Changes in bacterial and archaeal community structure along a eutrophication gradient in Loch Creran

Dr Melanie Sapp

Environment & Ecosystems

Ecosystem Quality Team

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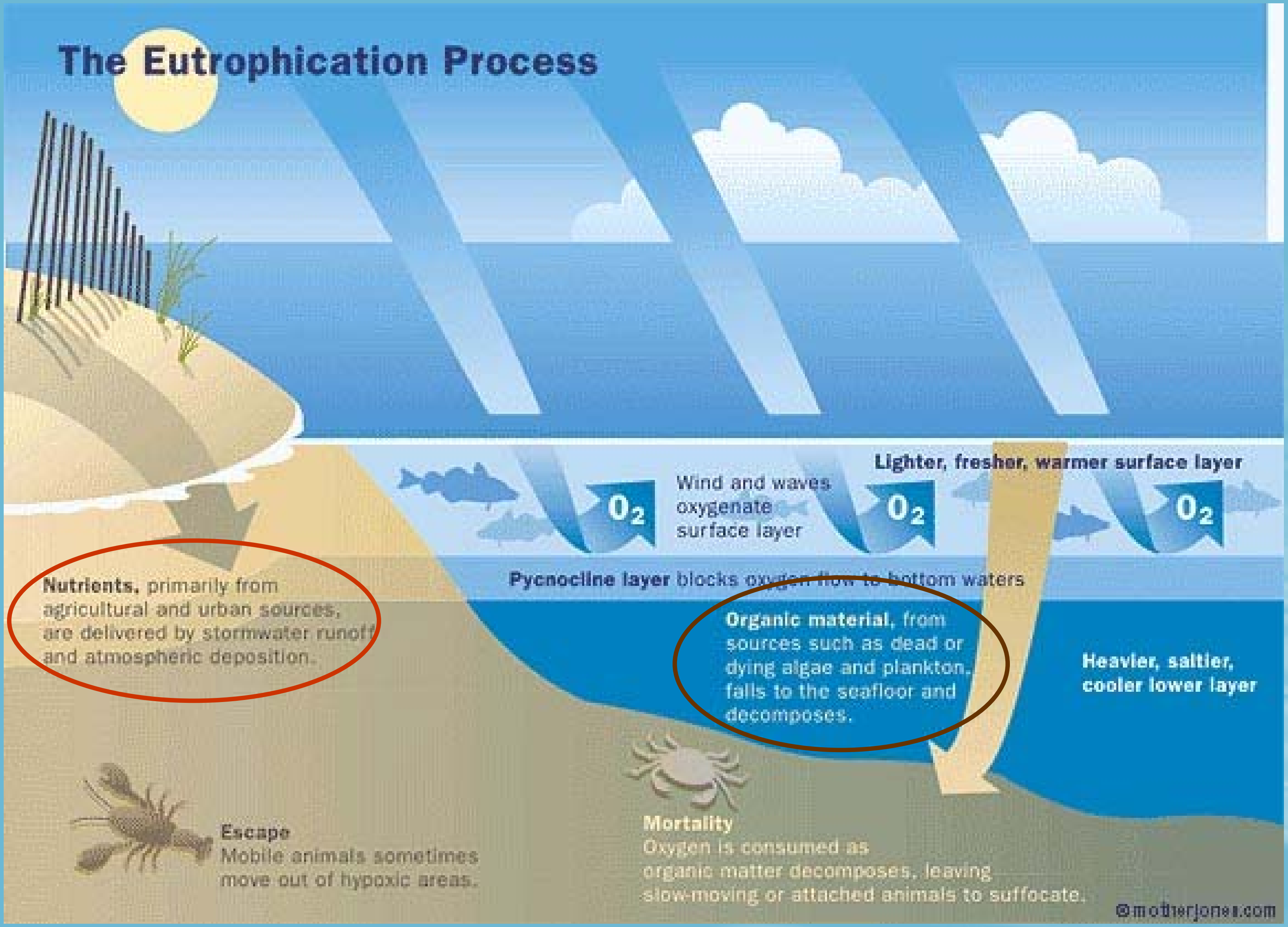
Microbial Ecology at Cefas

