Supplementary methods

Conservative vs. non-conservative tracer breakthrough

Given its non-conservative behavior, the lack of nitrate breakthough at the downgradient sampling well was insufficent to assess whether consumption tied to biological pathways was occuring. In contrast, the breakthrough of deuterium behaves in a conservative fashion, and as such, its appearance at a sampling well may be used to calculate the amount of injected nitrate that would be present in the absence of consumption. Subtracting the average pre-injection groundwater value (δD=-113.8‰) from that measured during the experiment and normalizing by the average tank composition yields the concentration of deuterium relative to the injectate (e.g., C/C_0; C/C_0 calculations are given below) at a given point in time; the complete lack of deuterium breakthrough yields C/C_0=0 whereas groundwater enriched with 50% of the δD label yields C/C_0=0 .50. Given the tank nitrate concentration of 2.5 mM, deuterium-derived C/C_0 values may then be used to quantify the concentration of nitrate that would have been measured at the monitoring well were consumption absent (e.g., C/C_0=0.50 implies a nitrate concentration of 1.25 mM). The difference between the measured and deuterium-derived nitrate concentration at the monitoring well thus represents the amount of nitrate lost to biological pathways.

Note that the small variations in injection wellhead difference designed to enhance the dispersal of injectate between the injection wells had negligible impact on aquifer transport properties and groundwater flow velocity or direction.

Calculations for C/Co values

The C/C_0 values were specifically calculated in the following manner:

1. Converting dD (‰) to parts per million values for the average nitrate tank

composition measured at three time points (average dD tank = 210.81‰) as follows:
dD = [(D/H-sample / D/H-standard) – 1)*1000] where D/H-standard = 0.000156

D/H-sample = [(dD/1000)+1] x 0.000156
D/H-sample in parts per million = D/H-sample x 1000000 = [(dD/1000)+1] x 156

D/H-average tank (ppm) = 188.89

2. Converting dD (‰) to parts per million values for the average pre-injection/background groundwater composition (dD = -113.78‰) measured at the six downgradient (CD01-CD05; CD18) wells prior to starting the injection and eleven time points at the upgradient/background well CU01: average pre-injection/background groundwater:

   D/H-average pre-injection/background groundwater (ppm) = 138.25

3. Subtract the pre-injection/background groundwater contribution from the average tank value to derive injection tank excess D:

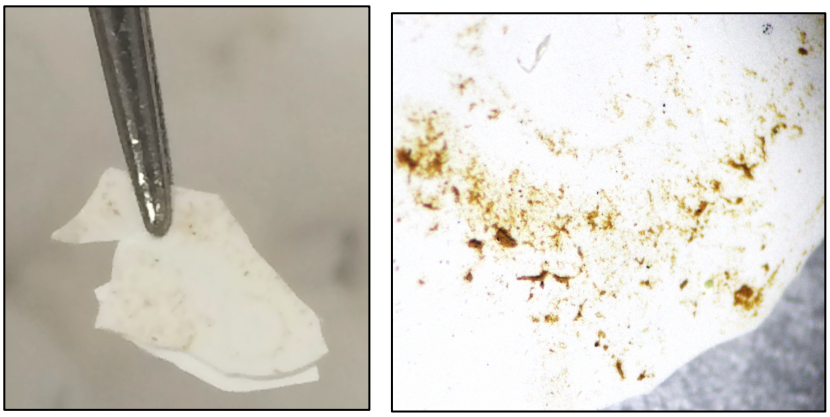
   D/H-tank minus background = D/H-average tank (ppm) - D/H-average pre-injection/background groundwater (ppm) = 50.64

4. Calculate C/C_0:

   (D/H-measured – D/H-average pre-injection/background groundwater)/D/H-tank minus background

Nucleic acid extraction

Genomic DNA and total RNA were co-extracted from 0.2-μm filters (orange spots, possibly iron oxides, were observed on several filter pieces; see below) using a modified version of the method described by Ivanov et al. (Ivanov et al 2009). Briefly, frozen filter pieces were directly placed into 2-mL Lysing Matrix E tubes (MP Biomedicals, Santa Ana, CA, USA) containing 700 μL extraction buffer (125 mM Miller phosphate buffer and 1% Miller SDS in TE, pH 7.4).



Filter piece with orange spots possibly indicating the presence of Fe(III) oxides.

After addition of 4.8 mM aluminum ammonium sulfate, the samples were disrupted in a FastPrep instrument (MP Biomedicals) for 30 seconds at 5.5 m/s. Proteinase K was added at 0.02 mg/mL, and the samples were incubated for 30 minutes at 37^{o}C with gentle agitation (200 rpm). The Lysing Matrix E tubes were centrifuged at 16k x *g* for 2 minutes at 4^{o}C and the supernatant was transferred to phase-lock gel tubes (QIAGEN, Venlo, Netherlands). The filters were then re-extracted as described above, excluding the incubation with proteinase K. After centrifugation, the supernatant was pooled with the first extraction and treated with 20% of 10% hexadecyltrimethylammonium bromide (CTAB) in 1 M NaCl. The samples were mixed by inversion for 1 min and then cleaned with equal volumes of phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (49:1), following standard techniques. The resulting supernatants were precipitated overnight at room temperature with 2.5 μL linear acrylamide and isopropanol alcohol equal to the sample volumes. After precipitation, the pellets were washed in 70% ethanol and re-suspended in Tris-EDTA, pH 8. Genomic DNA and total RNA were separated and

