# Supplementary Material

Anaerobic Hydrogen Consumption of nutrient-limited Aquifer Sediment microbial Communities examined by stable Isotope Analysis

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## Genetic Analyses

### **DNA Content**

DNA-content was measured on a Qubit Fluorometer 3.0 (Thermo Fisher, USA), shown in the following table. DNA-content was unusually low in replicate 1 of the biological control.

**Table S-1**: DNA content of all replicates and setups

|  |  |  |
| --- | --- | --- |
| Sample | Replicate | DNA [ng μl-1] |
| Measurement 1 | Measurement 2 |
| **Biological Control** | 1 | 0.055 | 0.436 |
| 2 | 8.00 | 3.68 |
| 3 | 3.75 | 1.30 |
| **Groundwater Ions** | 1 | 3.71 | 1.19 |
| 2 | 8.17 | 3.19 |
| 3 | 4.72 | 3.29 |
| **Trace Elements** | 1 | 4.20 | 1.44 |
| 2 | 28.4 | 5.09 |
| 3 | 4.73 | 3.35 |
| **Macro Nutrients** | 1 | 12.20 | 5.64 |
| 2 | 5.11 | 3.81 |
| 3 | 3.84 | 1.92 |
| Abiotic Control | 1 | n.d. | n.d. |
| 2 | n.d. | n.d. |

### **PCR programs**

The used programs for the PCR (polymerase chain reaction) of bacterial DNA and were as follows:

**Table S-2**: PCR-program for the amplification of the bacterial 16S rRNA gene

|  |  |  |
| --- | --- | --- |
| Temperature (°C) | Time (s) |  |
| 95 | 180 |  |
| 95 | 30 | 25 Cycles |
| 55 | 30 |
| 72 | 30 |
| 72 | 300 |  |

**Table S-3**: Two-step PCR-program for the amplification of the mcrA gene

|  |  |  |
| --- | --- | --- |
| Temperature (°C) | Time (s) |  |
| 95 | 180 |  |
| 95 | 20 | 4 Cycles |
| 48 | 20 |
| 72 | 15 |
| Ramp 48 - 72 |  |  |
| 95 | 20 | 24 Cycles |
| 55 | 20 |
| 72 | 15 |
| 72 | 600 |  |

### **Analysis of the mcrA gene**

The analysis of the mcrA showed that a *Methanbacterium* was dominant in all microcosms (Figure S-1).



Figure S-1: Distribution of bacterial species across biotic microcosms. Cut-off was 0.9 %. Labels are shortened according to the experimental names (SC: biological control, GW: groundwater ions, TE: trace elements, NSP: macro nutrients). Two technical replicates of each biological replicate were used. Dominating species (>90% relative abundance) was *Methanobacterium*.

## Isotope and Concentration Analyses

### Workflow

The microcosms were sampled and the samples stored in 10 ml vials flushed with He before measurement on a GC-IRMS setup.

Figure S-2: Schematic workflow from sampling to measurement with the GC-IRMS system. He: Helium, GC: Gas chromatograph, Isolink: device that contains and heats reactors for conversion of compounds; Conflo: Instrument which regulates flow into mass spectrometer; IRMS: isotope ratio mass spectrometer.

#### *Respikes*

|  |  |  |  |
| --- | --- | --- | --- |
| **Date** | **t [d]** | **amount [ml]** | **gas** |
| 23.05.2019 | 2 | 3 | H2 |
| 04.06.2019 | 14 | 3 | H2 |
| 19.06.2019 | 29 | 3 | H2 |
| 24.07.2019 | 64 | 1 | H2 |
| 31.07.2019 | 71 | 3 | H2 |
| 08.08.2019 | 80 | 3 | H2 |
| 21.08.2019 | 93 | 3 | H2 |
| 30.08.2019 | 102 | 3 | H2 |
| 02.09.2019 | 105 | 12 | H2 |
| 02.09.2019 | 105 | 1 | CO2 |

### Abiotic Controls

Concentration and isotope measurements of the abiotic control replicates are shown in Figure S-3. The isotope ratio was stable over the course of the experiment. Concentrations decrease due to continuous sampling. Slight overpressure lead to measurement of seemingly higher H2-concentrations in the beginning.



Figure S-3: δ2H- and δ13C values (A, C) and concentrations of CO2 and H2 (C,D) in the abiotic controls, shown here in two microcosm replicates (yellow, orange) as average with standard deviation of technical replicates.

### Concentration analyses

#### *CO2 and CH4*

CO2-concentrations decreased in biological replicates over time due to dissolution, loss and microbial consumption (cf. Figure S-4, Table S-4). The CO2 concentrations on day 121 are below the abiotic controls.



Figure S-4: Concentrations of CO2 of three biological replicates and two abiotic controls with standard deviation in the four setups. Onset of methane production is indicated with grey bar.

Methane concentrations varied in the different setups (Table S-4). Two groundwater ion replicates yielded no methane.