- **Ecosystem Status: Assessment relies on integration of response of** organisms at all levels of organisation
- **Environmental and ecosystem-related questions** best addressed applying a community-based approach, main topic: diversity changes
- Changes in microbial diversity: indicator for man-made activities affecting ecosystem processes, mediated by these organisms (?)
- **Contribution to wider ecosystem science**
  - biogeochemical cycling
  - food webs
  - pelagic or benthic habitats
  - ecological processes
- **Problem:** Lack of relevant data, microorganisms = ‘black box’ in models ignoring possible controls of processes by species or community interactions
- Focus on **benthic habitats**, importance of sediments for biogeochemical cycling and as sink for contaminants

Eutrophication



The Eutrophication Process

Nutrients, primarily from agricultural and urban sources, are delivered by stormwater runoff and atmospheric deposition.

Wind and waves oxygenate surface layer

Lighter, fresher, warmer surface layer

Pycnocline layer blocks oxygen flow to bottom waters

Organic material, from sources such as dead or dying algae and plankton, falls to the seafloor and decomposes.

Heavier, saltier, cooler lower layer

Escape
Mobile animals sometimes move out of hypoxic areas.

Mortality
Oxygen is consumed as organic matter decomposes, leaving slow-moving or attached animals to suffocate.

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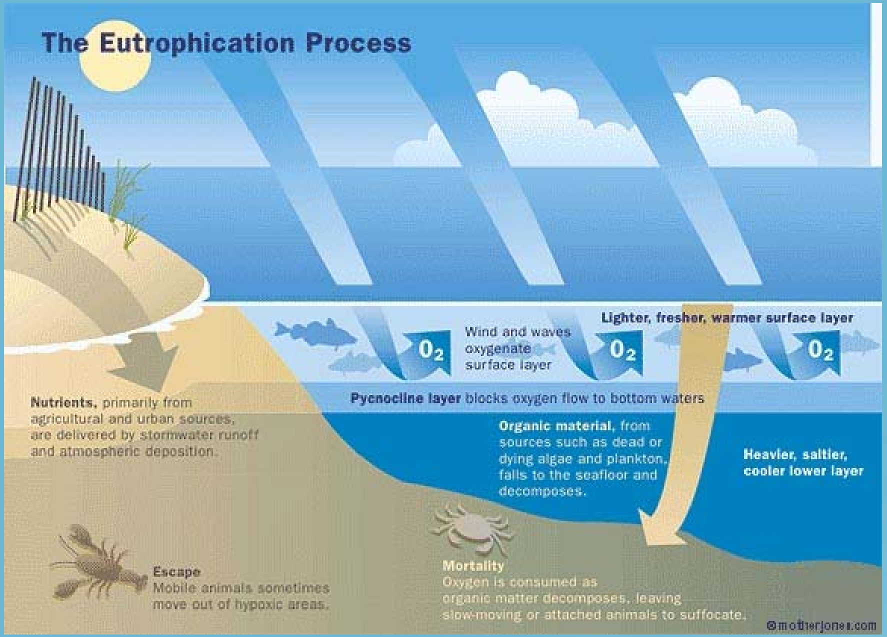
Eutrophication



Gulf of Mexico, coast off Florida

Fish farming

Aquaculture

- Discharge of nutrients, solid waste, medicines and antifoulants (eg copper based)
- Accumulation of waste feed and faeces from fish farms on the seabed under fish cages

Eutrophication - effects

- Changes in microbial community composition and function

→ Proliferation of harmful algal blooms accompanied by increased toxicity of these blooms

→ Altered routes and fluxes of organic and inorganic matter cycling

→ Disruption of food webs, often associated with increased bacterial abundance

→ Decreased functional diversity of aerobic microbial communities

→ Specific bacterial populations favoured by eutrophication whilst others are displaced eg Sulfate Reducing Bacteria

Effects on benthic communities

Authors	Study type	Analysis	Outcome / indication
Rossello-Mora et al (1999)	Mesocosm Addition of algal biomass	FISH, DGGE in combination with sequencing; bacteria	Changes in community structure, *Bacteroidetes* spp. ↑
Edlund et al (2006)	Baltic Sea, pollution gradient, *in situ*	T-RFLP archaea and bacteria	Changes in community structure
McCaig et al (1999)	Scottish fish farm, *in situ* (Loch Sunart)	DGGE and hybridization; ammonia-oxidizing bacteria	Change in community structure of marine *Nitrosomonas* group
Asami et al (2005)	Japanese shellfish farm, *in situ*	Clone library archaea and bacteria	δ-*Proteobacteria*, *Desulfobulbaceae* ↑
Bissett et al (2006/2007)	Tasmanian fish farm, *in situ*	DGGE, clone library bacteria	Community shifts, diversity ↑

