Supplementary Information

Methods

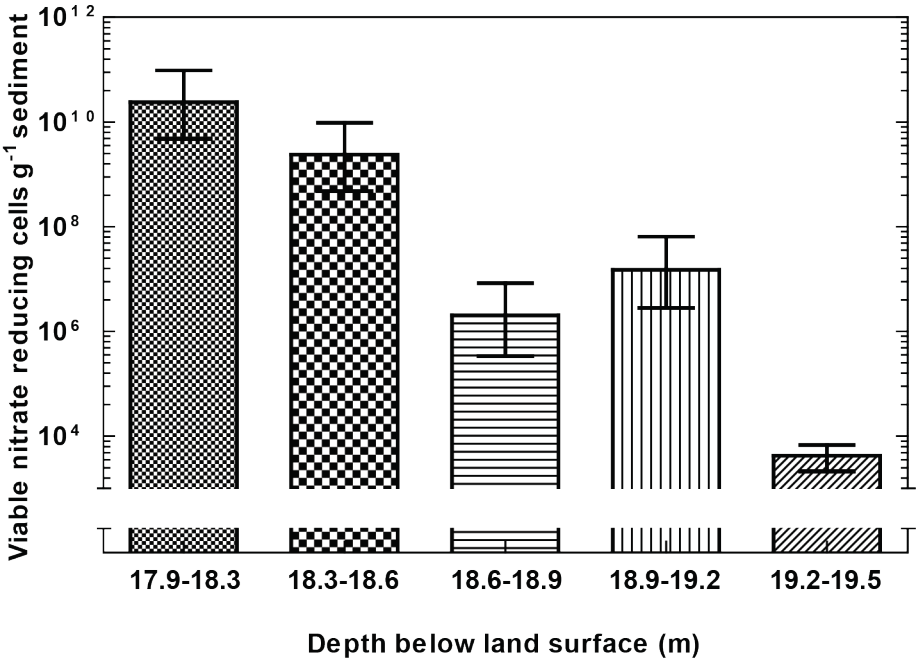
Most Probable Number (MPN) enumerations

MPN enumerations of core sections at various depth intervals were set up to enumerate the viable members of the heterotrophic nitrate reducing community (Weber et al. in prep). From the zone within the aquifer demonstrating the most abundant nitrate reducing microbial community a detailed MPN enumeration was conducted at 0.3m intervals. One gram of sediment was serially diluted 9mL of anoxic (headspace N_2:CO_2; 80:20) PIPES buffered (20mM, pH 6.4) artificial groundwater medium representing groundwater geochemistry (Snow 1996); (0.235g L^{-1} KH_2PO_4, 0.09g L^{-1} K_2HPO_4, 0.1g L^{-1} NH_4Cl, 0.26mM $MgCl_2$, 1.27mM $CaCl_2$. and 5ml/L of vitamin and mineral mix (Lovley and Phillips 1988) amended with acetate (final concentration 5mM), lactic acid (final concentration 6.8mM), and nitrate (final concentration 10mM). Prior to serial dilution, triplicate samples were amended with anoxic sodium pyrophosphate (0.1% final concentration) and gently shaken at room temperature for 1 hr. Tubes were statically incubated in the dark at room temperature for 8 weeks prior to enumeration. Tubes positive for nitrate reduction were determined by quantifying loss of nitrate from the culture medium relative to uninoculated controls. The Most Probable Number Calculator version 4.05 (Albert J. Klee, Risk Reduction Engineering Laboratory, US EPA [http://www.epa.gov/nerlcwww/other.htm]) was used to enumerate the nitrate reducing microbial community and calculate confidence limits.

