophysiology & Bioengineering Laboratory

AIMS

- 1) Insight into the microbial community structure of the hypoxic and anoxic niches of Rogoznica Lake
- 2) Identification of dominant members of the active prokaryotic communities
- 3) Evaluation of seasonal differences in ecology between the chemocline, monimolimnion and surface sediment
- 4) Identification of environmental factor(s) that influence particular microbial population(s)
- 5) Description of the ecophysiological interactions and elemental cycling

MATERIALS AND METHODS

- ❖ Samples were taken seasonally; water (chemocline and monimolimnion) by Niskin bottle; sediment (up to 10 cm) by gravity corer.
- ❖ T, S, O₂, OPR, pH measured *in situ* by CTD probe; nutrients (Strickland and Parsons, 1972); H₂S (Ciglencčki and Čosović, 1997); POC and DOC-high temperature catalytic oxidation method.
- ❖ Nucleic acid co-extraction by modified Griffiths et al. (2000) method.
- ❖ High-throughput 16S rRNA gene sequencing of DNA and cDNA (Illumina Mi-seq).
- ❖ qPCR assay targeting bacterial and archaeal 16S rRNA (Yu et al., 2005; McKeown et al., 2009).

STUDY SITE

Rogoznica Lake (Croatia) is a marine meromictic lake located on the Gradina Peninsula (43°32'N, 15°58'E) approximately 100 m from the open Adriatic Sea, close to village Rogoznica (Dalmatian coast). The lake has a circular shape with an area of 10,276m² and a maximum depth of 14 m. It is sheltered from the wind by 4–23 m high cliffs that prevent wind-shear mixing.