FISH: Fluorescence In Situ Hybridization
DGGE: Denaturing Gradient Gel Electrophoresis
T-RFLP: Terminal Restriction Length Polymorphism

Study Site (EU-COBO)

- Location: Loch Creran
- Typical fjordic sea loch
- Biogenic reefs of the calcareous tube-worm *Serpula vermicularis.* (World-wide distribution but development of reefs extremely rare)
- Fish farming produced eutrophication gradients over 3 years

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Sampling

- Stations sampled in triplicates along fish farming gradient
- Microbial community in top **0.5 cm**
- S1/S2/S3/S4 (less impacted than rest)
- S5 (intermediate site)
- S7 and S11 (highly impacted)

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Bowers and Connelly megacore including barrels of an internal diameter of 100 mm

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Methodology – nucleic acids

- **Environmental samples**
  → difficult DNA/RNA extraction from sediment due to humic acids/high load of organic substances
  - PowerSoil DNA kit MoBio (total community)
- **Fingerprinting - PCR**
  - Bacteria:
  - RISA (Ribosomal Intergenic Spacer Analysis)
    - ITS1 gene fragment (between 16S and 23S rRNA gene)
  - DGGE (Denaturing Gradient Gel Electrophoresis)
    - 16S rRNA gene fragment, sequencing of bands
  - Archaea:
  - DGGE (16S rRNA gene fragment, see above)

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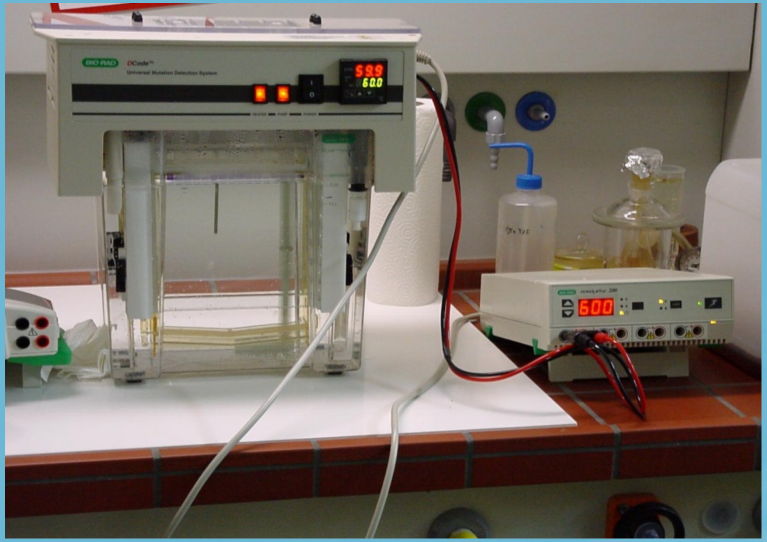
Fingerprinting



DCode system of BIO-RAD

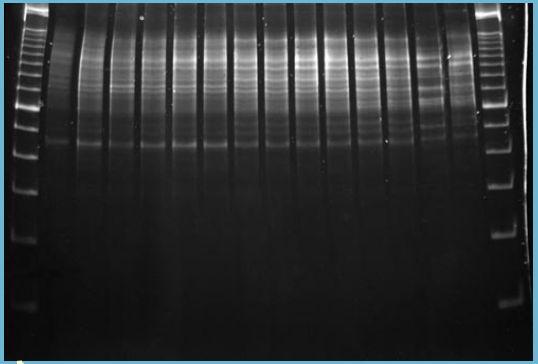
RISA (bacterial)

