

Supporting Information

Microbial Oxidation of Pyrite Coupled to Nitrate Reduction in Anoxic Groundwater Sediment

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Contains 6 pages: Figure S1; Figure S2; Figure S3; Table S1

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Figure S1

JØRGENSEN et al., 2008: Microbial Oxidation of Pyrite Coupled to Nitrate Reduction in Anoxic Groundwater Sediment

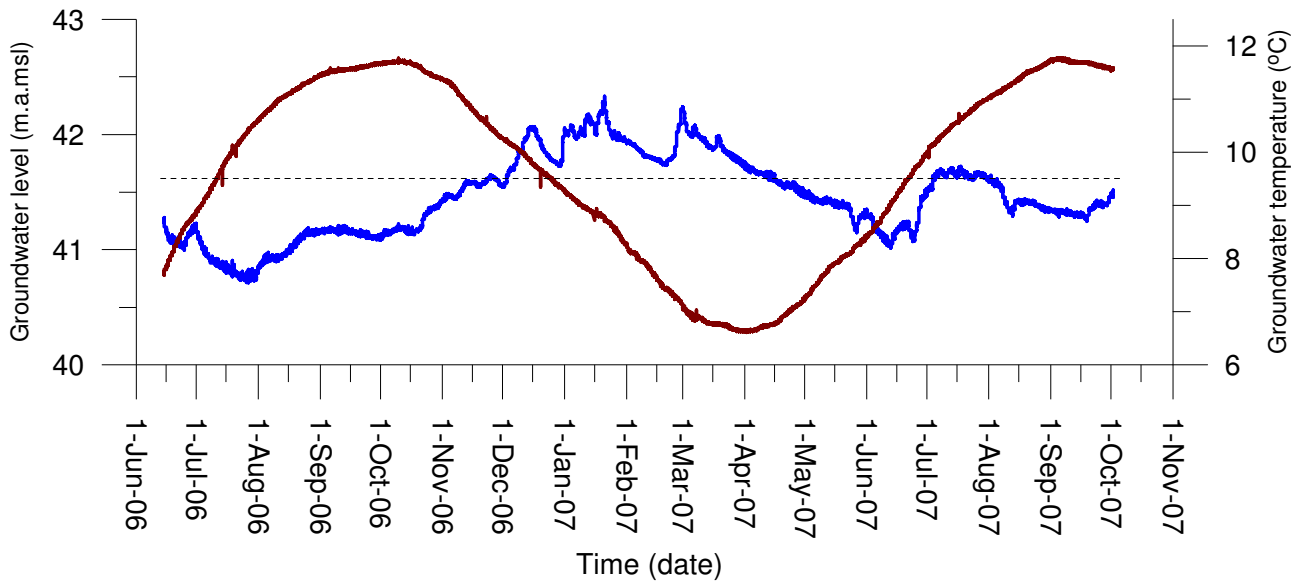


Figure S1: Daily values of groundwater level (blue line) and groundwater temperature (red line) at well P1 at Fladerne Bæk field site in the period 15. June 2006 to 1. October 2007. Ground surface is located at 43.6 meter above mean sea level (m.a.msl.) Groundwater temperature was measured at elevation 40.3 m.a.msl. Mean groundwater level during the period was 41.7 m.a.msl. and mean groundwater temperature was 9.3 °C as indicated by the dotted line.

