<u>Supplemental Information for</u>

Temporal Dynamics of In-Field Bioreactor Populations Reflect the Groundwater System and Respond Predictably to Perturbation

Andrew J. King[1#], Sarah P. Preheim[2#], Kathryn L. Bailey[1#], Michael S. Robeson II[3], Taniya Roy Chowdhury[1], Bryan R. Crable[1], Richard A. Hurt Jr. [1,4], Tonia Mehlhorn[1], Kenneth A. Lowe[1], Tommy J. Phelps[1], Anthony V. Palumbo[1], Craig C. Brandt[1], Steven D. Brown[1,4], Mircea Podar[1,4], Ping Zhang[8], W. Andrew Lancaster[9], Farris Poole[9], David B. Watson[1], Matthew W. Fields[5], John-Marc Chandonia[6], Eric J. Alm[7], Jizhong Zhou[8], Michael W. W. Adams[9], Terry C. Hazen[1,4], Adam P. Arkin[6], and Dwayne A. Elias[1,4*]

[1]Oak Ridge National Laboratory, Oak Ridge, TN, USA

[2]Johns Hopkins University, Baltimore, MD, USA

[3]Colorado State University, <u>Fort Collins, CO,</u> USA

[4]University of Tennessee, Knoxville, TN, USA
[5]Montana State University, Bozeman, MT, USA
[6]Lawrence Berkley National Laboratory, Berkley, CA, USA
[7]Massachusets Institute of Technology, Cambridge, MA, USA
[8]University of Oklahoma, Norman, OK, USA
[9]University of Georgia, Athens, GA, USA

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Number of Tables 1, Table S1.

Number of Figures 4, Figures S1-S4.

Supplemental Methods

Field site and experimental design.

The well casing was installed to 7.2 m. with the screen depth to 7.0 m. After a two week stabilization, a groundwater packer (Tigre Tierra, Aardvark Packers) positioned directly above a double bladder pump (P1150, QED Environmental Systems) was installed and placed at 5.5 m depth (the middle of the screened portion of the well), which was below the average groundwater depth of 4.0 m. The packer was used to seal off the groundwater from surface and rainwater contamination and contained a gas line for Argon gas to inflate the bladders. A liquid line was inserted through the bladder pump to deliver groundwater to the primary container and bioreactors which were located in a mobile laboratory (Figure S2).

The mobile laboratory was located 2.0 m from the well head. Groundwater was pumped into a sterile, covered 200 mL primary container with an overflow into a larger storage container. Pumping of the groundwater directly from the wells was not possible due to the low flow rate combined with the depth of the groundwater source. The bladder pump discontinuously delivered 500 mL of groundwater to the primary container every three minutes. Black viton lines (size 14; Masterflex) for each bioreactor were placed into the primary container so that groundwater was continually delivered to the bioreactors at a rate of 0.33 mL min^{-1} via a peristaltic pump. This correlated to a liquid phase replacement rate of 0.02/hr; 50 hour maximal generation time to retain a considerable fraction of the extant planktonic microbial community. This scheme of using the bladder pump and primary container ensured that the groundwater introduced into the bioreactor lines was outside of the well for no more than three minutes.

The sediment used in the biofilm coupons was obtained from the 5.5 m depth core that was drilled at the start of FW305 installation and kept at -80^{o}C until required. Sediment aliquots were pulverized while submerged in liquid N_2 using a Freezer Mill (model 6870, SPEX). Sediment aliquots (5 g) were then aseptically added to the biofilm coupons and a mesh and mesh holder screwed to the biofilm coupon holder (Figure S3). The bioreactors were assembled, covered in aluminum foil, autoclaved (121^{o}C, 20 min) and transported to the mobile laboratory. All lines and filters were packaged in aluminum foil, sterilized via autoclave and attached to the bioreactors in the mobile laboratory. The bioreactors also had a secondary glass wall so that temperature controlled water continually flowed around the sample and was adjusted at each sampling point to match the in-well groundwater temperature.