- Fragment size;
- differences in bacterial groups
- variable region ITS

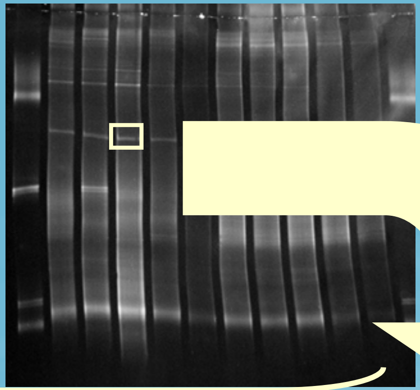
DGGE

- Base composition of fragment (melting behaviour)
- differences in species
- highly conserved region (16S rRNA gene)

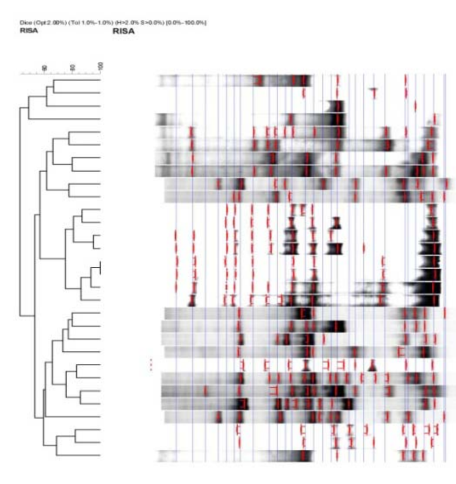
Methodology – Fingerprinting techniques



Ribosomal
Intergenic
Spacer
Analysis



Denaturing
Gradient
Gel
ophoresis

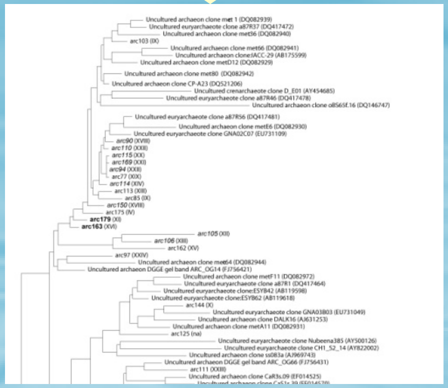


Peak/Bandmatching
→ Band classes

→ **Clustering, Ordination**

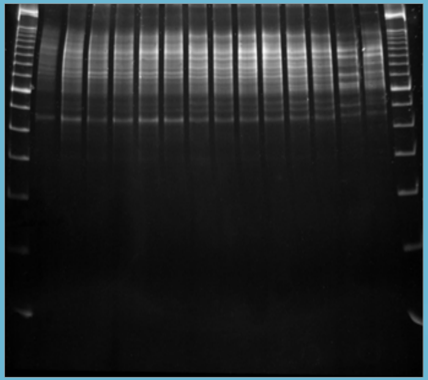
→ β-Diversity
Species richness

→ **Canonical Correspondence Analysis** (CCA)
e.g.

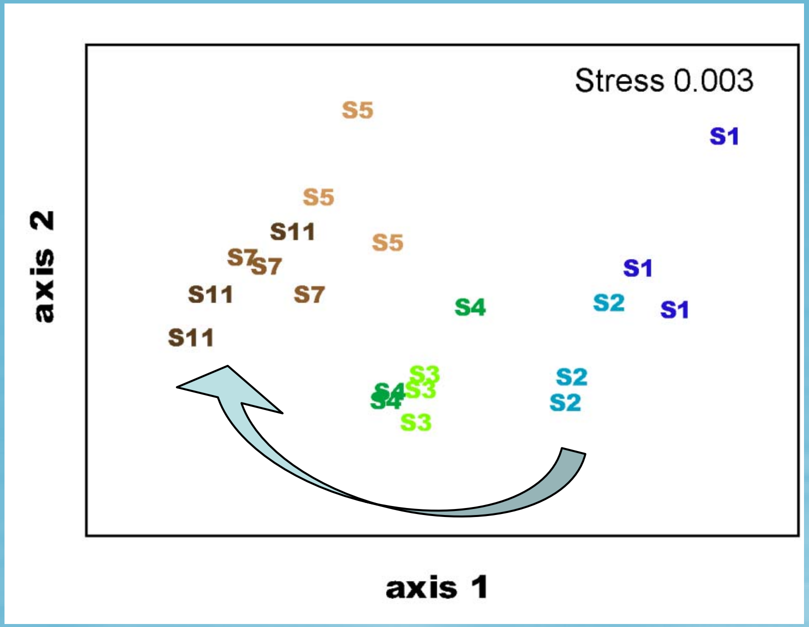


Sequence information from DGGE bands (500 bp), NCBI
→ Phylogeny (16S rRNA gene), ARB