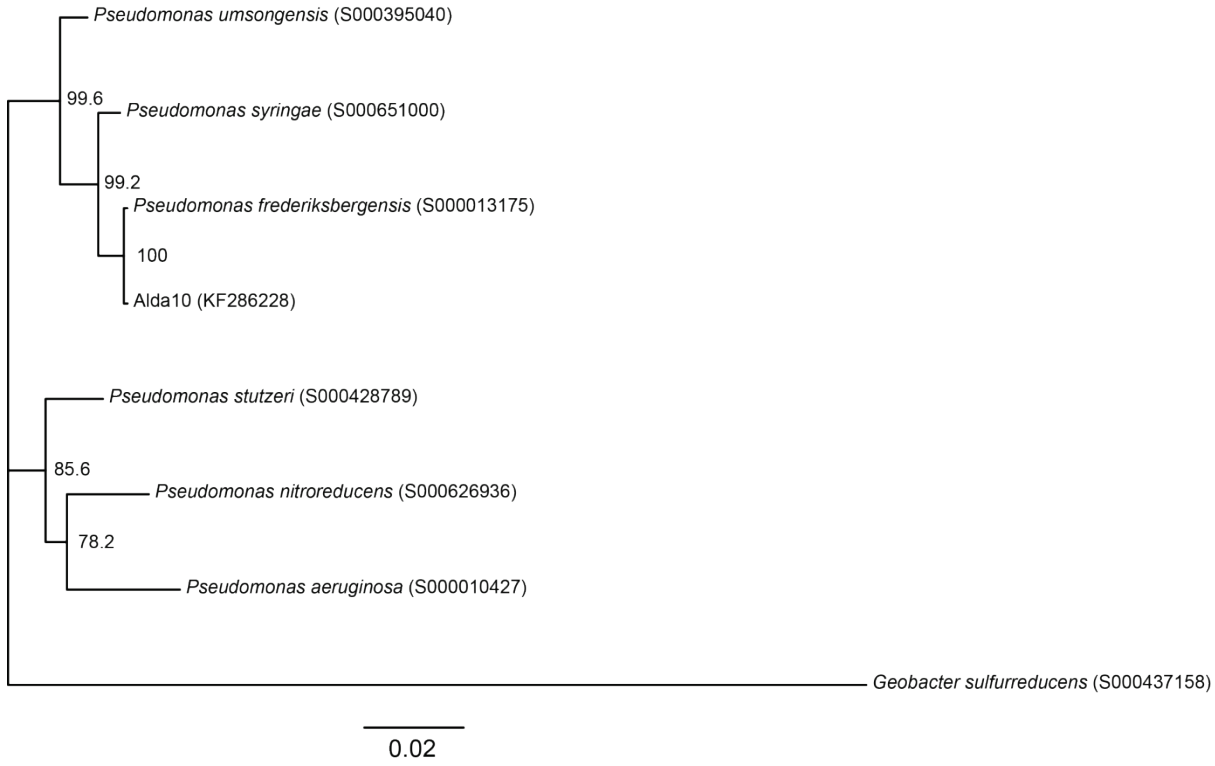
Isolation of nitrate reducing microorganism and analysis of putative VLPs

Subsurface sediment from a nitrate contaminated aquifer (30-37 mg/L) harbored an abundant heterotrophic nitrate reducing microbial community ($4.3 \times 10^3 - 2.4 \times 10^{10}$ cells g^{-1} sediment)

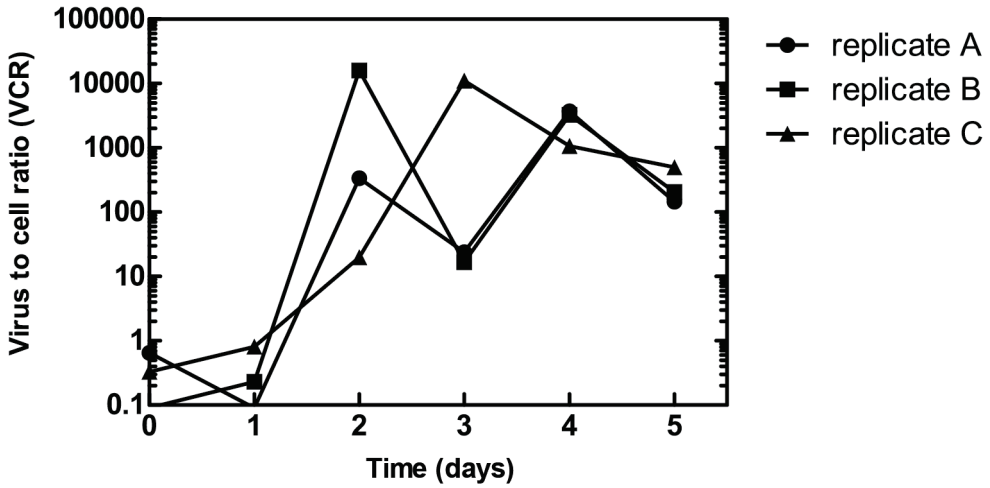
(Supplementary Figure S1). The region exhibiting the highest abundance of nitrate reducing bacteria (17.9 – 18.6m below land surface) was selected for isolation of a heterotrophic nitrate reducing bacterium. Isolation of colony isolates were obtained from serial dilutions of positive MPN tubes amended with anoxic noble agar (final concentration 0.7%). Ten colonies were isolated in an anaerobic glovebag (Coy; Grass Lake, MI, USA). All isolated colonies were identified as *Pseudomonas frederiksbergensis* with 99% 16S rRNA gene sequence similarity and one isolate was selected for further study.