purified using a DNA/RNA AllPrep kit (QIAGEN) following the manufacturer's instructions, with the addition of 1% beta mercaptoethanol in Buffer RPT. Total RNA was DNase-treated on-column following manufacturer's instructions (QIAGEN). Genomic DNA concentration and quality were measured with a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA) and NanoDrop 2000c UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Total RNA concentration and quality were measured with Qubit and a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) with RNA Pico Chips. The samples were stored at -80^{o}C until further processing.

Metagenomic library construction and sequencing

Sequencing libraries (ca. 400-bp inserts) were constructed at the Functional Genomics Lab (FGL), a QB3-Berkeley Core Research Facility at UC Berkeley. At the FGL, a S220 Focused-Ultrasonicator (Covaris, Woburn, MA, USA) was used to fragment DNA, and the fragmented DNA was cleaned & concentrated with the MinElute PCR Purification kit (QIAGEN). The library preparation was performed on Apollo 324TM with PrepX™ ILM 32i DNA Library Kit (WaferGen Biosystems, Fremont, CA) and sequenced using an Illumina HiSeq 2500 (San Diego, CA, USA) with paired-end, 150-bp reads.

Metatranscriptomic library construction and sequencing

The total RNA samples were treated with a RiboZero rRNA Removal Kit for Bacteria (Illumina) following the manufacturer's instructions. The sample input varied from 70 to 2050 ng total RNA. Depletion of rRNA was confirmed with a Bioanalyzer 2100 trace, using an RNA 600 Pico kit (Agilent Technologies). The bar-coded cDNA

libraries were constructed from the rRNA-depleted RNA using the TruSeq RNA Sample Preparation Kit v1 (Illumina), with a single modification: the protocol began with the "Elute, Prime, and Fragment " step by adding 13 μL of the "Elute, Prime, and Fragment Mix" to 5 μL of RiboZero-treated RNA. Bar-coded samples were quantified with a Qubit fluorometer, normalized to 5 nM, and the size range confirmed with a Bioanalyzer trace using DNA 500 LabChip chips (Agilent Technologies). The samples were pooled to five per library and sequenced by QB3 on an Illumina HiSeq 2500 with single-end, 150-bp reads.

Metagenome sequencing quality control, trimming, and filtering

Each sample was sequenced using the Illumina platform to obtain paired-end, 150-bp reads with 400-bp inserts. The quality score profile of each sample was checked using FastQC, and based on those results, each sequence was trimmed 10 bp from the 5' end and 15 bp from the 3' end to remove regions with sequencing biases. The mean Q score for all trimmed sequences was > 30.

Low-complexity sequences were removed using the *dust* approach (Hancock and Armstrong 1994) the percent ambiguous bases set to <50% and maximum allowed *dust* score set to 50 using prinseq (http://sourceforge.net/projects/prinseq/files/standalone/). The orphan reads (i.e., with only one pair passing the filtering process) were removed using an in-house script. The total amount of sequence and their Q score statistics per sample that were inputted into the metagenome assembly were as follows:

Table S2A. Metagenome assembly statistics

Sample ID	Filter Size (μm)	Sampling Date	Gb	Q score (μ +/- σ)
2A	0.1	11/13/13	9.509	35.19 +/- 7.59
2B	0.1	12/4/13	8.277	35.06 +/- 7.80
2C	0.2	12/4/13	8.553	34.93 +/- 7.99
2D	0.1	12/18/13	4.69	34.79 +/- 8.13

2E	0.2	12/30/13	7.386	34.69 +/- 8.32
3A	0.2	11/13/13	7.943	38.79 +/- 5.27
3B	0.2	12/18/13	8.199	38.64 +/- 5.59
3C	0.1	12/30/13	9.641	38.91 +/- 5.05
3D	0.1	1/15/14	8.783	38.73 +/- 5.45

Metagenome co-assembly

Trimmed and filtered sequences from all samples were concatenated into a single fastq file, giving a total of 72.98 Gb of sequence from 9 samples (note that only the DNA and RNA results for the four 0.2-μm filter samples are reported in this study). These sequences were coassembled using an in-house iterative metagenome assembly pipeline. In each iteration, the assemblies were performed using Ray Meta (Boisvert et al 2012), the mapping of reads to the scaffolds using Bowtie 2 (Langmead and Salzberg 2012), the post processing of sequence alignments using samtools (Williams et al 2009) and customized R and python scripts.

The iterative assembly consisted of the following steps:

(1) First, we determined the bins of coverage in the dataset with an initial assembly using a k-mer size that covered the whole range of genome abundances, and hence coverages. Each coverage bin corresponded to one or more genomes. For this initial assembly, we set the k-mer size to ca. 1/3 of the mean read length (k=39). In practice, a few different iterations of k-mer sizes were used to provide an accurate estimate of coverage bins. Ray-meta was run with the scaffolding option turned off and the minimum contig length was set to 1 kb.