Effects of fish farming on the benthic microbial community – bacteria I



RISA fingerprint

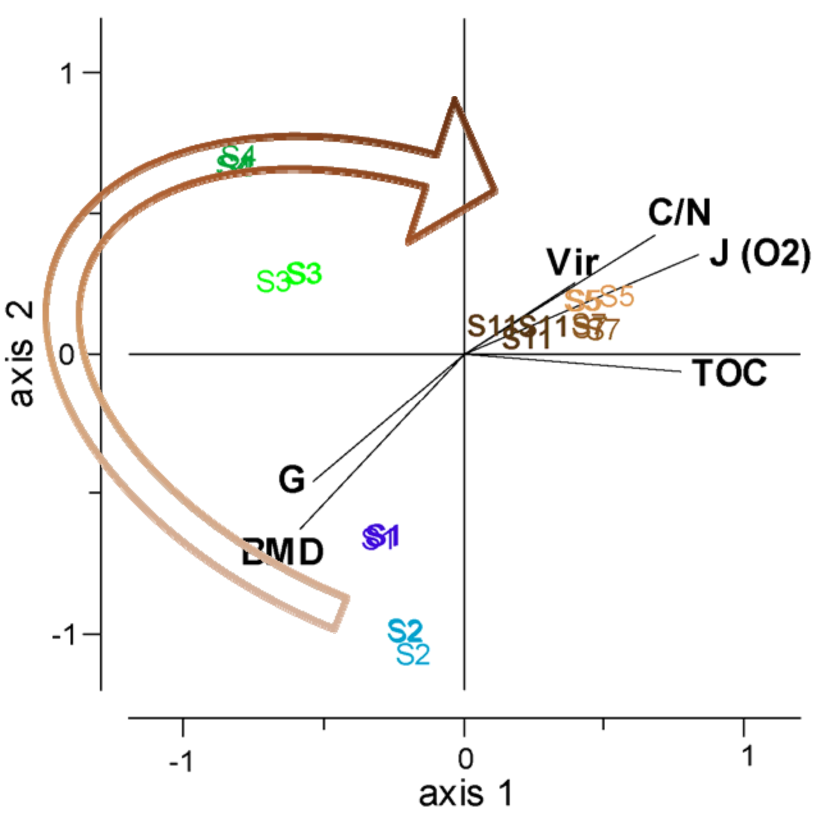


nMDS plot based on Bray–Curtis similarities of **bacterial communities** (RISA fingerprints).

- Gradient from low impact (S1) to high organic carbon impact (S11)
- Clear shift of communities in response to the eutrophication impact (arrow)
- Analysis of similarities (ANOSIM) with sample statistic **R=0.77** (P=0.001).

Effects of fish farming on the benthic microbial community – bacteria II

- Sum of all canonical eigenvalues: **0.66**
- Monte Carlo F=1.9961, p=0.003
- Significant factors TOC and C/N
- Sites S1 and S2 correlated with BMD and G



CCA plot **bacterial communities** (DGGE fingerprints)

Effects of fish farming on the benthic microbial community – bacteria III

Influence by factors

\+ TOC and J (O_2)

