Evidence for a typical microbial community signature associated with hypoxia

Dynamics of benthic microbial communities and organic matter preservation associated with hypoxia

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Organic Matter (OM)

water column

OM

Bottom-water oxygen concentration

sediments
Benthic Ecosystem response to hypoxia

With increasing oxygen depletion the microbial size spectrum dominates benthic energy fluxes.

Any change in the **microbial community structure** may have repercussions on the flux of energy and matter through the ecosystem.
Do variations in oxygen supply cause differences in the preservation of OM and shifts in the microbial community structure?

- Biogeochemistry
- Microbiology

MICROBIAL HABITAT

Picture from inside submarine JAGO, right before the immersion in the waters of the Black Sea.

D. Donis
Campaign overall scientific aim

Response of Black Sea ecosystems to oxygen availability

MSM 15/1 cruise (Apr-May 2010)

Crimean Shelf (Black Sea)

R/V Maria S. Merian

Natural laboratory to study hypoxic conditions
Bottom-water oxygen concentration (µM)

Oxygen regimes:
- Oxic
- Oxic/hypoxic
- Anoxic/hypoxic
- Anoxic

Water column depth along the transect

10 km
Organic Matter input

Similar primary productivity in the area ~ 170 g C m\(^{-2}\) yr\(^{-1}\) (Grégoire & Beckers 2004; Yunev 2011)
Bottom-water oxygen concentration

Organic matter input
Similar surface productivity... very different ecosystem developed

**Anoxic**

**Oxic**

How does hypoxia influence OM degradation and microbial communities?
Does variations in oxygen supply cause differences in the preservation of OM?

- Total Organic Carbon (TOC)
- Pigments
- Total hydrolysable Amino acids

Ratios between amino acids
- Indicative of degradation state of POM (Dauwe et al. 1999)

Degradation Index (DI) → (lower scores) more degraded → (higher scores) less degraded

Does variations in oxygen supply cause shifts in the microbial community structure?

- Microbial abundance (AODC)
- Microbial diversity (fingerprinting and high-throughput sequencing)
More degradation under oxic conditions

The dynamic sites, with oxic and anoxic periods, resemble the preservation potential under anoxic conditions
Chlorophyll-a in surface sediments (0-1 cm b.s.f.)

- Proxy for the deposition of phytodetrital materials

→ Accumulation of phytodetrital material under variable to anoxic conditions

→ Hypoxic stations show less phytodetritus degradation at similar particle flux rates compared to the oxic stations
Degradation index (DI) in surface sediments (0-1 cm b.s.f.)

→ Based on the change in relative abundance of 20 amino acids

→ Higher degradation under permanent oxic conditions

→ More fresh material accumulated towards anoxic conditions
Anoxic sediments show higher preservation potentials compared to oxic sediments

Selective preservation of OM was found in areas characterized by dynamic or permanent hypoxia

The amino acid based degradation index shows that under oxic conditions, labile material is degraded faster than under anoxic conditions

WHY?
Canfield, Chem. Geol. (1994)

- Oxygen penetration (microelectrodes)
  - 104 m

- Oxygen dependent
- Oxygen independent

- % Carbon Preserved

- Burial rate (g cm$^{-2}$ yr$^{-1}$)
  - 0.04 g cm$^{-2}$ yr$^{-1}$

- Categories:
  - anoxic
  - anoxic/hypoxic
  - oxic/hypoxic
  - oxic

- Diagram:
  - Canfield, Math. Geol. (1994)
Total cell abundance (AODC) in surface sediments (0-1 cm b.s.f.)

\[ \text{AODC} \times 10^9 \text{ (cells cm}^{-3} \text{ sediment)} \]

- **Anoxic**
- **Anoxic/hypoxic**
- **Oxic/hypoxic**
- **Oxic**

→ Different oxygen regimes host similar microbial numbers
Increase in richness by rare types under hypoxic conditions, typical for perturbed microbial systems

→ Similar trend in other ecosystems subjected to hypoxia
Relative abundances of the most abundant bacterial families

Flavobacteriaceae dominate along the transect, consistent with its metabolic plasticity and high dominance in organic rich sediments.

