**Supporting information**

**Geochemical and microbial community determinants of reductive dechlorination at a site biostimulated with glycerol**

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**Sampling procedure**

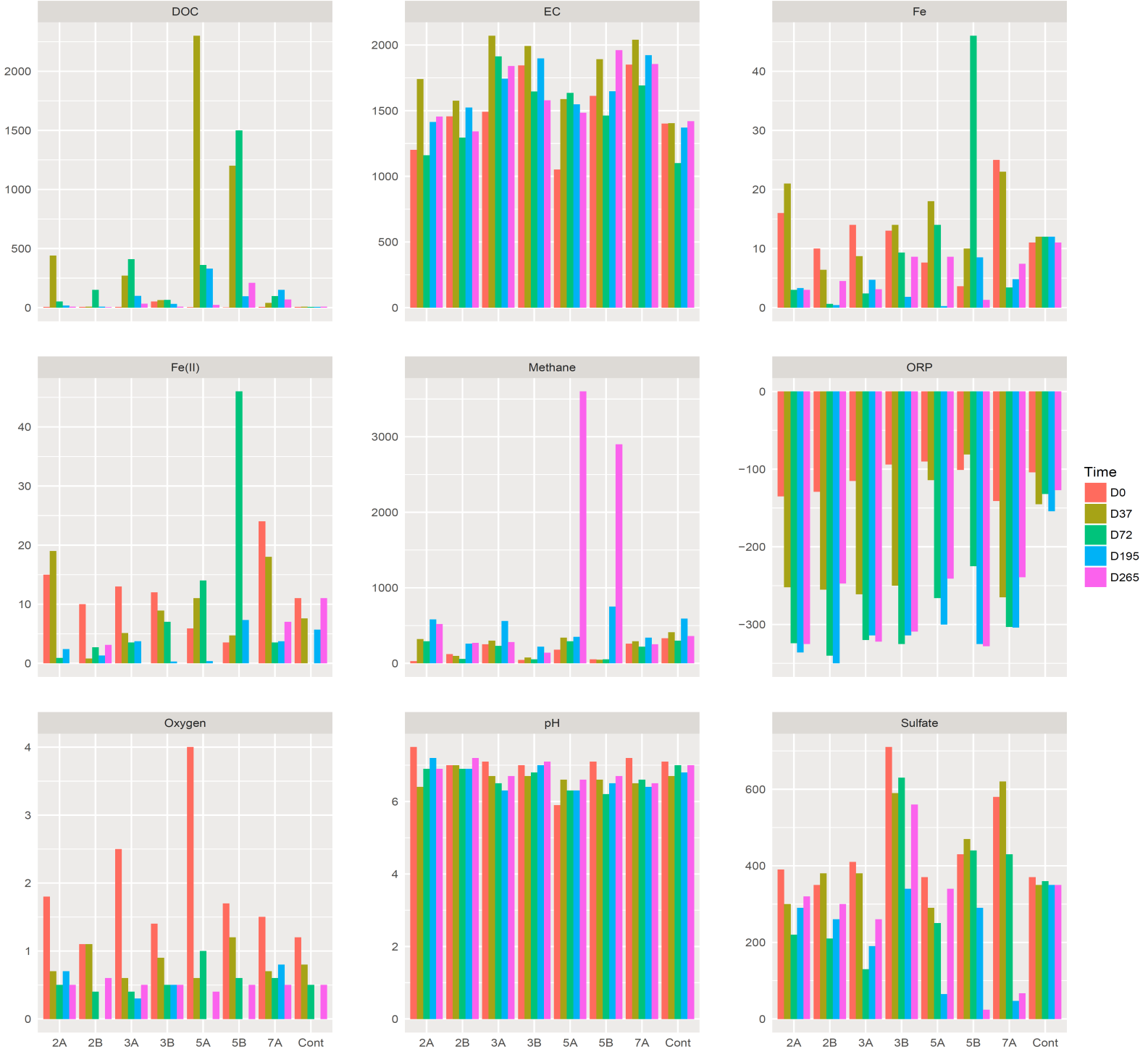
All samples were collected using a peristaltic pump in polyethylene tubes (Eijkelkamp, Giesbeek, The Netherlands), following purging until electrical conductivity, pH, dissolved oxygen (DO), temperature and oxidation-reduction potential (ORP) parameters stabilized. Those parameters were measured using a flow-through cell (Eijkelkamp, Giesbeek, The Netherlands), equipped with a multimeter (MultiLine F/SET3, WTW, Weilheim, Germany) and suitable electrodes for determining temperature and electrical conductivity (TetraCon 325, WTW, Weilheim, Germany), pH (Sen Tix 41, WTW, Weilheim, Germany), DO (CellOx 325, WTW, Weilheim, Germany) and ORP (Oxitrode Platinum Hamilton, Bonaduz, Switzerland). Samples for stable carbon isotope analysis were collected in 250 mL amber bottles without leaving headspace, and immediately treated with NaOH pellets to reach a pH above 11 to stop microbial activity.