Flavobacteria

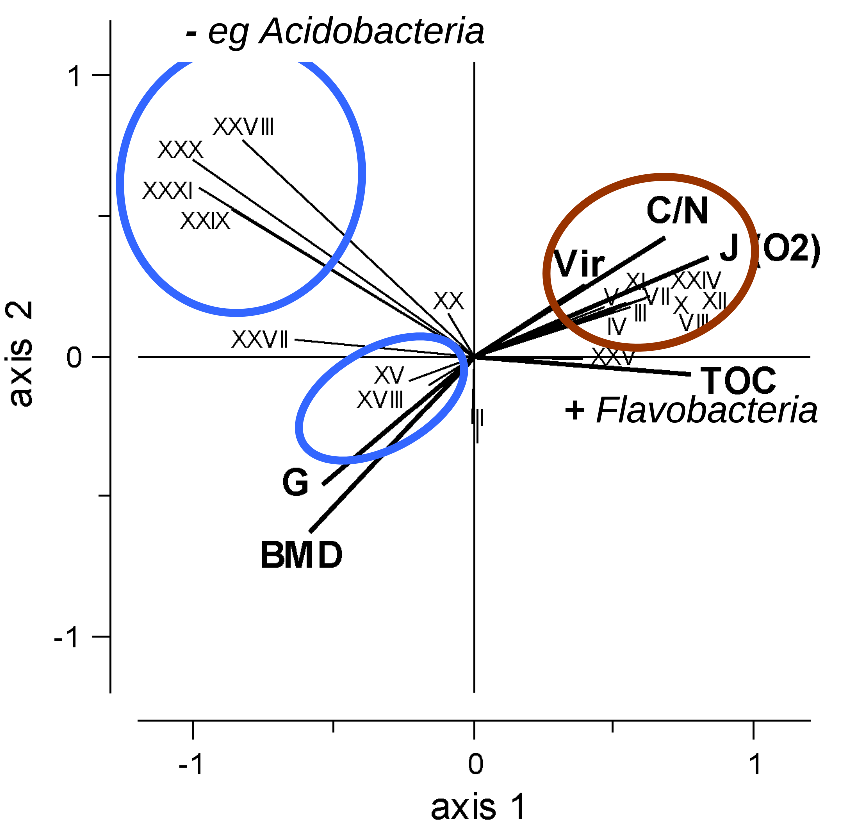
\+ G and BMD (- J(O_2))

δ- and γ-

Proteobacteria

\- TOC

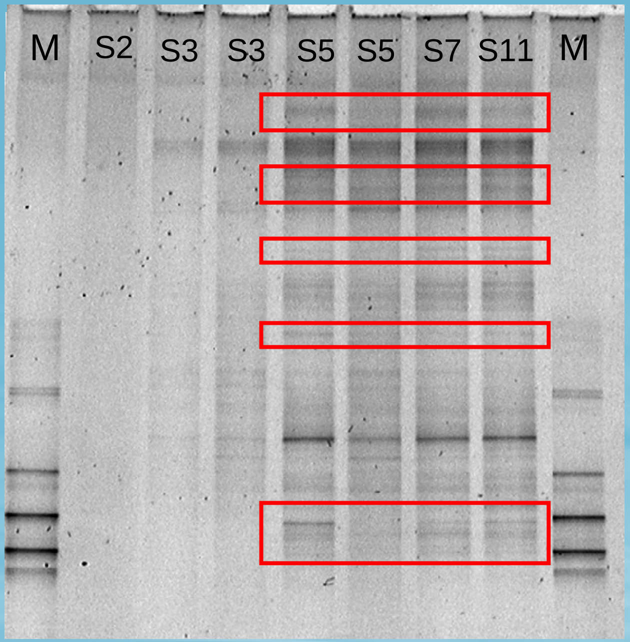
eg *Acidobacteria*



CCA plot **bacterial communities** (DGGE fingerprints)