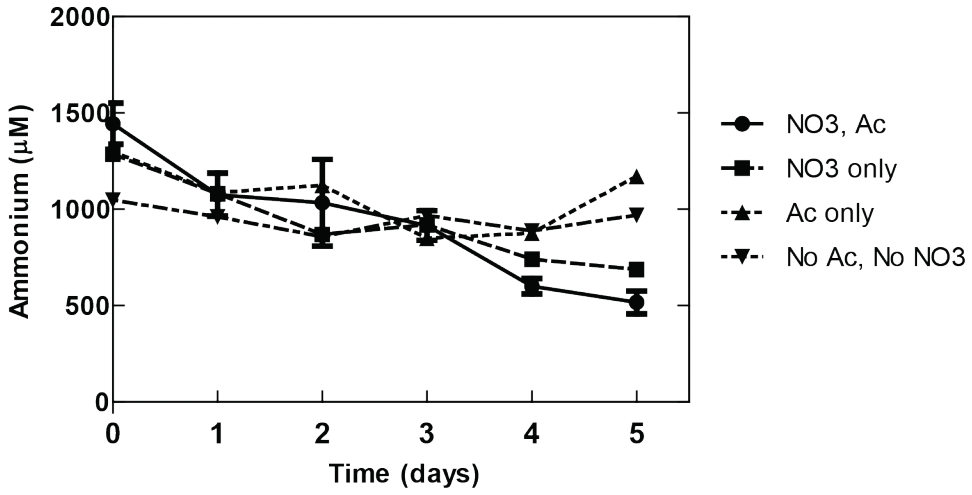
Supplementary Figure S1. Most probable number enumeration of viable heterotrophic nitrate reducing cells from sections of the collected sediment core from 17.9 – 19.5m below surface. Error bars represent 95% confidence intervals.



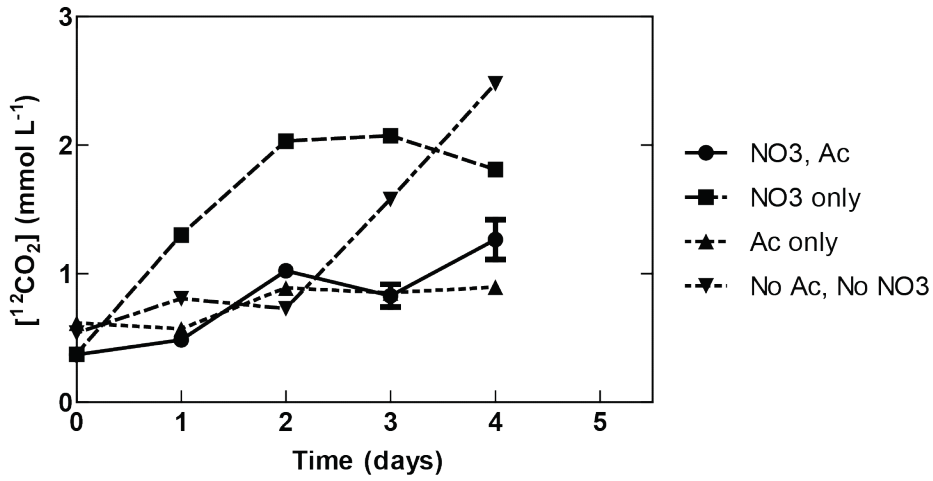
Supplementary Figure S2. Neighbor-joining phylogenetic tree of near complete 16S rRNA gene sequences from a nitrate-reducing bacterium, *Pseudomonas* sp. Alda10, isolated from the most probable number dilution series and closely related *Pseudomonas* spp.. *Pseudomonas* Alda10 is closely related to *P. frederiksbergensis* (99% 16S rRNA gene sequence similarity). *Geobacter sulfurreducens* was selected as the outgroup. Sequences were aligned using ClustalW, and distances were calculated using the Jukes-Cantor model. Tree was bootstrapped with 1000 subsamples. Accession numbers appear after species names.