## Carbon isotope analysis

The stable carbon isotope composition of chlorinated ethenes (CEs) in the groundwater samples were determined on a gas chromatograph-combustion-isotope ratio mass spectrometer (TRACE GC Ultra, GC combustion interface, MAT 253, all from Thermo Fisher Scientific, Bremen, Germany). Each sample was analyzed in duplicate via purge-and-trap, cryofocussing and subsequent analysis on a VOCOL 60 m x 0.25 mm ID. Authentic laboratory standards were used for identification of chlorinated compounds and to improve the accuracy of 13C/12C analyses by linear correction of raw isotope values. Isotope ratios (R) of samples are expressed in the delta notation (δ13C) in per mil [‰] relative to the international Vienna Pee Dee Belemnite standard (VPDB, 13C/12C = (11237.2 ± 2.9) × 10‑6) according to:



**Fig. S1.** Groundwater geochemical parameters. Scales on *Y*-axes are: dissolved organic carbon (DOC) (mg/L), electrical conductivity (EC) (µS/cm), Fe (mg/l), Fe(II) (mg/l), methane (µg/l), oxidation-reduction potential (ORP) (mV), oxygen (mg/l), sulfate (mg/l). Cont: control well.



**Fig. S2.** Predicted (Chao 1) and observed OTU richness, and phylogenetic diversity at each filter over time (five samples per filter). D: day; Cont: control well.

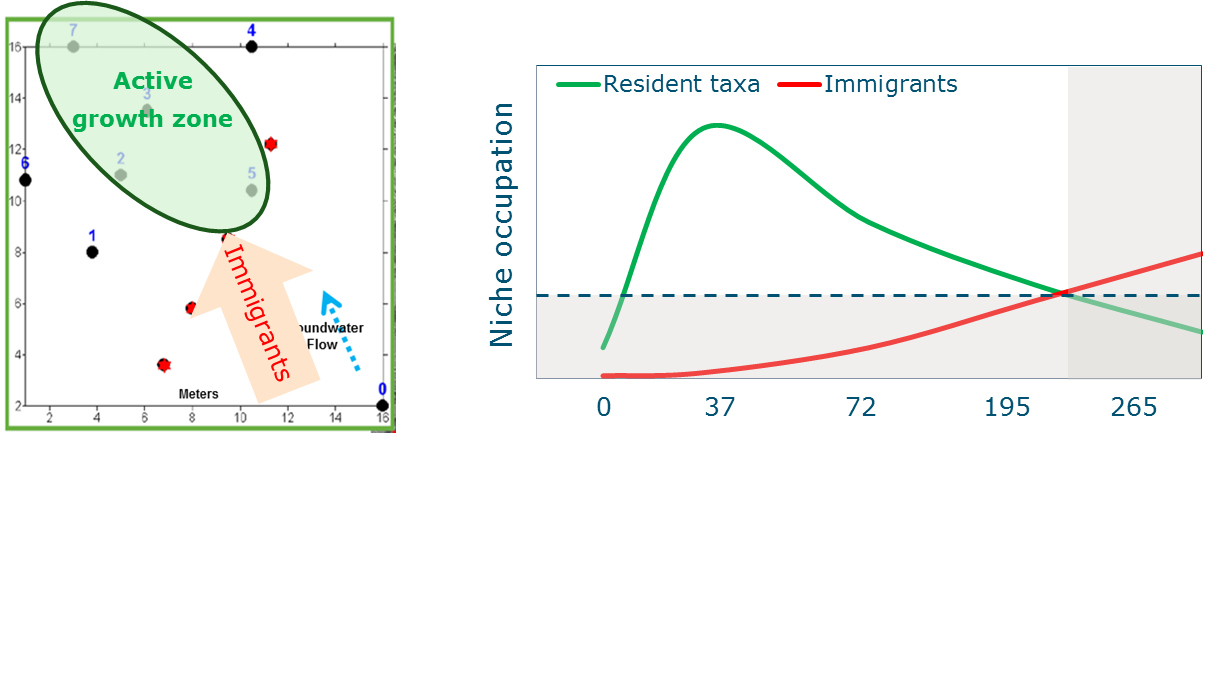


**Fig. S3.** Ordination of community composition by nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distances. D: day; Cont: control well.