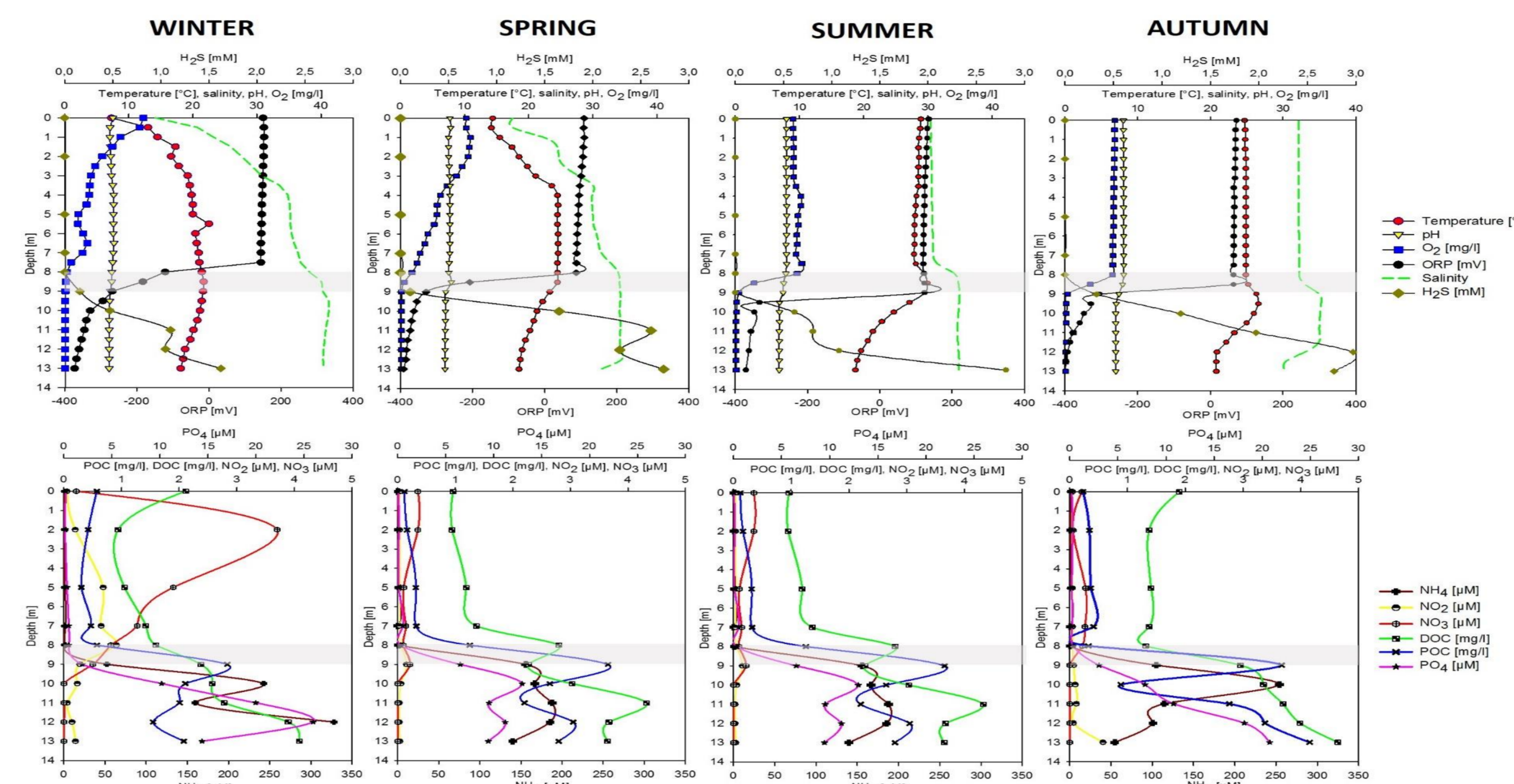


Fig. 1: Vertical profiles of physicochemical parameters of Rogoznica Lake during the investigation seasons. Samples for high-throughput 16S rRNA gene sequencing were taken from the chemocline (shaded areas) and monimolimnion (12 m).

