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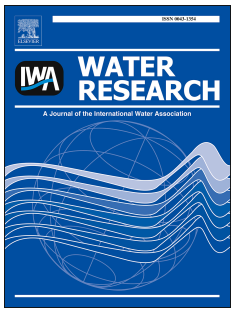

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PII: S0043-1354(18)30582-7

DOI: 10.1016/j.watres.2018.07.039

Reference: WR 13938

To appear in: *Water Research*

Received Date: 28 April 2018

Revised Date: 9 July 2018

Accepted Date: 16 July 2018

Please cite this article as: Beganskas, S., Gorski, G., Weathers, T., Fisher, A.T., Schmidt, C., Saltikov, C., Redford, K., Stoneburner, B., Harmon, R., Weir, W., A horizontal permeable reactive barrier stimulates nitrate removal and shifts microbial ecology during rapid infiltration for managed recharge, *Water Research* (2018), doi: 10.1016/j.watres.2018.07.039.

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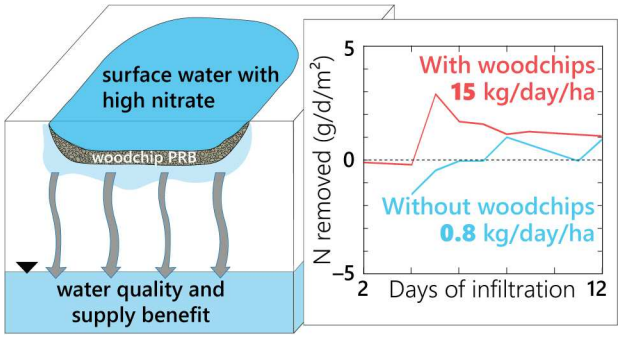

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A horizontal permeable reactive barrier stimulates nitrate removal and shifts microbial ecology during rapid infiltration for managed recharge

Sarah Beganskas[a*], *Galen Gorski*[a], *Tess Weathers*[a], *Andrew T. Fisher*[a], *Calla Schmidt*[c], *Chad Saltikov*[b], *Kaitlyn Redford*[b], *Brendon Stoneburner*[b], *Ryan Harmon*[a1], *Walker Weir*[a2]

[a]Earth and Planetary Sciences, University of California Santa Cruz, Santa Cruz, California, 95064

[b]Microbiology and Environmental Toxicology, University of California Santa Cruz, Santa Cruz, California, 95064

[c]Environmental Science, University of San Francisco, San Francisco, California, 94117

[1]Present address: Hydrologic Science and Engineering, Colorado School of Mines, Golden, Colorado, 80401

[2]Present address: Hydrologic Sciences, University of Nevada Reno, Reno, NV, 89557

*Corresponding author, sbeganskas@ucsc.edu

Declarations of interest: none

ORC IDs

Sarah Beganskas: 0000-0002-5146-9736

Galen Gorski: 0000-0003-0083-4251

Tess Weathers: 0000-0002-8152-2702

Andrew T. Fisher: 0000-0003-2102-8320

Calla Schmidt: 0000-0003-2120-9877

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ABSTRACT

We present results from field experiments linking hydrology, geochemistry, and microbiology during infiltration at a field site that is used for managed aquifer recharge (MAR). These experiments measured how a horizontal permeable reactive barrier (PRB) made of woodchips impacted subsurface nitrate removal and microbial ecology. Concentrations of dissolved organic carbon consistently increased in infiltrating water below the PRB, but not in un-amended native soil. The average nitrate removal rate in soils below the PRB was 1.5 g/m^2/day NO_3-N, despite rapid infiltration (up to 1.9 m/d) and a short fluid residence time within the woodchips (≤6 h). In contrast, 0.09 g/m^2/day NO_3-N was removed on average in native soil. Residual nitrate in infiltrating water below the PRB was enriched in $\delta^{15}N$ and $\delta^{18}O$, with low and variable isotopic enrichment factors that are consistent with denitrification during rapid infiltration. Many putative denitrifying bacteria were significantly enhanced in the soil below a PRB; *Methylotenera mobilis* and genera *Microbacterium*, *Polaromonas*, and *Novosphingobium* had $\log_2$ fold-changes of +4.9, +5.6, +7.2, and +11.8, respectively. These bacteria were present before infiltration and were not enhanced in native soil. It appears that the woodchip PRB contributed to favorable conditions in the underlying soil for enhanced nitrate removal, quantitatively shifting soil microbial ecology. These results suggest that using a horizontal PRB could improve water quality during rapid infiltration for MAR.

