

Changes in bacterial and archaeal community structure along a eutrophication gradient in a Scottish sea-loch

Introduction

Eutrophication is generally caused by an increased organic matter supply to an ecosystem. Reasons for this can be inorganic nutrient enrichment, input of allochthonous organic carbon or pollutants to the system. The ecological consequences include inter alia the proliferation and increased toxicity of harmful algal blooms, hypoxia and anoxia, altered routes and fluxes of organic and inorganic matter cycling, the disruption of food webs and alteration of bacterial community structure (Roselló-Mora *et al.* 1999; Edlund *et al.* 2006). Fish farming is one important source of anthropogenic nutrient and pollution enrichment in coastal waters and it was suggested recently that microorganisms display a gradual response towards this impact. In our study we investigated if archaeal assemblages are also altered by organic carbon input and to what extent changes in community structure can be linked to the enrichment gradient or to factors such as habitat heterogeneity.



Figure 1 Study Site Loch Creran, Scotland, 56°31'N/5°21'W

Methods

Subsamples from sediment cores taken from the first 0.5 cm of sediment surface; Seven replicated stations covered a gradient from unimpacted conditions (S1) to high organic carbon impact (S11).

Community Analyses

- Bacterial community**
 - RISA (Ribosomal Intergenic Spacer Analysis): ITS1 gene fragment¹
 - DGGE (Denaturing Gradient Gel Electrophoresis): 16S rRNA gene fragment¹; Gradient: 25-60%
- Archaeal community**
 - DGGE, 16S rRNA gene fragment¹; Gradient 20-50%

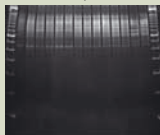


Figure 2 RISA profiles of ITS1 gene fragment, bacterial community of stations S1-S5

- Bacterial cell and virus like particle counts via epifluorescence microscopy
- Macrofauna obtained from five replicate sediment cores, identification to species level; total biomass (blotted wet weight) per species and sample;
- Elucidation of biogenic mixing depth, measurement of oxygen profiles in sediment cores → oxygen flux;
- Characterisation of sediment type including porosity, grain size, C/N ratio as well as total organic carbon;
- Environment-Community relationships analysed by multivariate statistics namely nonmetric Multidimensional Scaling (nMDS) and Canonical Correspondence Analysis (CCA)

Results

Differences between stations in bacterial and archaeal community with strong shifts from a more pristine situation to highly impacted sites (Figures 3 and 4).

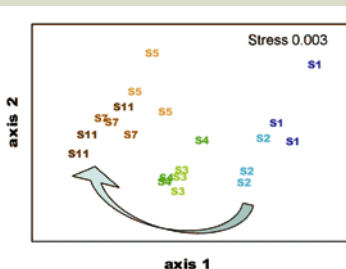


Figure 3 nMDS plot based on Bray-Curtis similarities of bacterial communities (RISA). The ordination shows a clear shift of communities in response to the eutrophication impact (see arrow). This is confirmed by the analysis of similarities (ANOSIM) with sample statistic $R=0.77$ ($P=0.001$)

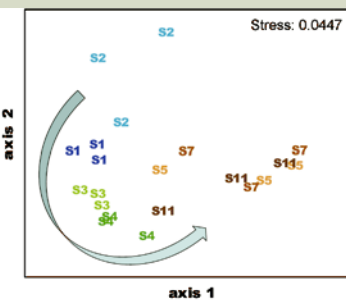


Figure 4 nMDS plot based on Bray-Curtis similarities of archaeal communities (DGGE). The ordination shows a clear shift of communities in response to the eutrophication impact (see arrow). This is confirmed by the analysis of similarities (ANOSIM) with sample statistic $R=0.486$ ($P=0.002$)

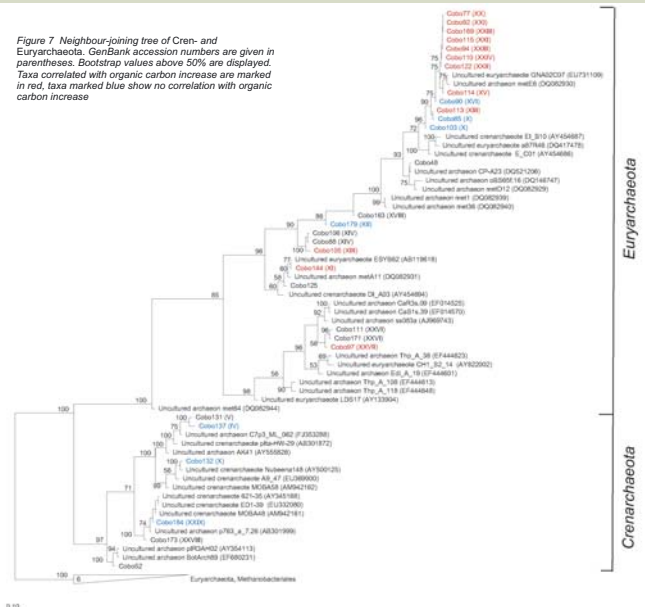


Figure 7 Neighbour-joining tree of Cren- and Euryarchaeota. GenBank accession numbers are given in parentheses. Bootstrap values above 50% are displayed. Taxa correlated with organic carbon increase are marked in red, taxa marked blue show no correlation with organic carbon increase