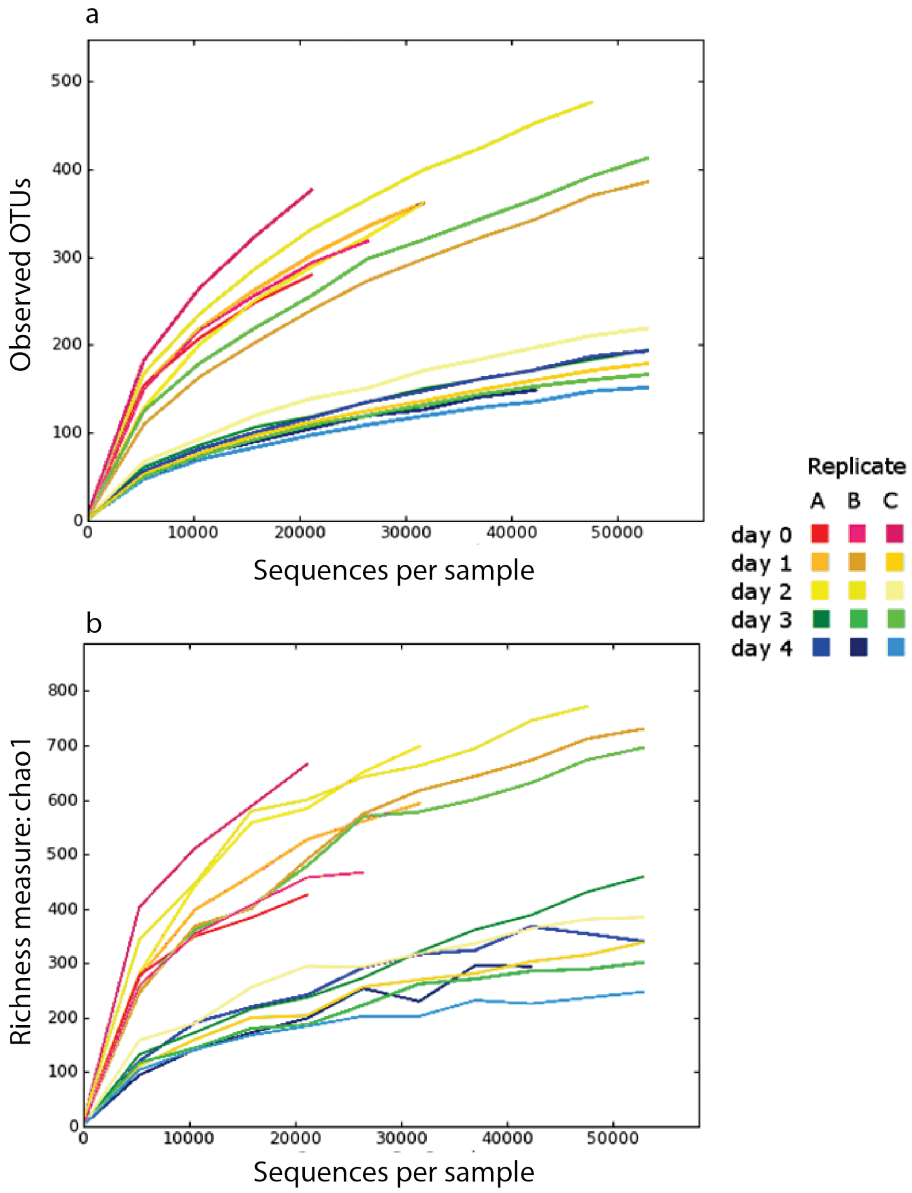
Supplementary Figure S3. Individual replicate treatments of the virus to cell ratio (VCR) (Figure 1f) over the course of acetate and nitrate stimulation. Variability was observed due to differences in the rate of response of a single replicate treatment that exhibited a one day lag.



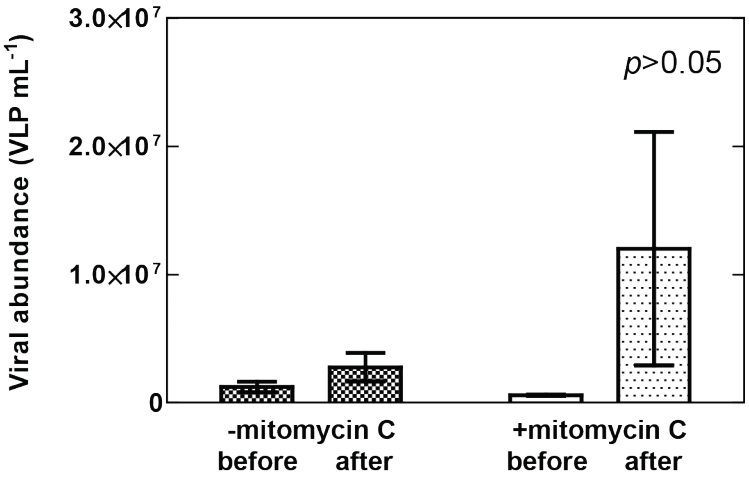
Supplementary Figure S4. Ammonium concentrations over the course of acetate and nitrate stimulation (Figure 1), sediment slurries demonstrate ammonium consumption rather than production. Slurries were amended with 10mM ^{13}C-acetate and 10mM nitrate and compared to singular control treatments amended with 10mM nitrate only, 10mM ^{13}C-acetate only, or without any electron donor or acceptor. Symbols represent the mean of triplicate samples with error bars denoting standard error of measure.