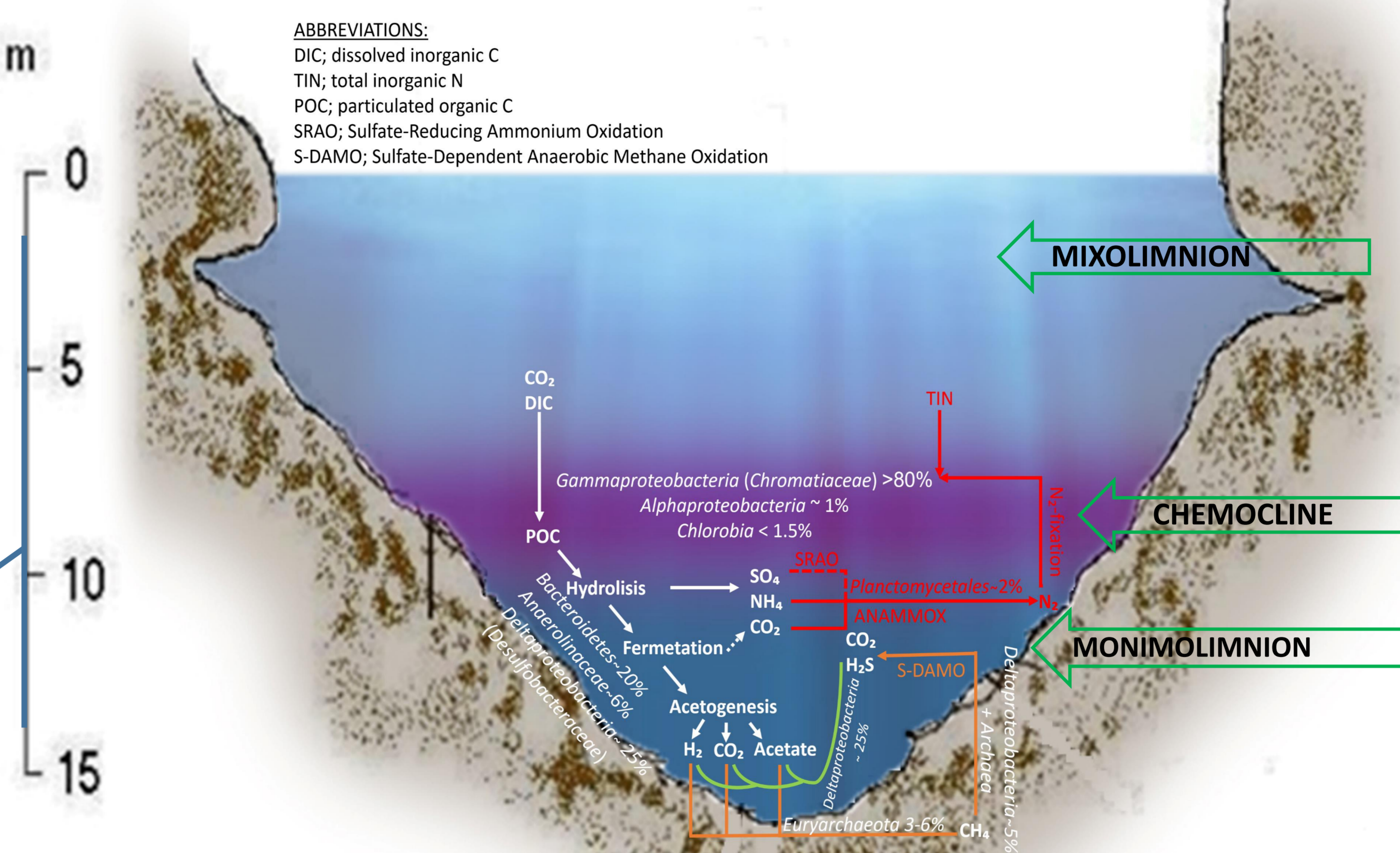


Fig 8: Schematic view of the potential biogeochemical processes in the chemocline, monimolimnion and sediment of Rogoznica Lake, inferred from the phylogenetic affiliations of the cDNA sequences. Relative abundance of dominant (>1%) taxa is given for each pathway.

RESULTS

- Physicochemical parameters showed that the water column was vertically stable throughout the year (Fig. 1) as a result of thermohaline and chemical stratification, typical for meromictic lakes. Seasonal changes were less expressed below the oxic zone.
- Long-term data showed that the temperature, ammonium and sulfide concentration in the lake are increasing (Fig. 2).
- Clear vertical structure of microbial community (Fig. 6) was observed, with *Gammaproteobacteria* dominating the chemocline, *Deltaproteobacteria* dominating the monimolimnion and significantly ($p < 0.05$) higher archaeal abundance was found in the sediment (Fig. 3).
- Quantitative PCR confirmed that their abundance remained stable, with no significant ($p > 0.05$) changes over the sampling seasons (Fig. 4).
- Community was significantly influenced by temperature, dissolved organic carbon, sulfide and phosphates (Fig. 5).
- In contrast to findings in similar environments, diversity in Rogoznica Lake was found to be very low in both, DNA and RNA gene pools (Fig 7), thus a biogeochemical cycling of carbon, nitrogen and sulfur is distributed among limited number of taxa (Fig. 8). No evidences of potential nitrifiers and denitrifiers and a very weak potential for methane oxidation and anammox reaction was found in this sulfur-dominated environment.

CONCLUSION

The extreme euxinic chemistry severely curtails the ecological and metabolic diversity, which converges on a small group of dominant taxa underpinning the functioning of the lake. Loss of, or very weak, metabolic pathways were probably caused by development of extremely specialized taxa which makes this environment quite vulnerable as some of them might be extruded as a result of oscillation in environmental conditions and climate change.