Supplementary Figures

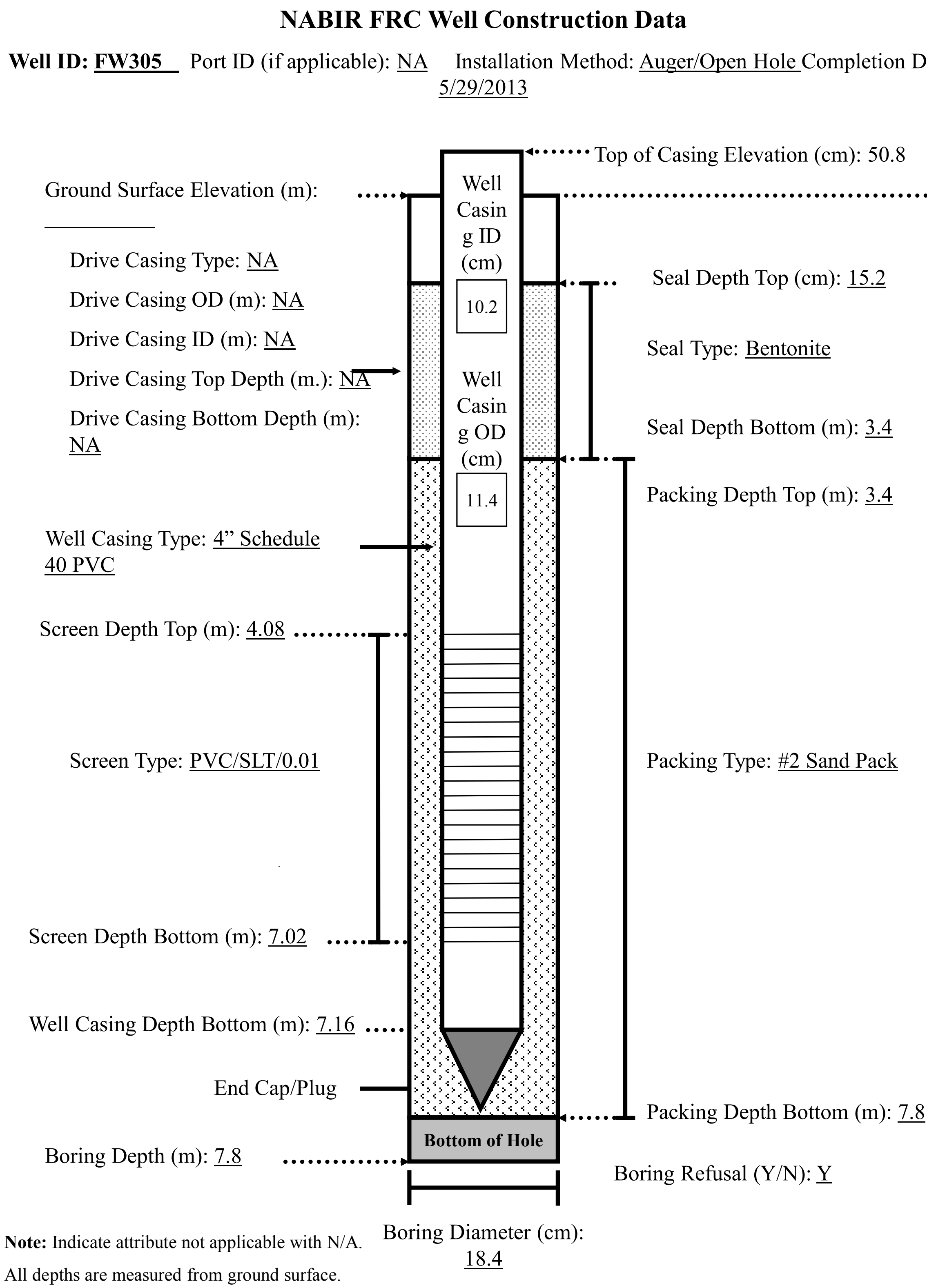

Figure S1: Detailed schematic diagram of well FW305 installation and materials used.