KEYWORDS

denitrification, managed aquifer recharge, permeable reactive barrier, nitrate reduction, infiltration, soil microbiology

47 ABBREVIATIONS

48 MAR, managed aquifer recharge; PRB, permeable reactive barrier; NS, native soil; DOC,
49 dissolved organic carbon; TOC, total organic carbon; TN, total nitrogen; OTU, operational
50 taxonomic unit

51

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1. INTRODUCTION

As global fresh water demand increases, climate changes, and land use shifts, groundwater is an increasingly important and vulnerable resource (Wada et al., 2010). Managed aquifer recharge (MAR) is a strategy employed in many settings to increase groundwater supply, introducing surface water into aquifers using a variety of techniques (Bouwer, 2002). Water used for MAR can come from diverted surface flows, hillslope runoff, or treated wastewater (Beganskas & Fisher, 2017; Bekele et al., 2011; Schmidt et al., 2011).

MAR can impact water quality as well as water supply (Hartog & Stuyfzand, 2017; Ma & Spalding, 1997). Groundwater quality can be improved if introduced water dilutes lower-quality groundwater or if solutes undergo beneficial geochemical transformations during infiltration, including oxidation/reduction reactions, precipitation, adsorption, and biodegradation (Johnson et al., 1999; Wilson et al., 1995). Alternatively, reactions during infiltration and/or recharging contaminated water could degrade groundwater quality (Tedoldi et al., 2016).

Globally, nitrate (NO_3^-) is the most widespread nonpoint source groundwater pollutant; elevated nitrate concentrations in streams and groundwater put human health and aquatic ecosystems at risk (Gurdak & Qi, 2012). Denitrification is the most-studied nitrate removal mechanism and involves progressive reduction of NO_3^- to NO_2^-, NO, N_2O, and finally N_2 (Korom, 1992). Anammox and dissimilatory nitrate reduction to ammonium are additional nitrate removal pathways in soils and aquatic systems (Shan et al., 2016). All three processes are microbially mediated and may occur concurrently (Long et al., 2013). Denitrification is favored under suboxic to anoxic conditions and requires abundant electron donors (often organic carbon); these

conditions may exist in shallow soils during infiltration for MAR (Wang et al., 2018) and related management strategies aquifer storage and recovery (ASR) and soil aquifer treatment (SAT) (Mienis & Arye, 2018; Pan et al., 2017; Vanderzalm et al., 2013, 2018). Temperature, pH, saturation, vegetation, and other factors have also been shown to influence denitrification rates (Hiscock et al., 1991; Rao & Malini, 2014; Xiong et al., 2017).

Several approaches have been developed to improve water quality by promoting denitrification and other nitrogen removal pathways. Bioreactors and denitrification beds containing reactive, carbon-rich material (e.g., woodchips, plant debris, biochar) have been deployed to treat surface water with elevated nutrient concentrations (Christianson & Schipper, 2016; Moorman et al., 2010). Denitrification beds (large tanks of reactive material) have been particularly effective at treating agricultural runoff before it reaches a stream (Warneke et al., 2011). For impaired groundwater, a vertical permeable reactive barrier (PRB) made of carbon-rich material can be installed perpendicular to groundwater flow (Obiri-Nyarko et al., 2014; Robertson et al., 2005). PRBs are typically used to remove one or more specific contaminants and have successfully remediated plumes of metals, organic compounds, and nutrients (Ludwig et al., 2002; Thiruvenkatachari et al., 2008). Denitrification rates in PRBs and bioreactors vary over time and as a function of inflowing [NO_3^-], residence time within the reactive material, temperature, and other factors (Addy et al., 2016; Roberston et al., 2008).