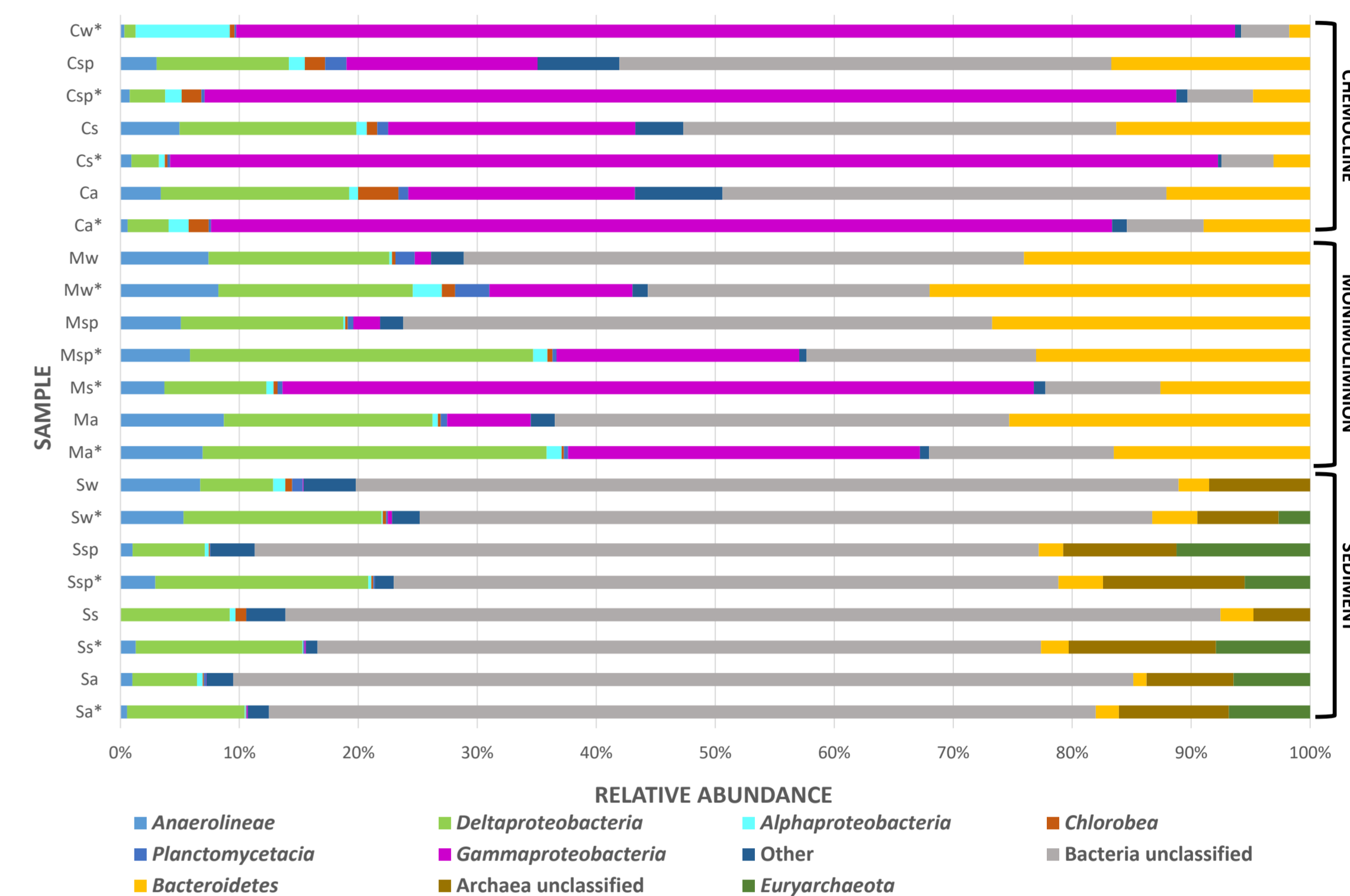


Fig. 3: Relative abundance of bacterial and archaeal communities. Capital letters C, M and S represent chemocline, monimolimnion and sediment, respectively. Lower case letters stand for: w-winter; sp-spring; s-summer; a-autumn. cDNA samples are marked with asterisk (*). Represented taxa occurred at >1% abundance in at least one sample.