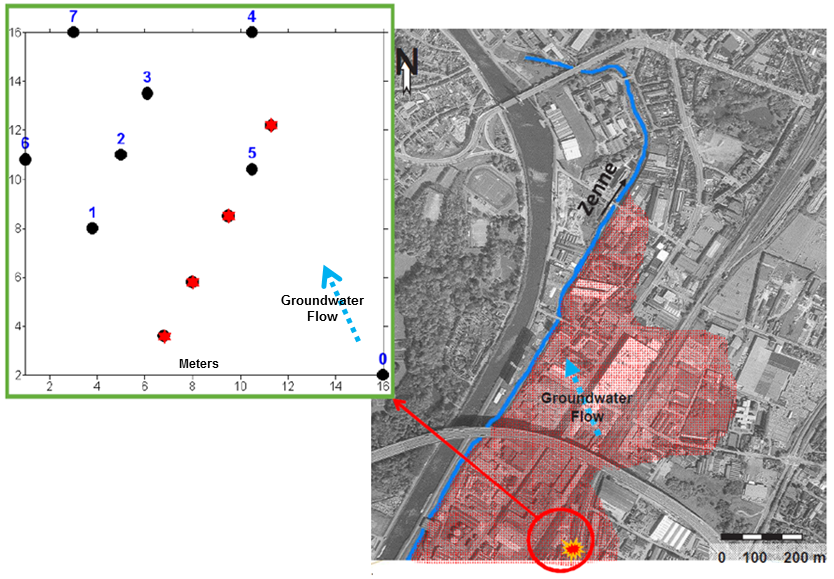


**Fig. S4.** Distribution of the most abundant phyla (> 1% average relative abundance across all samples, left panel) and genera (> 0.7% average relative abundance across all samples, right panel) in the control (Cont) and impacted filters. D: day.

**Fig. S5.** Niche occupation concept. According to phylogenetic instability in the control well (well 0 in left figure), groundwater brings in immigrant microbes (the arrow from the control well towards the stimulated zone or active growth zone). The resident taxa in the stimulated wells exploit the stimulated conditions and occupy the available niches in the active growth zone. However, they were exposed to the newly arriving taxa brought by natural groundwater flow but it is only towards the end of the experiment (and hence fading stimulation) that the immigrants pass the threshold after day 195 (dashed line in right figure) and become established in the stimulated wells by day 265.



**Fig. S6.** Schematic presentation of the study site close to the Zenne River. Monitoring wells (black circles) and injection wells used for glycerol delivery (red stars) at the site are indicated. Well 0, located upstream of the injection wells, is taken as control. Samples were taken from the monitoring wells impacted by glycerol injection (shown in the dashed oval) i.e. well 2, 3, 5 and 7 (only shallow filter, 7A) as well as for the control well throughout the study.



**Table S4.** Significance tests of the differences of microbial communities at order level. The groups with no significant differences (*P*>0.05) are shown in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample groups | PERMANOVA a | |  | ANOSIM b | |
| *F* | *P* |  | *R* | *P* |
| Control vs Day 0 | 2.0930 | 0.0090 |  | 0.3862 | 0.0101 |
| Control vs Day 37 | 1.5131 | 0.0480 |  | 0.2535 | 0.0404 |
| Control vs Day 72 | 2.0912 | 0.0007 |  | 0.5631 | 0.0013 |
| Control vs Day 195 | 1.8299 | 0.0218 |  | 0.3991 | 0.0215 |
| **Control vs Day 265** | **1.1284** | **0.2397** |  | **0.1152** | **0.1301** |
| Day 0 vs Day 37 | 3.0799 | 0.0001 |  | 0.5607 | 0.0006 |
| Day 0 vs Day 72 | 4.8358 | 0.0004 |  | 0.9485 | 0.0006 |
| Day 0 vs Day 195 | 4.6262 | 0.0007 |  | 0.8387 | 0.0006 |
| Day 0 vs Day 265 | 2.9800 | 0.0001 |  | 0.5267 | 0.0006 |
| **Day 37 vs Day 72** | **1.4551** | **0.0717** |  | **0.1633** | **0.0793** |
| Day 37 vs Day 195 | 2.0512 | 0.0200 |  | 0.3848 | 0.0192 |
| Day 37 vs Day 265 | 1.6617 | 0.0180 |  | 0.2459 | 0.0146 |
| **Day 72 vs Day 195** | **1.3986** | **0.1152** |  | **0.1118** | **0.1166** |
| Day 72 vs Day 265 | 2.1443 | 0.0004 |  | 0.4956 | 0.0006 |
| **Day 195 vs Day 265** | **1.529** | **0.0651** |  | **0.1681** | **0.0810** |
| a Permutational multivariate analysis of variance using distance matrices. Significance tests were performed by *F* test based on sequential sums of squares from permutations of the raw data.  b Analysis of similarities. Statistic *R* is based on the difference of mean ranks between groups and within groups. | | | | | |