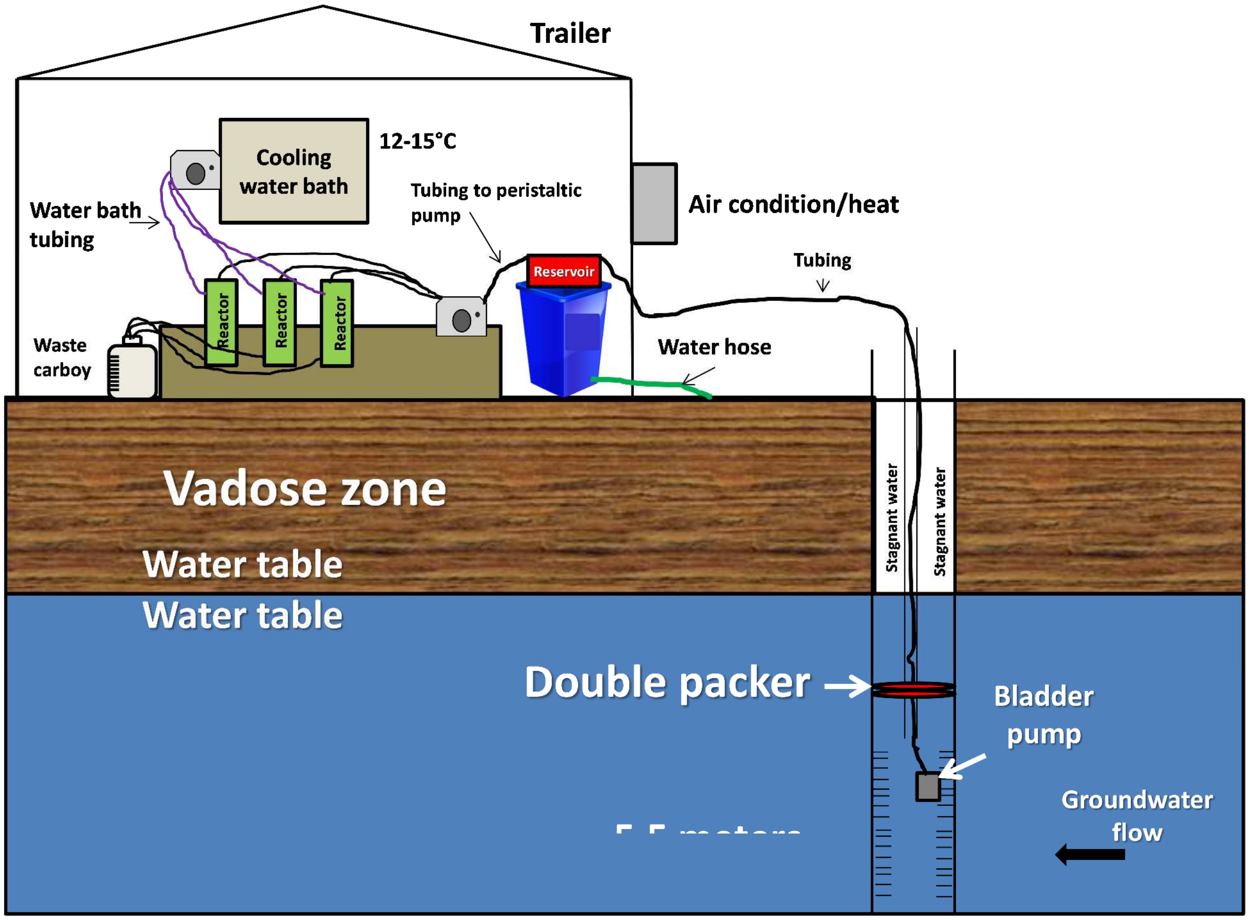

Figure S2: Cartoon depicting the bioreactor experimental setup. A packer isolated the groundwater from contamination and limited groundwater inflow for the experiment to the well casing regions with openings to allow in fresh subsurface groundwater. Therefore no stagnant, standing water in the wells infiltrated the experiment.