Figure S2

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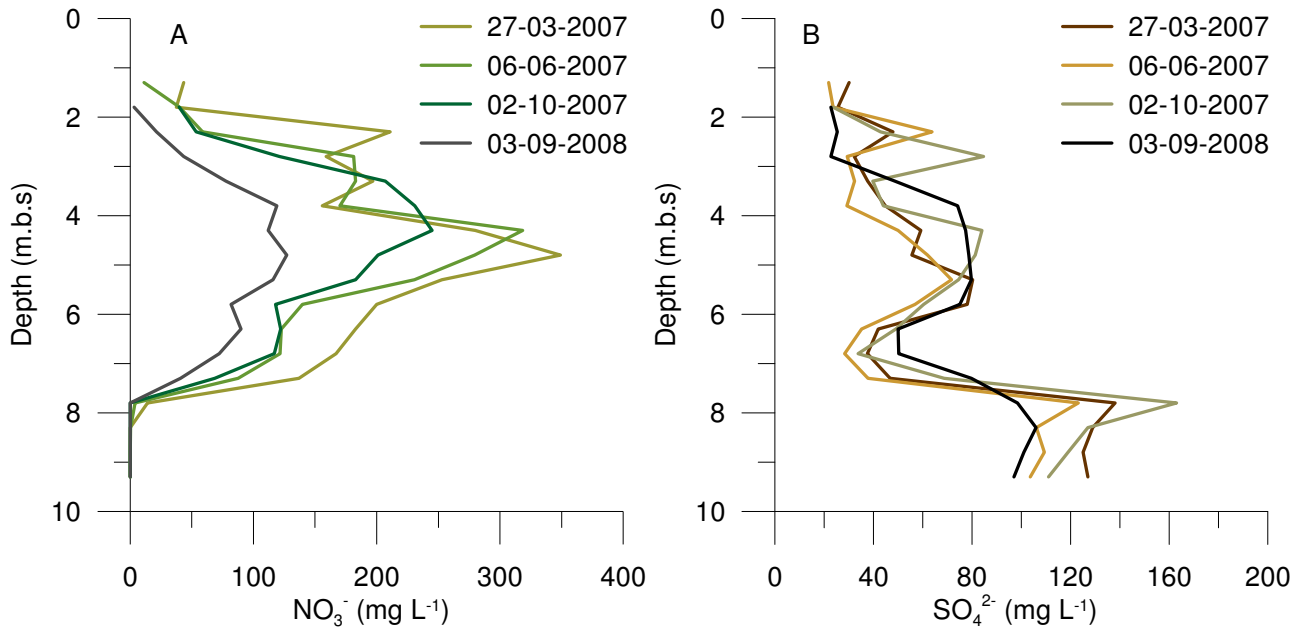


Figure S2 A & B: Groundwater nitrate (NO_3^-) and sulfate (SO_4^{2-}) concentrations at 4 different points in time. X-axis show element concentrations and y-axis show depth in meter below surface. Supporting figure 2 A (left) shows marked variability in NO_3^- concentrations over time and may be explained by both groundwater flow and/or nitrate reduction in the aquifer. A sharp decrease in NO_3^- concentrations is observed in all four profiles at approximately 8 meters below surface terrain corresponding to the depth where natural pyrite appears. Systematic increases in sulfate concentrations are observed in supporting figure 2 B (right) at the exact same depth as where nitrate disappears.

Figure S3

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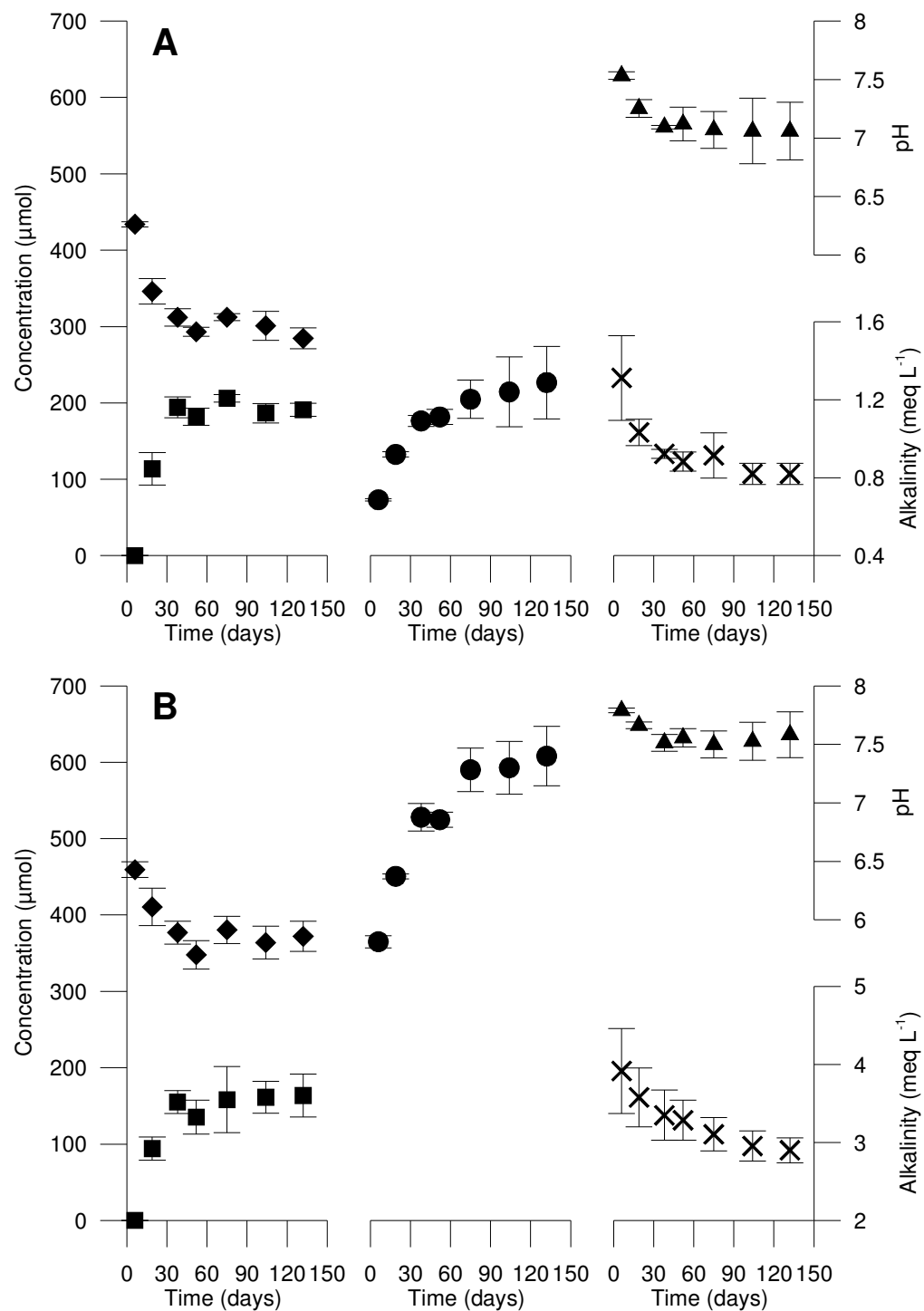