Correlation with environmental variables

- Variation in community data explained significantly (Monte Carlo permutation)
- Bacterial community: 31% variation explained
 - Biogenic mixing depth and macrofaunal biomass → Communities from more pristine situation
 - C/N ratio and total organic carbon content → Communities from highly impacted sites
- Archaeal community: 87% variation explained (Figure 5)
 - Grain Size → Communities from more pristine situation
 - Organic carbon content, station and total organic carbon → Communities from highly impacted sites



Figure 8 Bowers and Connolly megacore including barrels of an internal diameter of 100 mm

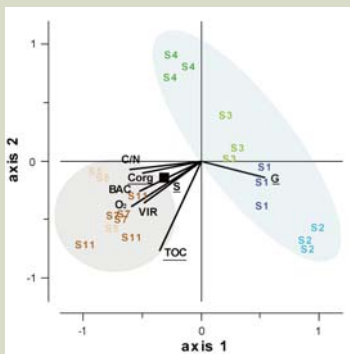


Figure 5 CCA biplot of intersample correlations of archaeal community using grain size (G), total organic carbon (TOC), oxygen flow (O₂), virus like particles (VIR) and bacterial (BAC) counts, organic carbon content (Corg) and C/N ratio (C/N). Significant variables are underlined, sample groups are highlighted (blue for more pristine sites S1 to S4, brown for impacted sites S5 to S11)

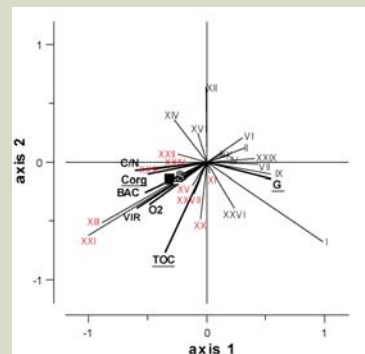


Figure 6 CCA biplot of interspecies correlations of DGGE band classes (- archaeal taxa) using grain size (G), total organic carbon (TOC), oxygen flow (O₂), virus like particles (VIR) and bacterial (BAC) counts, organic carbon content (Corg) and C/N ratio (C/N). Significant variables are underlined, DGGE band classes correlated with increase in organic carbon content are marked in red

Conclusions and Outlook

- Eutrophication caused by fish farming showed a strong effect on both bacterial and archaeal community structure;
- Especially changes in archaeal community could be linked with allochthonous carbon input showing
 - Increased presence of Euryarchaeota
 - Decreased presence of Crenarchaeota
- Crenarchaeota assumed to be more susceptible for organic carbon input than Euryarchaeota

Next steps

- Statistical analyses of bacterial DGGE fingerprints followed by sequence analyses of respective DGGE bands
- Synthesis of results for bacterial community based on RISA and DGGE fingerprints
- Elucidation of potential use of specific microbial taxa as indicators for eutrophication caused by fish farming

Acknowledgements

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References

- Roselló-Mora R, Thamdrup B, Schäfer H, Weller R, and Amann R (1999) The response of the microbial community of marine sediments to organic carbon input under anaerobic conditions. *Syst. Appl. Microbiol.* 22:237-248;
- Edlund A, Soule T, Sjöling S, and Jansson JK (2006) Microbial community structure in polluted Baltic Sea sediments. *Environ. Microbiol.* 8:223-232.

¹ITS1 gene fragment: BAC1392t: 5'-GYA CAC ACC GCC CGT-3' and BAC235r: 5'-GGG TTB CCC CAT TCR G-3'
¹¹⁶S rRNA gene fragment: BAC341t: 5'-(clamp) CC TAC GGG AGG CAG CAG-3' and BAC907m: 5'-CCG TCA ATT CMT TTR AGT TT-3'
¹¹⁶S rRNA gene fragment: ARC344t: 5'-(clamp) AC GGG GTG CAG CAG GCG CGA-3' and ARC915r: 5'-GTG CTC CCC CGC CAA TTC CT-3'