**Table S5.** Overview of primer sequences and thermal cycling conditionsused for qPCR in this study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Primer | Oligonucleotide sequence (5´-3´) | Thermal profile | Number  of cycles | Target | Reference |
| Dco728F | AAGGCGGTTTTCTAGGTTGTCAC | 95 °C 10 min | 1 | *Dcm* | (Smits *et al* 2004) |
| Dco944R | CTTCATGCATGTCAAAT | 95 °C 15 s, 50 °C 30 s, 72 °C 30 s | 40 |  |  |
|  |  |  |  |  |  |
| Vcr1022F | CGGGCGGATGCACTATTTT | 95 °C 10 min | 1 | *vcrA* | (Ritalahti *et al* 2006) |
| Vcr1093R | GAATAGTCCGTGCCCTTCCTC | 95°C 15 s, 58°C 60 s | 40 |  |  |
|  |  |  |  |  |  |
| Bvc925F | AAAAGCACTTGGCTATCAAGGAC | 95 °C 10 min | 1 | *bvcA* | (Ritalahti *et al* 2006) |
| Bvc1017R | CCAAAAGCACCACCAGGTC | 95°C 15 s, 58°C 60 s | 40 |  |  |
|  |  |  |  |  |  |
| TceA1270F | ATCCAGATTATGACCCTGGTGAA | 95 °C 10 min | 1 | *tceA* | (Ritalahti *et al* 2006) |
| TceA1336R | ATCCAGATTATGACCCTGGTGAA | 95°C 15 s, 60°C 60 s | 40 |  |  |

**Table S6.** Overview of MiSeq primers and Unitags and thermal cycling conditions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primers and Unitags | | | Thermal cycling conditions | |
| Primera | Oligonucleotide sequence (5´-3´)b | Reference | PCR step | Thermal profile |
| 27F-DegS | GTTYGATYMTGGCTCAG | (van den Bogert et al 2011) | PCR-1 | 98°C for 30 s  25 cycles of denaturation at 98°C for 10 s, annealing at 56°C for 20 s and elongation at 72°C for 20 s  final extension at 72°C for 10 min |
| 338R-I | GCWGCCTCCCGTAGGAGT | (Daims *et a*l 1999) |
| 338R-II | GCWGCCACCCGTAGGTGT | (Daims *et al* 1999) |
| Unitag1 | GAGCCGTAGCCAGTCTGC | (Tian *et al*, under review) | PCR-2 | 98°C for 30 s  5 cycles of denaturation at 98°C for 10 s, annealing at 52°C for 20 s and elongation at 72°C for 20 s  final extension at 72°C for 10 min |
| Unitag2 | GCCGTGACCGTGACATCG | (Tian *et al*, under review) |

a Primer names may not correspond to original publication

b M = A or C; R = A or G; W = A or T; Y = C or T

**References**

Daims H, Brühl A, Amann R, Schleifer KH, Wagner M (1999). The domain-specific probe EUB338 is insufficient for the detection of all bacteria: Development and evaluation of a more comprehensive probe set. *Syst Appl Microbiol* **22:** 434-444.

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van den Bogert B, de Vos WM, Zoetendal EG, Kleerebezem M (2011). Microarray analysis and barcoded pyrosequencing provide consistent microbial profiles depending on the source of human intestinal samples. *Appl Environ Microbiol* **77:** 2071-2080.