Assembled Reactor **Top of reactor** **Biofilm coupon**

Figure S3: Custom designed in-field bioreactors. (Left) An assembled, double-walled bioreactor to maintain consistent temperature with eight biofilm coupons containing ground sediment from FW305 coring, (middle) bioreactor head plate showing inlet lines and eight removable bioreactor coupon holders, (right) one of the eight per bioreactor biofilm coupon holders showing the mesh covered opening at the bottom that contained the ground and sterile sediment.

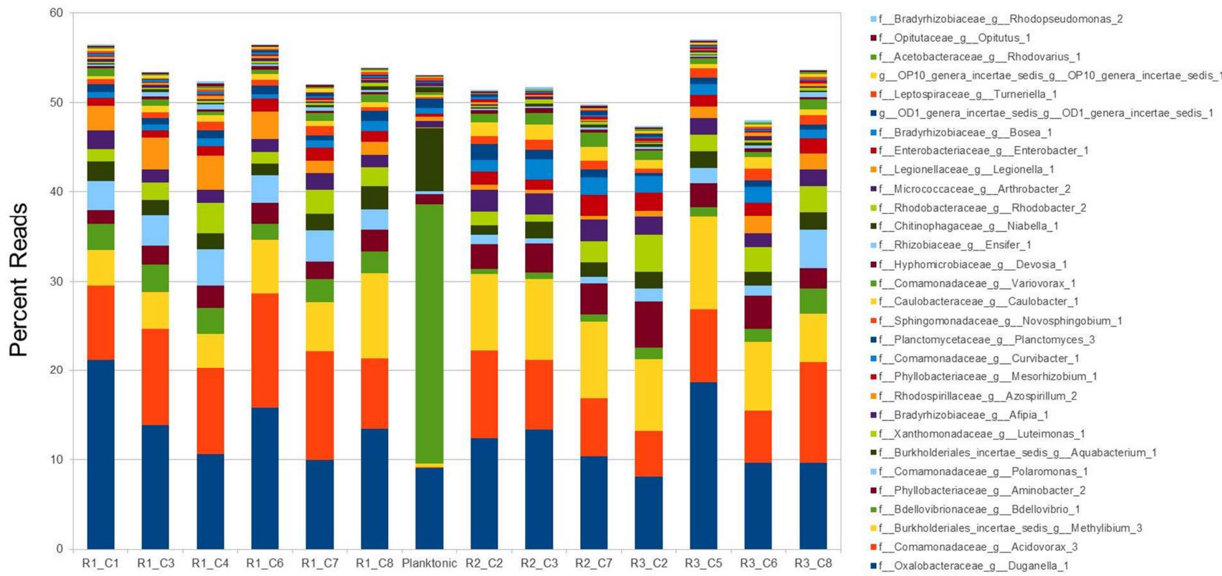

Figure S4. The 30 most abundant genera from OTUs that were observed in all biofilm coupons. Coupons are labeled by reactor and then coupon replicate. Each color is a separate genera and the genus species label is followed by the number of OTUs contributing to that genus.