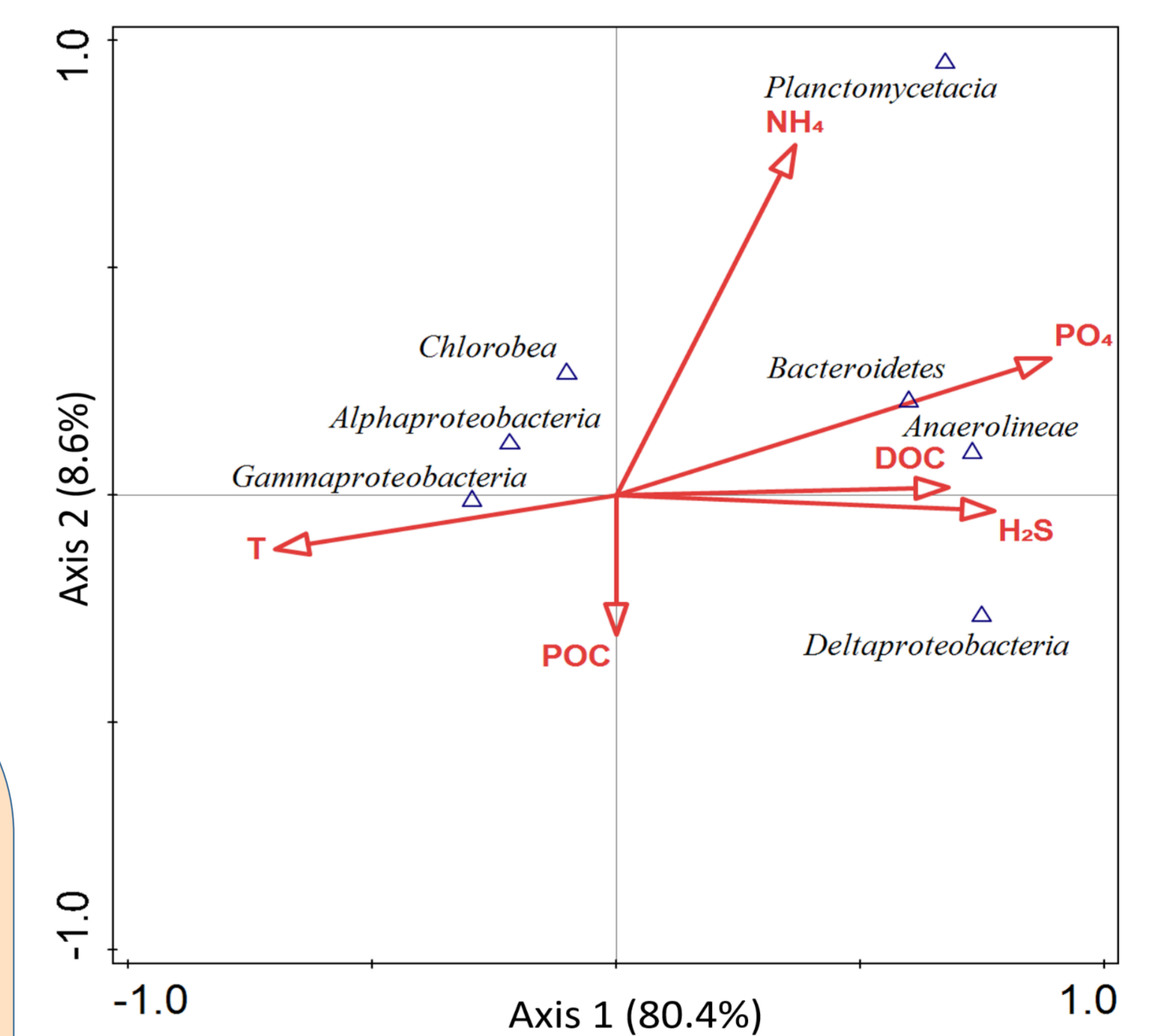


Fig. 5: Canonical correspondence analysis (CCA) ordination diagram of bacterial taxa related to environmental variables of temperature (T), particulated (POC) and dissolved organic carbon (DOC), sulfide (H₂S), phosphate (PO₄) and ammonium (NH₄).