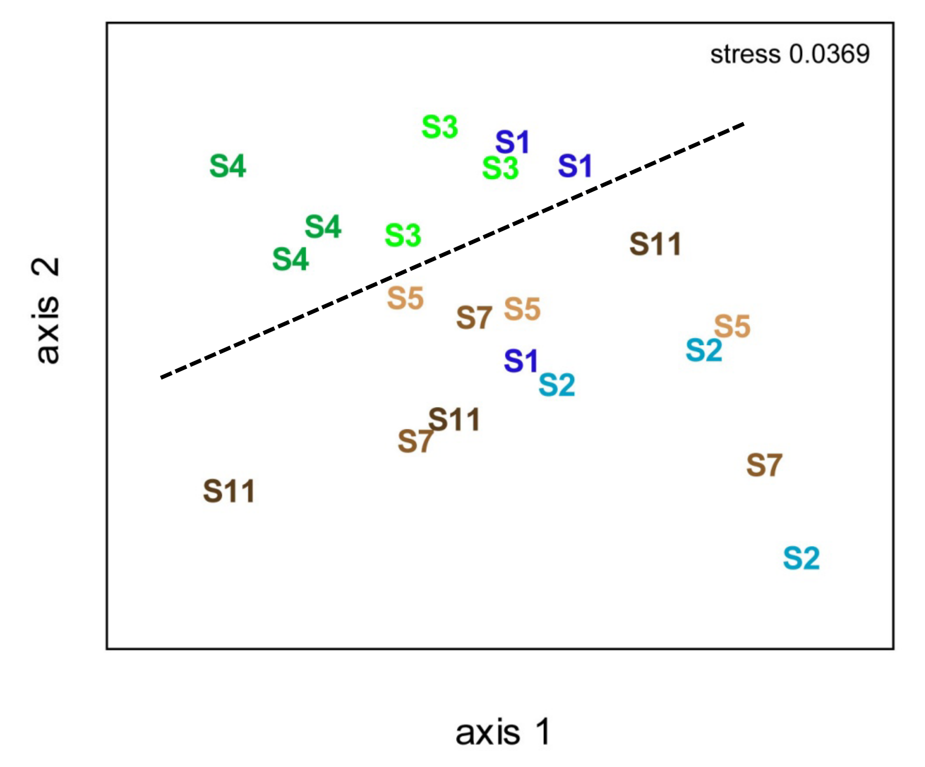
Effects of fish farming on the benthic microbial community – SRP

- Analysis of functional gene *apr A* via DGGE
  - alpha subunit of the dissimilatory APS reductase
  - APS to sulfite in sulfate-reducing prokaryotes
- Sulfate-reducing prokaryotes (δ-*Proteobacteria*) show increased diversity at impacted sites (*Desulfobacterales*)



Fingerprint of *apr A* gene: **sulfate reducing prokaryotes**

Effects of fish farming on the benthic microbial community – archaea I



nMDS plot based on Bray–Curtis similarities of **archaeal communities** (DGGE fingerprints).

- Weak gradient from less impacted conditions (S1) to high organic carbon impact (S11)
- Shift of communities in response to the eutrophication impact, S2: outliers
- Analysis of similarities (ANOSIM) with sample statistic **R=0.560** (P=0.001)

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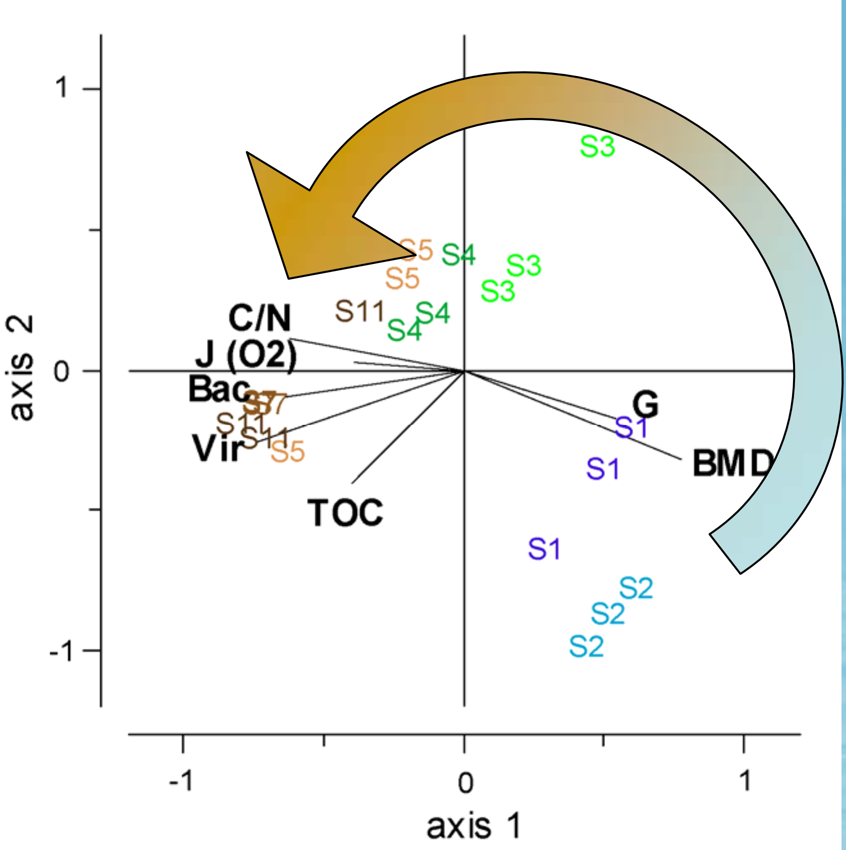
Effects of fish farming on the benthic microbial community – archaea II