Sulfate reducers (Desulfo bacteraceae and Desulfo bulbaceae) showed higher abundances when oxygen decreases → Rates of sulfate reduction detected on hypoxic to anoxic conditions.
Bottom-water oxygen concentration
Bottom-water oxygen concentration
Funding:

- hypox
- Max Planck Institute for Marine Microbiology

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TA’s from the HGF-MPG Group for Deep Sea Ecology and Technology (MPI- Bremen)

Lab GO (Lilian Nuñez, Paulina Concha)
Thank you for your attention
Sequence frequency (454 MPTS), for the most abundant archaeal classes
• oxygen penetration (oxygen microprofiles)

**104 m**

Oxygen dynamics at the Crimean Shelf

**135 m**

→ Drop of >150 µM day⁻¹ oxygen

Anna Lichtschlag et al., in preparation
35783 tags, ca. 50% singletons, 4670 OTUs

Bacteroidetes and Proteobacteria were the most abundant taxa

130 OTUs shared among oxygenation regimes - representing 50% of all sequences

Dominance of Deltaproteobacteria, Gammaproteobacteria and Flavobacteria in agreement with previous studies in ecosystems subjected to hypoxia

Prevalence of these clades along different hypoxic ecosystems suggest the central role of these taxa in sediments exposed to hypoxia
Variable conditions resemble anoxic conditions
High turnover between variable to anoxic conditions

Number of shared bacterial types (OTUs) and dissimilarity (0-1 cm b.s.f.)

- Variable conditions: 939 (0.68)
- Anoxic conditions: 437 (0.58)
- Anoxic/hypoxic conditions: 587
- Oxidized/hypoxic conditions: 457
- Oxidized conditions: 196 (0.61)
- Oxidized/hypoxic conditions: 275
- Oxidized/hypoxic conditions: 304
- Oxidized/hypoxic conditions: 300 (0.71)
Permanent oxic conditions showed lowest richness and evenness

Increase in rare types in variable conditions

Similar trend in pelagic bacterial communities from Oxygen Minimum Zones

<table>
<thead>
<tr>
<th></th>
<th>Oxic/Hypoxic</th>
<th>Oxic/Hypoxic</th>
<th>Oxic/Hypoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed number of OTUs (sobs)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>572</td>
<td>19 (18-21)</td>
<td>980 (869-1131)</td>
<td>58</td>
</tr>
<tr>
<td>1238</td>
<td>126 (116-137)</td>
<td>3002 (2678-3399)</td>
<td>41</td>
</tr>
<tr>
<td>1211</td>
<td>139 (128-152)</td>
<td>2573 (2321-2883)</td>
<td>47</td>
</tr>
<tr>
<td>894</td>
<td>44 (40-49)</td>
<td>1853 (1648-2114)</td>
<td>48</td>
</tr>
</tbody>
</table>

Bacterial diversity index based on 454 MPTS (including confidence intervals)

(OTU\textsubscript{97\%} sub sampling 4000 sequences, 0-1 cm b.s.f.)
Bacterial community structure
Non Metric Multidimensional Scaling (Bray-Curtis) of the relative abundances (OTUs)

→ Changes in bacterial community structure when data is grouped according to the oxygen dynamics

→ Permanent oxic condition is significantly different from the rest

→ Highest difference between end members (ANOSIM; $R=0.9$, $P<0.05$).

→ Oxygen regimes (categories) explains 11% of the variability ($P < 0.01$)
Total organic carbon (%) in surficial sediments (0-1 cm b.s.f.)

→ % TOC in the range of other OMZ systems

→ Effects of varying oxygenation on preservation
ARISA – **Automated Ribosomal Intergenic Spacer Analysis**

1. Environmental sample
2. DNA extraction, purification & quantification
3. 16S-ITS PCR amplification (triplicate)

**Diagram:**

```
16S rDNA  ITS 1  23S rDNA  ITS 2  5S rDNA
```

**Primers:**

- ITSF
- ITSReub

**Labeled ITS fragments:**

- **Internal standard profile**
- **Sample profile**

4. Capillary electrophoresis

Sketch: S. Schöttner
**454 MPTS** Massively parallel tag sequencing

Extracted DNA

PCR (targeting the v4-V6 region of the bacterial 16S rRNA gene)

Assemblies onto a bead + sequencing reagents

Nucleotides are flowed sequentially (TACG)

The addition of a nucleotide results in a reaction generating a light signal
Outlook

→ Group turnover from oxic to anoxic sites

→ Archaea (454 MPTS data)

→ Is the signature of oxygen variation in surface sediments preserved in deeper layers?

→ DOM variation along biogeochemical gradient

**FT-ICR-MS** (Ultrahigh-resolution mass spectrometer - 15 Tesla Fourier-Transform ion cyclotron resonance mass spectrometer)
Conclusions

Does hypoxia produce differences in the OM preservation?

→ Variations in oxygen supply cause significant differences in the preservation of OM, more OM and of biochemically better quality accumulates under variable to low oxygenation at the Crimean Shelf

Do variations in oxygen supply cause shifts in the microbial community structure?

→ Bacterial communities were influenced by spatial variation in oxygen content, increases in rare types under hypoxic conditions

→ On the Family level microbial community composition seems very similar, differences at high taxonomic level (OTUs)