Table S1: List of OTU's that co-occur with DO/pH alteration or with Nitrite

Clade Taxonomy	
Co-occurring Oxygen Correlated	**Nitrite Correlated**
Clostridia Clostridiales	Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae Sphingobium
Deltaproteobacteria	Bacteroetes Sphingobacteria Sphingobacteriales
Unidentified Bacterium	Proteobacteria Alphaproteobacteria Rhizobiales
Unidentified Bacterium 1	Proteobacteria Alphaproteobacteria Rhizobiales Hyphomicrobiaceae Pedomicrobium
Unidentified Bacterium 2	Proteobacteria Alphaproteobacteria Rhizobiales Phyllobacteriaceae Aminobacter
Unidentified Bacterium 3	Proteobacteria Alphaproteobacteria Sphingomonadales Sphingomonadaceae Sphingobium 1
Unidentified Bacterium 4	Acidobacteria Gp6
Alphaproteobacteria	Verrucomicrobia Opitutae Opitutales Opitutaceae Opitutus
Unidentified Bacterium 5	Spirochaetes Spirochaetes Spirochaetales Leptospiraceae Turneriella
Betaproteobacteria	Proteobacteria Alphaproteobacteria Rhizobiales Bradyrhizobiaceae Afipia
Unidentified Bacterium 6	Proteobacteria Alphaproteobacteria Caulobacterales Caulobacteraceae Brevundimonas
Clostridia	Proteobacteria Betaproteobacteria Burkholderiales Comamonadaceae Acovorax
Alphaproteobacteria 1	Proteobacteria Betaproteobacteria Burkholderiales Oxalobacteraceae Massilia
Acidobacteria_Gp4	Proteobacteria Betaproteobacteria Rhodocyclales Rhodocyclaceae
Unidentified Bacterium 7	
Gammaproteobacteria	
Gammaproteobacteria Aeromonadales Aeromonadaceae Aeromonas	
Anaerolineae Anaerolineales Anaerolineaceae	
Epsilonproteobacteria Campylobacterales Campylobacteraceae Arcobacter	
Bacilli Lactobacillales Carnobacteriaceae Carnobacterium	
Sphingobacteria Sphingobacteriales Chitinophagaceae Flavisolibacter	
Betaproteobacteria Burkholderiales Comamonadaceae Comamonas	
Betaproteobacteria Burkholderiales Comamonadaceae Comamonas 1	
Actinobacteria Actinomycetales Corynebacteriaceae Corynebacterium	
Gammaproteobacteria 1	
Deltaproteobacteria Desulfuromonadales Geobacteraceae Geobacter	
Betaproteobacteria Hydrogenophilales Hydrogenophilaceae Thiobacillus	
Betaproteobacteria Hydrogenophilales Hydrogenophilaceae Thiobacillus 1	
Betaproteobacteria Hydrogenophilales Hydrogenophilaceae Thiobacillus 2	
Alphaproteobacteria Rhizobiales Hyphomicrobiaceae Rhodoplanes	
Clostridia Clostridiales Incertae	
Clostridia Clostridiales Incertae 1	
Clostridia Clostridiales Incertae 2	
Actinobacteria Actinomycetales Intrasporangiaceae Janibacter	
Clostridia Clostridiales Lachnospiraceae	
Betaproteobacteria Nitrosomonadales Nitrosomonadaceae Nitrosomonas	
Betaproteobacteria Nitrosomonadales Nitrosomonadaceae Nitrosomonas 1	
Nitrospira Nitrospirales Nitrospiraceae Nitrospira	
Clostridia Clostridiales Peptostreptococcaceae	
Alphaproteobacteria Rhizobiales Phyllobacteriaceae Aquamicrobium	
Bacteroidia Bacteroidales Porphyromonadaceae Petrimonas	
Gammaproteobacteria Pseudomonadales Pseudomonadaceae Pseudomonas	
Alphaproteobacteria Rhizobiales Rhizobiaceae Ensifer	
Alphaproteobacteria Rhodobacterales Rhodobacteraceae	
Betaproteobacteria Rhodocyclales Rhodocyclaceae Azoarcus	
Betaproteobacteria Rhodocyclales Rhodocyclaceae	
Betaproteobacteria Rhodocyclales Rhodocyclaceae Thauera	
Betaproteobacteria Rhodocyclales Rhodocyclaceae Azoarcus 1	
Deinococci Deinococcales Trueperaceae Truepera	
Alphaproteobacteria Rhizobiales Xanthobacteraceae Pseudolabrys	
Gammaproteobacteria Xanthomonadales Xanthomonadaceae	
Gammaproteobacteria Xanthomonadales Xanthomonadaceae 1	