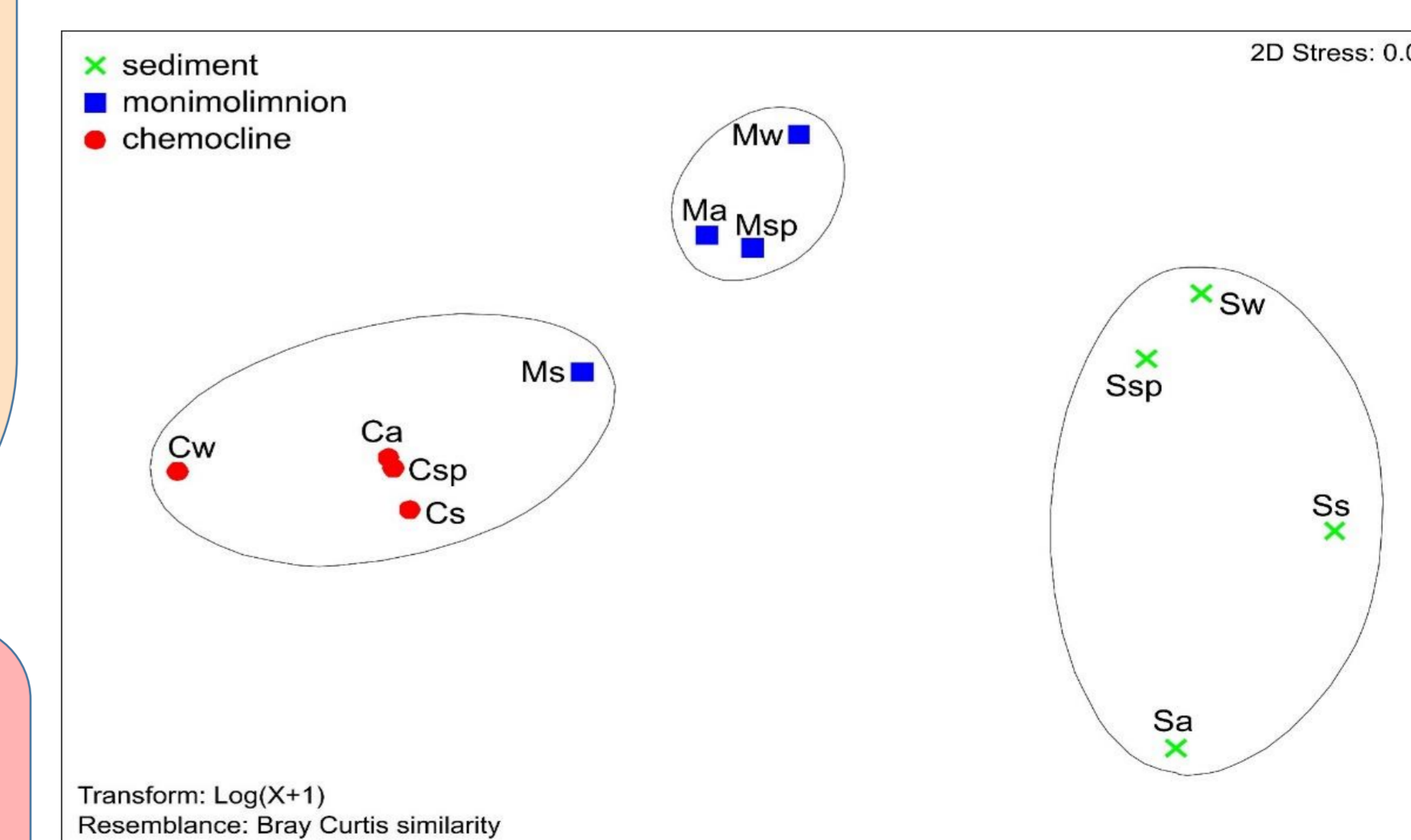


Fig. 6: nMDS plot showed 3 distinct groups of cDNA samples separated based on the results of the cluster analysis (similarity 70%). The capital letters C, M and S represent chemocline, monimolimnion and sediment, respectively. The lower case letters stand for: w-winter; sp-spring; s-summer; a-autumn.