Figure S3: Preliminary results on anoxic pyrite oxidation coupled to nitrate reduction by pure culture strain *Thiobacillus Denitrificans* (DSM 12475, www.DSMZ.de) in (A) sterile Milli-Q solution and (B) sterile tap water solution at pH 7. Diamonds = NO_3^- , squares = NO_2^- , dots = SO_4^{2-} , triangles = pH and crosses = alkalinity. The facultative anaerobe chemolithoautotroph bacterial strain DSM 12475 *Thiobacillus denitrificans* was purchased as an actively growing culture at the German Collection of Microorganisms and Cell Cultures. All handling of the facultative anaerobes were carried out in an anaerobe glove box with a palladium catalyst. The bacteria were inoculated anoxically in sterile thiosulfate maintenance medium 113 at 30 °C and pH 7.0. A sterile glass bottle with a volume of 585 ml with a butyl rubber septum and aluminum crimp was used as growth reactor. The MilliQ water and tap water pH 7.0 (tap water adjusted to pH 7 with 0.1M HCl) reactors consist of 585 ml glass bottles added 450 ml water which was sterilized and deoxygenated by autoclaving at 121 °C 15 psi. for 30 minutes. One gram of sterile acid washed pyrite ($0.37 \text{ m}^2 \text{ g}^{-1}$) was subsequently added along with $50 \text{ mg L}^{-1} \text{ NO}_3^-$, 25 ml of thiosulfate omitted medium 113 along with 0.75 ml of *Thiobacillus denitrificans* pure culture solution. The sterile growth medium was only injected into the reactors at the start of the experiment and not replenished during the course of the incubation period. All experiments were conducted in triplicate and results are shown as mean values \pm standard deviation of the mean. The SI figures 3 A & B indicate decreasing nitrate concentrations with more or less congruent sulfate and nitrite productions. Decreases of pH and alkalinity are noticed over time in both figures which likely reflects the consumption of the inorganic carbon from the dissolved bicarbonate by the bacteria during growth. The elevated sulfate and alkalinity concentrations in SI figure 3B are due to natural tap water concentration of these solutes. The decreasing reaction rates most likely reflect the relative depletion of growth medium concentration over time where one or more of the growth medium element in the scarcest supplies will be rate limiting. This assumption could be tested by either a continuous replenishment of nutrients over time or by a stepwise addition or removal of a single element or compound at a time. Another factor which has the potential of limiting bacterial respiration and/or growth could be the release of biotoxic trace metals incorporated in the pyrite (see SI table 1). A third factor which could limit the reduction of nitrate to less oxygenized nitrogen compounds is the production of specific enzymes used in the denitrification process, such as nitrate reductase, nitrite reductase, nitric oxide reductase and so forth. If one or more of these catalyzing enzymes cannot be produced the reduction sequence will stop and a build up of denitrification intermediates will build up. In the experiment, something is apparently inhibiting the phase shift and further reduction of aqueous nitrite. The preliminary results indicate however that *Thiobacillus denitrificans* is able to utilize pyritic sulfide S_2^{2-} for the reduction of nitrate to nitrite.

Table S1

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Element	Content (wt. %)	Element	Content (ppm)	Element	Content (ppm)
Fe	48	Pb	350	Cu	15
S	50	Zn	6300	Mn	40
Σ Silicates	1	Ni	< 5	As	40

Table S1: Chemical composition of the pyrite used in the incubation experiment.