(2) Next, the coverage distribution plot (i.e. # bp vs. k-mer coverage) of the contigs from the initial assembly was calculated and plotted using custom R scripts. The peaks of coverage bins in the coverage distribution plot were identified and each of the coverage

bins was assembled in a separate iteration from the highest to lowest coverage bin using estimated optimized assembly parameters for each. The parameter set for each bin included k-mer size, minimum-seed coverage depth, and maximum-seed coverage depth. For each bin, whenever feasible, we chose a k-mer size giving a k-mer coverage between 30-50 under the constraint that k≥31.

(3) Iterative assembly: Each iteration consisted of an assembly of unmapped reads from the previous iteration with the optimized parameters turning on the scaffolding option. The scaffolds from the current bin of coverage were accumulated into the final set of scaffolds.

The final assembly was in 53426 scaffolds with an N50 size of 9.5 kb and had a total length of 150 Mb. The largest scaffold length was 1.15 Mb.

All of the final scaffolds were tested for the presence of chimeric assemblies using paired-end read and depth-of-coverage consistency with in-house scripts.

Binning, bin evaluation and curation

The final set of quality-controlled scaffolds were binned into genome bins using a Gaussian Mixture Model (GMM) clustering using tetranucleotide frequencies and depth of coverage across 9 samples as features. The depth of coverage profiles for the scaffolds were estimated based on mapping of reads from each sample to the scaffolds using Bowtie 2 (with options --sensitive, max# of mismatches N=1) and calculating the mean coverage for each scaffold from each of the samples. Tetranucleotide frequencies were calculated using in-house scripts. GMM clustering was performed using CONCOCT (Alneberg et al 2014) with number of clusters set to 500 (-c 500) to obtain preliminary bins.

Bin completion and purity was evaluated using a set of single-copy ribosomal proteins. Scaffolds having 16 single-copy genes encoding ribosomal proteins (*rplB, rplC, rplD, rplE, rplF, rplN, rplO, rplP, rplR, rplV, rplX, rpsC, rpsH, rpsJ, rpsQ, rpsS*) were determined based on the scoring by the corresponding TIGRFAM (Haft et al 2003) and Pfam (Finn et al 2014) models. Preliminary bins were checked for over- and under-binning and manually curated using DNA depth of coverage, GC content, and complementarity of the single-copy ribosomal gene set.

Functional Annotation

Open reading frames (ORFs) were predicted from the final set of scaffolds using Prodigal's (Hyatt et al 2010) meta procedure (-p meta). Sequence similarity searches of protein sequences from the predicted ORFs were performed using USEARCH (-ublast -query_cov 0.5 –target_cov 0.5 –id 0.97) (Edgar 2010) against UniRef100 (Suzek et al 2015). Protein domain annotations were predicted with InterProScan (Mulder and Apweiler 2007). Non-coding RNAs were predicted using Infernal (with cmsearch using default options against Rfam 11) (Nawrocki and Eddy 2013).

Metatranscriptomic Sequence Data Analysis

Each sample was sequenced using the Illumina platform to obtain single-end, 150-bp reads. Quality control, trimming, and filtering of low-complexity sequences were carried out in the same manner as for metagenome sequences. The total amount of sequence and their Q score statistics per sample were as follows:

Table S2B. Metatranscriptome assembly statistics for 0.2-μm filters

SampleID	Sampling Date	Raw reads	Raw Gb	QC'ed reads	QCed Gb	Mapped reads	% Mapped reads	Q score (μ +/- σ)
S10	11/13/13	25588239	3.83	25408399	3.201	1545630	6.083	37.86 +/- 7.27
S12	12/4/13	24575011	3.68	23461697	2.956	10377914	44.233	39.54 +/- 4.17
S14	12/18/13	22452862	3.36	22358679	2.817	10344120	46.264	38.07 +/- 6.91
S16	12/30/13	28334826	4.25	27299354	3.43	5899771	21.611	38.22 +/- 4.85

Estimation of transcript abundances

Transcriptomic reads were mapped to all of the predicted ORFs from the metagenome assembly using Bowtie 2. Raw mapping results were processed using samtools and the number of raw reads for each ORF was normalized to reads per kilobase per million reads mapped (RPKM) using in-house scripts. The percent contribution of each bin of interest to the full metatranscriptome was calculated as the ratio of the RPKM sum of all reads within a particular bin to the RPKM sum of the total metatranscriptome.

Additional bioinformatic analyses

Genes of interest for specific metabolic pathways were identified in a custom metagenomic database created for this study with BLAST 2.2.30+ (Camacho et al 2009) and BLASTP searches (Altschul et al 1990) against the NCBI non-redundant database. Where applicable, JGI IMG-ER was used to determine synteny of gene neighborhoods to known species (Markowitz et al 2013). Alignments were performed with Clustal Omega (Sievers et al 2011) using default settings.

Supplemental Methods References

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