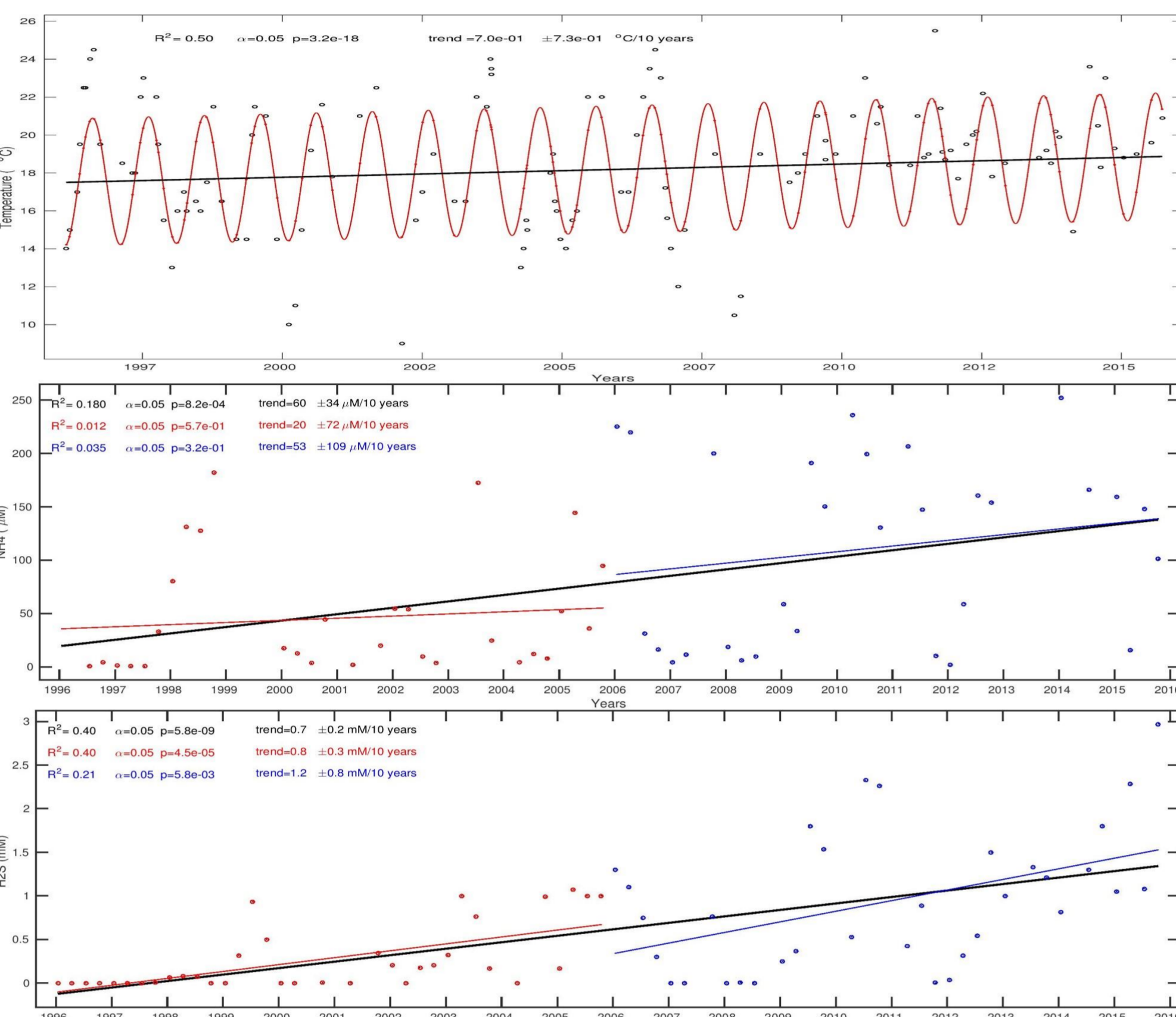


Figure 2. Long-term (1996-2015) trends of temperature (A), ammonium (B) and sulfide (C) in the monimolimnion of Rogoznica Lake.



Fig. 4: Abundance of bacterial (solid line) and archaeal (dashed line) 16S rRNA genes in the chemocline, monimolimnion and sediment of Rogoznica Lake. Vertical error bars represent standard deviation.