- Sum of all canonical eigenvalues: **0.87**
- Monte Carlo

  F=2.4072 (p=0.001)

Community changes are driven by

- TOC
- Biogenic mixing depth



CCA plot **archaeal communities** (DGGE fingerprints)

Effects of fish farming on the benthic microbial community – archaea III

Influence by factors

+ C/N

mostly *Euryarchaeota*

+ TOC

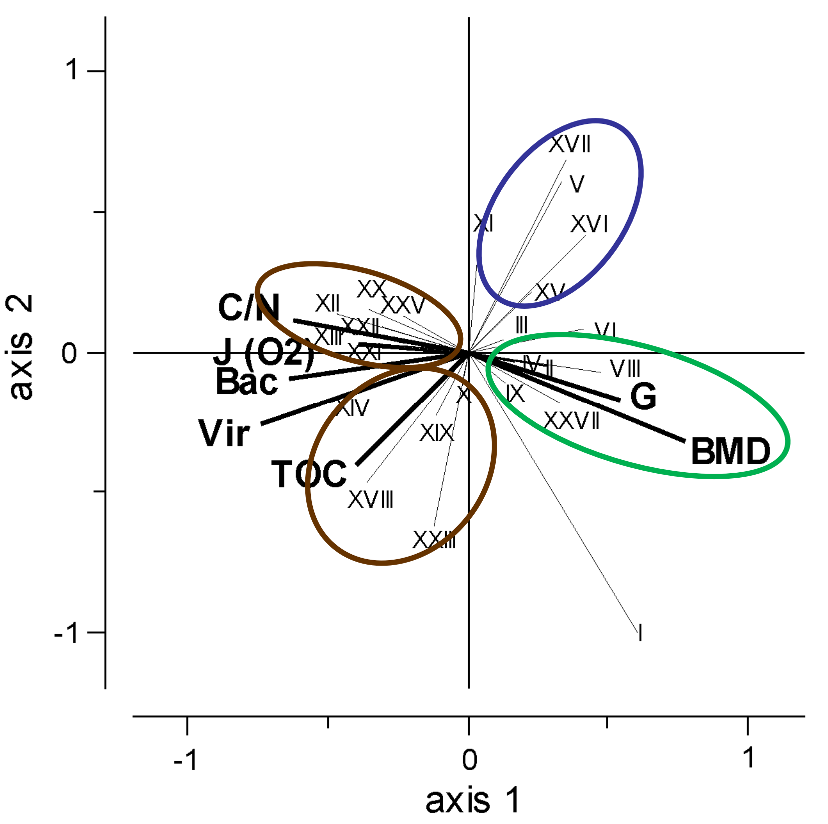
Euryarchaeota

+ BMD and G (and – C/N)

mostly *Crenarchaeota*

\- TOC

Euryarchaeota and unknown phylotypes



CCA plot **archaeal communities** (DGGE fingerprints)

Conclusions

- **Bacterial community shift** → TOC
  - Changes explained to 66%
  - *Flavobacteria* (*Bacteroidetes*) ↑, important in initial biopolymer degradation of organic matter (Bissett 2008)
  - Sulfate reducing prokaryotes (δ-*Proteobacteria, Desulfobulbaceae*) ↑, first study to show clear shift apparent at intermediate site
- **Archaeal community shift** → TOC, BMD
  - Good representation by environmental factors (87%)
  - Intermediate communities already strongly affected
  - Different phylotypes influenced by different factors
- **Recovery** of ecosystem?

Acknowledgements

Elanor M. Bell
Lois Calder (SAMS)

Eric Breuer (NOAA)

Jasmin Godbold UNIVERSITY OF ABERDEEN

Michaela Schratzberger
Freya Goodsir
Veronique Creach
Cefas

Jan Kuever
Markko Remesch
MPA Bremen
BREMEN INSTITUTE FOR MATERIALS TESTING

Thank you for your attention!