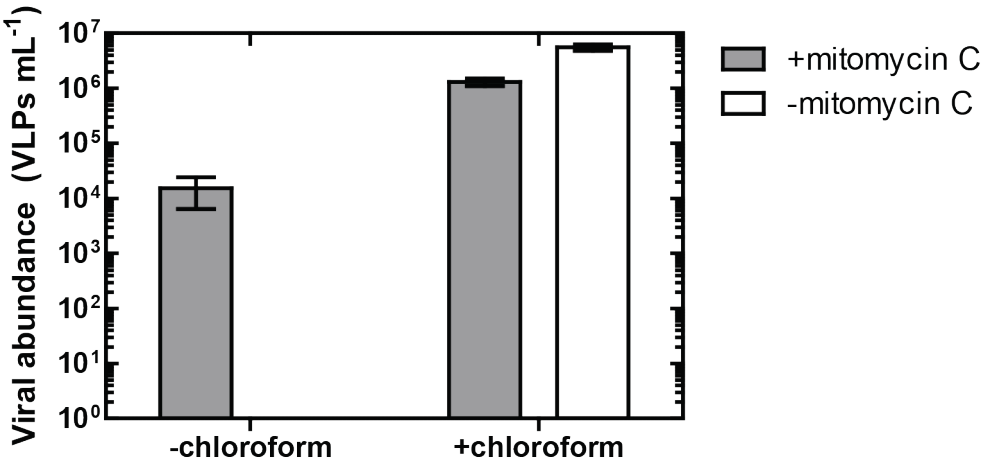
Supplementary Figure S5. $^{12}CO_2$ concentrations over the course of the stimulation reveal that there is a supply of endogenous reducing equivalents within the sediments. Experimental treatments conducted in triplicate were amended with 10mM ^{13}C-acetate and 10mM nitrate and compared to singular control treatments amended with 10mM nitrate only, 10mM ^{13}C-acetate only, or without any electron donor or acceptor. Symbols represent the mean of triplicate samples with error bars denoting standard error of measure.



Supplementary Figure S6. Rarefaction curves (**a**) of bacterial community samples based on observed OTUs collected over the course of four days of stimulation with acetate and nitrate indicate a decrease in observed OTUs over the course of the stimulation. Ten sampling repetitions were performed without replacement. Chao1 estimated sample richness (**b**) of the same samples show that richness decreases over the course of the incubation. Figures were modified from images generated by QIIME.



Supplementary Figure S7. Viral abundance in sediment slurries (10% w/v inoculum amended to AGW medium) before and after a 24 hour incubation with addition of mitomycin C (1.0 µg/mL). Sediment slurries in which mitomycin C was omitted served as the negative control. Analysis of Variance did not indicate a statistically significant difference between treatments (Two-way ANOVA; Time factor P=0.2319, Mitomycin C factor P=0.4004, Interaction factor P=0) indicating that the majority of the natural viral population is not lysogenic. Bars represent the mean of triplicate samples with error bars denoting standard error of measure.



Supplementary Figure S8. Viral abundance in cultures of *Pseudomonas* sp. Alda10 after a 24 hour incubation with mitomycin C (1.0µg/mL) and chloroform lysis of cultures. Incubation with 1.0µg/mL mitomycin C resulted in significant production of viruses relative to a control treatment in which mitomycin C was omitted (Two-way ANOVA; mitomycin C factor P=0.0055, chloroform factor P=0.0009, interaction P=0.0053). However, when cells were chloroform-lysed in both experimental and control treatments both resulted in a significant increase in viral abundance. (Two-way ANOVA; chloroform factor 53.27% of total variation; mitomycin C factor 20.46% of total variation).. . Bars represent the mean of triplicate samples with error bars denoting standard error of measure.

References Cited

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