Recently, PRBs have been installed horizontally, rather than vertically, to target infiltrating water before it reaches groundwater. A horizontal PRB in an infiltration basin can enhance organic contaminant removal (Valhondo et al., 2018); in this study, we examined how a horizontal

98 woodchip PRB could enhance nitrate removal during rapid infiltration for MAR. Specifically, we
99 seek to address the following questions: (a) Is denitrification during rapid infiltration enhanced
100 by brief passage through a carbon-rich PRB? (b) How does infiltration through a PRB affect
101 microbial ecology in shallow soils? (c) How might using a horizonal PRB improve water quality
102 during MAR? To address these questions, we conducted a novel series of plot-scale field
103 experiments to represent shallow soil conditions during infiltration for MAR, collecting co-
104 located and contemporaneous hydrologic, geochemical, and microbial samples. These
105 interdisciplinary experiments were designed to compare nitrate removal processes in native soils
106 to those within and below a horizontal woodchip PRB, with applications for simultaneously
107 improving groundwater supply and quality via MAR.
108
109 2. MATERIALS AND METHODS
110 *2.1 Field site.*
111 Field work was completed within the 2.5-ha Harkins Slough MAR infiltration basin in the Pajaro
112 Valley, central coastal California, USA (**Figure S1**). Land use in the Pajaro Valley is a mix of
113 agricultural, urban, residential, and undeveloped, but most groundwater use supports agricultural
114 activities. Nitrate concentrations in major surface water bodies and groundwater often exceed the
115 U.S. Environmental Protection Agency's Maximum Contaminant Level of 10 mg/L NO_3-N (Los
116 Huertos et al., 2001; Pajaro Valley Water Management Agency, 2016).
117
118 The experimental site is located on eolian (dune) and alluvial deposits, and shallow soils are
119 characterized as Baywood loamy sand, typically having ~81% sand, 16% silt, and 2% clay
120 (USDA, 2014). The Harkins Slough MAR system is operated by a local agency that diverts

121 water from a nearby wetland when flows and water quality are sufficiently high. Diverted water
122 passes through a rapid sand filter before entering the infiltration basin. Recharged water is
123 subsequently recovered from a network of shallow wells, mixed with recycled water (tertiary
124 treated and disinfected) and groundwater from farther inland, and distributed to local customers
125 in lieu of pumping from the regional aquifer. Earlier studies of the Harkins Slough MAR system
126 examined infiltration dynamics and water quality during infiltration through native soils,
127 focusing on the central, deeper part of the infiltration basin (Racz et al., 2011; Schmidt et al.,
128 2011); the experiments presented in this study were located at higher elevation near the edge of
129 the infiltration basin and explored how a PRB may stimulate nutrient cycling (**Figure S1**).
130
131 *2.2 Plot construction and operation.*
132 We constructed four hand-excavated infiltration plots, each 1 m^2 in area. Each plot was lined
133 with fiberglass walls, caulked at the corners, and backed by bentonite (**Figure 1A,B**). Two plots
134 (NS1 and NS2) contained only native soil. In plots PRB1 and PRB2, a horizontal PRB consisting
135 of a 30-cm-thick layer of redwood chips (0.5–2 cm in dimension and rough in shape) was
136 installed above native soils. Woodchips were added to the plots by hand and gently consolidated,
137 then covered with a coarse nylon screen held down by washed, rounded river rocks to prevent
138 floatation. A hose delivered groundwater with elevated [NO_3^-] to the plots from a nearby well. A
139 float switch and solenoid valve controlled water delivery, keeping the water level within a
140 limited range (**Figure 1C**). For each test, water infiltrated continuously for 14–15 days; we ran
141 four tests in series over a 10-week period.
142

Experimental plots were designed to represent a small-scale MAR infiltration basin. Some lateral infiltration occurs in field-scale MAR systems (Bouwer, 2002), but a larger fraction of lateral flow was expected to occur below the 1-m^2 plots, especially near the edges (see **Supporting Information**). Accordingly, all instruments for subsurface sampling and measurements were installed within the central 0.16 m^2 of each plot, where flow is dominantly vertical (**Figure 1D**). All infiltration rate and geochemical load calculations are based on data and samples collected from this region.