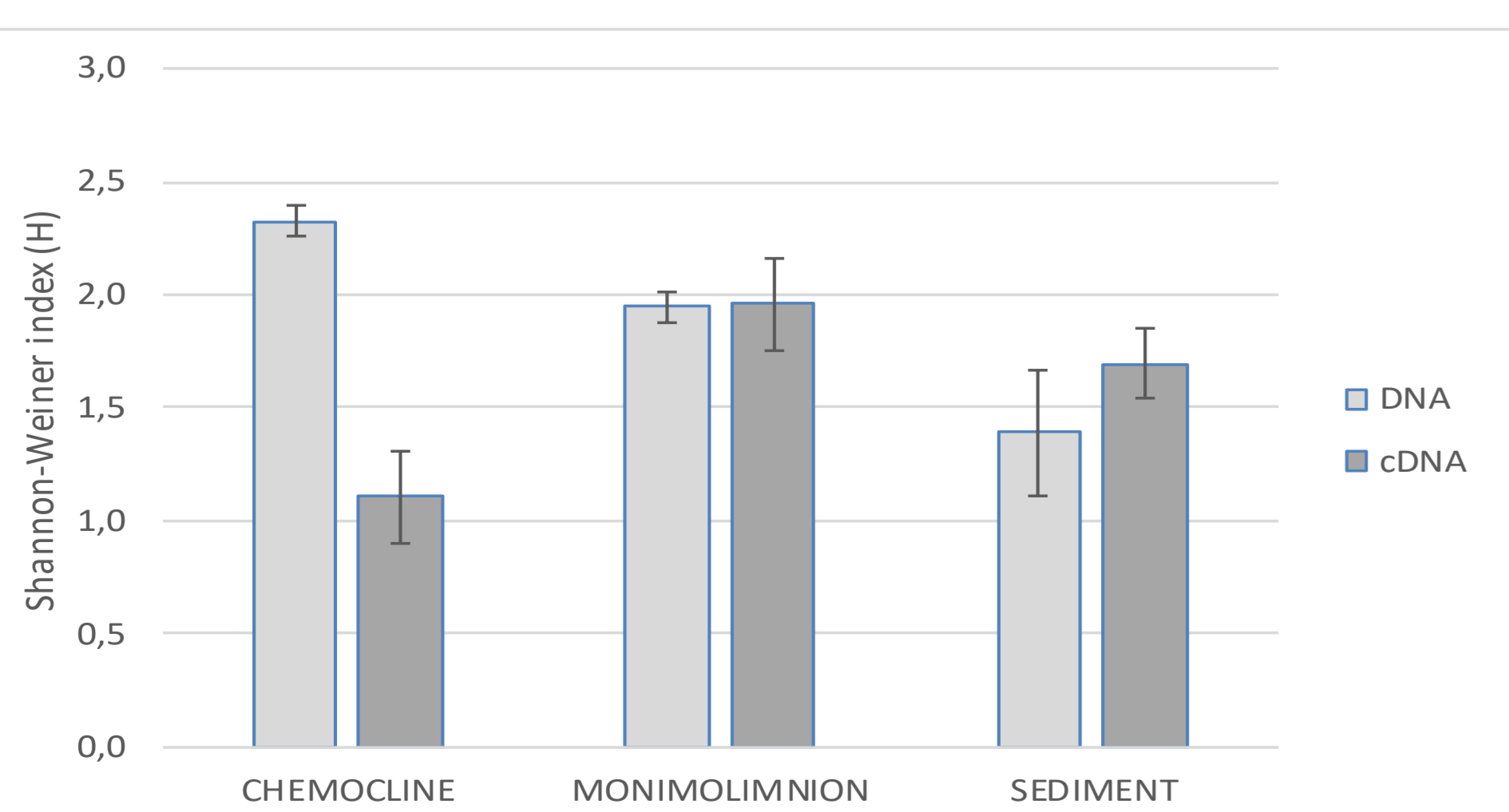


Fig. 7: Shannon-Weiner diversity index (averaged) for the chemocline, monimolimnion and sediment samples based on DNA and cDNA OTUs. Vertical error bars represent standard deviation.

REFERENCES

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ: Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Appl Environ Microb* 2000, 66(12):5488-5491.
 Yu Y, Lee C, Kim J, Hwang S: Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol Bioeng* 2005, 89(6):670-679.
 McKeown RM, Scully C, Enright AM, Chinaila FA, Lee C, Mahony T, Collins G, O'Flaherty V: Psychrophilic methanogenic community development during long-term cultivation of anaerobic granular biofilms. *ISME J* 2009, 3(11):1231-1242.
 Strickland JD, Parsons TR: A practical handbook of seawater analysis. 1972.
 Ciglencčki I, Čosović B: Electrochemical determination of thiosulfate in seawater in the presence of elemental sulfur and sulfide. *Electroanal* 1997, 9(10):775-780