**Table S-4**: CO2 concentrations of biotic microcosm at day 0 and day 121, as well as CH4-concentrations on day 121. Shown is the average of triplicate measurements with standard deviation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Replicate** | **CCO2, t=0 [%]** | **CCO2, t=121d [%]** | **CCH4 t=121d [%]** |
| average | stdev | average | stdev | average | stdev |
| **biological control** | 1 | 12.4 | ± 0.7 | 2.2 | ± 0.1 | 3.4 | ± 0.1 |
| 2 | 14.9 | ± 0.7 | 1.9 | ± 0.1 | 2.7 | ± 0.1 |
| 3 | 13.6 | ± 1.1 | 2.4 | ± 0.1 | 3.7 | ± 0.1 |
| **ground-water ions** | 1 | 14.6 | ± 0.6 | 0.6 | ± 0.0 | n.d. |
| 2 | 13.0 | ± 0.2 | 3.8 | ± 0.2 | n.d. |
| 3 | 12.0 | ± 0.6 | 2.0 | ± 0.0 | 3.6 | ± 0.1 |
| **trace elements** | 1 | 12.9 | ± 0.3 | 5.4 | ± 0.1 | 0.2 | ± 0.0 |
| 2 | 14.1 | ± 1.3 | 3.6 | ± 0.1 | 1.8 | ± 0.0 |
| 3 | 13.7 | ± 0.6 | 3.9 | ± 0.1 | 1.6 | ± 0.0 |
| **macro nutrients** | 1 | 20.3 | ± 0.8 | 3.5 | ± 0.1 | 3.0 | ± 0.1 |
| 2 | 18.0 | ± 1.7 | 3.7 | ± 0.1 | 2.8 | ± 0.1 |
| 3 | 23.1 | ± 0.4 | 4.9 | ± 0.1 | 2.2 | ± 0.0 |

n.d.: not determined, below detection limit

#### *H2*

Before the re-spike on day 93, only a few samples contained residual H2 and on the last day of the experiment, only replicate 2 of the *groundwater ions* setup and replicate 1 of *the trace element* setup contained remaining H2 (Table (S-5).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Replicate** | **CH2, t=92d [%]** | **CH2,t=93d [%]** | **CH2, t=94d [%]** | **CH2, t=105d [%]** | **CH2, t=109d [%]** | **CH2, t=121d [%]** |
| av. | stdev | av. | stdev | av. | stdev | av. | stdev | av. | stdev | av. | stdev |
| **biological control** | 1 | 6.9 | ± 0.6 | 9.2 | ± 0.2 | 7.5 | ± 0.3 | 10.4 | ± 0.9 | 4.8 | ± 0.5 | n.d. |
| 2 | n.d. | 2.0 | ± 0.1 | 0.6 | ± 0.0 | 5.6 | ± 0.5 | n.d. | n.d. |
| 3 | n.d. | 1.3 | ± 0.1 | 0.7 | ± 0.1 | n.d. | 0.9 | ± 0.1 | n.d. |
| **ground-water ions** | 1 | n.d. | 7.2 | ± 0.2 | 4.4 | ± 0.2 | 14.0 | ± 1.5 | n.d. | n.d. |
| 2 | n.d. | 6.7 | ± 0.3 | 1.6 | ± 0.0 | 14.4 | ± 3.3 | 8.8 | ± 0.9 | 9.3 | ± 0.9 |
| 3 | 3.1 | ± n.d. | 6.5 | ± 0.6 | 1.7 | ± 0.1 | 16.1 | ± 1.4 | 2.8 | ± 0.3 | n.d. |
| **trace elements** | 1 | 6.6 | ± 0.2 | 15.0 | ± 0.9 | 11.4 | ± 0.3 | 5.9 | ± 0.4 | 7.2 | ± 1.1 | 5.9 | ± 0.4 |
| 2 | 9.4 | ± 0.3 | 17.0 | ± 0.8 | 11.7 | ± 0.6 | 4.3 | ± 0.6 | n.d. | n.d. |
| 3 | 5.0 | ± 0.1 | 12.8 | ± 0.2 | 7.1 | ± 0.5 | 16.2 | ± 0.8 | 5.7 | ± 0.1 | n.d. |
| **macro nutrients** | 1 | n.d. | 7.1 | ± 0.8 | 2.7 | ± 0.2 | 11.1 | ± 0.9 | n.d. | n.d. |
| 2 | n.d. | 6.9 | ± 0.5 | 2.4 | ± 0.2 | 4.4 | ± 0.7 | n.d. | n.d. |
| 3 | n.d. | 6.8 | ± 0.2 | 2.5 | ± 0.2 | 7.6 | ± 0.1 | n.d. | n.d. |
|  |  |  |  | re-spike |  |  | re-spike |  |  | end |

**Table S-5**: Average concentration of H2 of three biological replicates with standard deviation in the four different setups. Setups were spiked again at days 92 and 105, respectively. Samples are shown in percent of headspace volume.

n.d.: not determined, below detection limit

## General Coping Mechanisms for Nutrient Limitations

Microbes often carry optimised proteins and enzymes, which require less potentially limited elements. Additionally, the entire genome is never expressed, which enables structural and functional adjustment. Still, natural environments are characterised by the available nutrients, which implies lack of others. There are four general strategies followed by microorganisms when faced with nutrient limitations: acquisition, mobilisation, sparing and recycling [1]. The first step is usually change in *acquisition* of the limited element or compound. Here, additional uptake systems are employed. Low-affinity uptake systems are replaced by high-affinity ones that come with higher energy demand [2–4]. At the same time, stored resources are *mobilised*. If these measures were not effective, *sparing* is the next step. This is an umbrella term for austerity measures, which aims to reduce cellular demand. Here, non-essential proteins and macromolecules might be selectively repressed or the limited element might be substituted [1,3]. The last measure microorganisms can employ is *recycling*. Elements are recovered via autophagy. When limitations are lifted and the nutrient is available again, catabolisation can be fast [3].

# References

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[4] Sim MS, Ono S, Bosak T. Effects of iron and nitrogen limitation on sulfur isotope fractionation during microbial sulfate reduction. Appl Env Microbiol. 2012;78:8368–8376.