The inflowing water supply was intermittent during test NS2 (**Figure S2**), which prevented maintenance of saturated conditions in the shallow soil, so we focused on data from NS1 as a no-treatment control for comparison with results from tests PRB1 and PRB2. For each test, the float switch was installed high in the plot (water level ~0.5 m) and then lowered (water level ~0.3 m) after a period of initial infiltration. We lowered the float switch on day 4 during test PRB1, day 7 during test NS1, and day 8 during test PRB2. See **Supporting Information** for details on plot construction, operation, and sampling.

2.3 Sampling.

Soil samples were collected using a hand auger before and after infiltration at 10–20 cm intervals down to 110 cm below plot base (cm-bpb); soil aliquots for microbiological analysis were collected with ethanol-rinsed spatulas. While experiments were running, fluid samples were collected using six piezometers in two nests of three with screen centers at 30, 55, and 80 cm-bpb. Additional fluid samples were drawn from within the PRB during tests PRB1 and PRB2. We sampled surface and subsurface water 10–12 times during each test, pumping the fluid

166 through a 0.45 µm cellulose acetate filter into acid-washed, pre-rinsed polyethylene and glass
167 bottles. Samples were put on ice immediately and stored at –20°C until analysis.
168
169 *2.4 Infiltration rates.*
170 We converted absolute pressure at the plot base to water level, correcting for local barometric
171 pressure. During periods when the solenoid valve was closed (every 10–20 min), preventing
172 water from flowing into the plot, we calculated the bulk infiltration rate as change in water level
173 over time. We independently calculated the vertical component of infiltration in the center of the
174 plot with thermal probes installed in the soil. This technique uses heat as a tracer to quantify the
175 vertical infiltration rate from the amplitude reduction with depth of periodic temperature
176 fluctuations (Hatch et al., 2006). Thermal probes do not provide reliable infiltration data during
177 the first and last few days of each test due to edge effects equivalent to the order of the filter
178 applied to the thermal data (2–3 days); thus we focused our analysis on days 4–12 of each test.
179
180 *2.5 Soils characterization*.
181 To determine soil texture, splits from pre-infiltration samples were digested with 30% hydrogen
182 peroxide (to remove organic carbon), freeze dried, suspended in an eluent with 4 g/mL of sodium
183 hexametaphosphate, $(NaPO_3)_6$, as a deflocculant, and analyzed in a Beckman Coulter LS 13320
184 Particle Size Analyzer. Running commercial and internal lab standards and repeat analyses of
185 field samples indicated repeatability and precision of 3–5% (relative deviation) for each of 92
186 grain-size bins across a range of ≤0.4 µm to 2 mm. Total organic carbon (TOC) and total
187 nitrogen (TN) were analyzed in pre-infiltration samples using a Thermo Fisher Flash 2000.
188 Samples were homogenized, oven-dried, vapor acidified for 24 hours, and oven-dried again.

189 They were packed into tin capsules, crushed into cubes, and analyzed. A certified soil reference
190 material was analyzed every ten samples, giving a relative standard deviation of <3%.
191
192 *2.6 Water chemistry*.
193 $[NO_3^-]$, $[NO_2^-]$, and $[NH_4^+]$ were determined by colorimetric flow injection analysis on a Lachat
194 Instrument QuickChem 800. Dissolved organic carbon (DOC) was measured by combustion
195 catalytic oxidation using a Shimadzu TOC-VCSH total organic carbon analyzer. Regular
196 analyses of sample splits, blanks, and laboratory standards indicate accuracy for both instruments
197 of 3–5%. Concentrations of chloride, sulfate, bromide, and phosphate were analyzed using a
198 Dionex ICS 2100. Standards were run every ten samples and all had errors ≤10%, with most
199 ≤5%. We calculated daily nitrate load reduction (g N/m^2/day) as:
200 $$\left[\mathrm{NO}_{3surface}^-\right] IR_V - \left[\mathrm{NO}_{3deepest}^-\right] IR_V$$
201 where IR_V is the vertical infiltration rate measured at the center of the plot. Normalized nitrate
202 removal rates (day^{-1}) were calculated as:
203 $$\frac{\left[\mathrm{NO}_{3surface}^-\right] - \left[\mathrm{NO}_{3deepest}^-\right]}{T_R\left[\mathrm{NO}_{3surface}^-\right]}$$
204 where T_R is the residence time of water between the surface and the deepest subsurface sample,
205 calculated using IR_V.
206
207 A subset of 36 water samples from NS1 and PRB1 were analyzed for $\delta^{15}N$ and $\delta^{18}O$ of nitrate
208 (relative to air and Vienna Standard Mean Ocean Water, respectively) using bacterial
209 denitrification at University of California, Davis's Stable Isotope Facility (Casciotti et al., 2002).
210 This facility uses a ThermoFinnigan GasBench + PreCon trace gas concentration system

211 interfaced to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer. Twelve
212 standards were run at regular intervals and showed no discernible drift, with standard deviations
213 of .06‰ for $\delta^{15}N$ and .15‰ for $\delta^{18}O$. For each day, nitrogen and oxygen enrichment factors were
214 calculated using an approximation of the Rayleigh equation.
215
216 *2.7 Phylogenetic sequencing.*
217 Soil DNA was extracted with the PowerSoil DNA Isolation Kit (QIAGEN) and quantified with a
218 Qubit 4 Fluorometer (Invitrogen). Partial 16S rRNA genes (V4 and V5 variable regions) were
219 amplified using primers modified to contain 5' sequencing adapters for barcoding and
220 sequencing using the Illumina MiSeq platform. Samples were analyzed by agarose gel
221 electrophoresis to confirm the presence of ~550 bp amplicons. An amplicon sequencing pipeline
222 was adapted from the Illumina MiSeq platform protocol for 16S metagenomic libraries (Illumina
223 Inc., 2013). The overall pipeline included the primary PCR using 16S rRNA gene primers
224 (Parada et al., 2016), PCR clean-up, library preparation (adding unique sequencing indices
225 [barcodes] to each PCR amplicon), normalizing DNA concentrations of each library, and library
226 pooling. The pooled library was sequenced on the Illumina MiSeq (600 cycles v3 PE300 flow
227 cell kit) at the University of California, Davis Genome Center. See **Supporting Information** for
228 primer specifications, amplification protocols, and additional method details.
229
230 Paired-end sequence post-processing was performed with QIIME version 2018.2 (Caporaso et
231 al., 2010) based on the analytical pipeline steps specified in Weathers et al. (2016) and using the
232 QIIME2 plugins described below. Demultiplexing was summarized using demux
233 (https://github.com/qiime2/q2-demux) and DADA2 (Callahan et al., 2016) was used for

234 truncating and denoising. Truncation thresholds were calculated as in Parada et al. (2016)
235 ensuring the average quality score for a 50 bp sliding window remained above 33. The QIIME2
236 plugin feature-table (McDonald, Clemente, et al., 2012) was used to create visual summaries of
237 sequences per sample. We trained a Naive Bayes classifier to our specific primers and assigned
238 taxonomy with Greengenes reference database version 13_8 with 99% OTUs (McDonald, Price,
239 et al., 2012) using the feature-classifier plugin (https://github.com/qiime2/q2-feature-classifier).
240 An additional comparison with SILVA 128 99% OTUs did not yield significant taxonomic
241 differences after filtering, therefore the results presented herein were generated with the
242 Greengenes reference database. See **Supporting Information** for details regarding plugins for
243 taxonomy visualization, alignment, tree generation, diversity, and differential abundance.
244
245 All samples were filtered so the minimum total observed percent per OTU summed across all
246 samples was 0.1%. OTUs are reported at the lowest identified taxonomic level. Log_2 fold-
247 changes were calculated per treatment as log_2(average abundance after / average abundance
248 before) to quantify each OTU's enhancement or inhibition during infiltration (Love et al., 2014).
249 Sequence data have been submitted to the National Center for Biotechnology Information
250 Sequence Read Archive database (SRA accession: SRP151895).
251
252 3 RESULTS
253 *3.1 Soils.*
254 Grain size data revealed predominantly sandy soils in all plots (**Figure S3**), consistent with
255 regional geology and mapped soil units. For all soil samples, d_{10} (10% finer) was >100 µm and
256 d_{50} >245 µm. TOC was <6% and TN was <0.07% by weight for all samples. TOC and TN did

not vary significantly with depth or differ in samples from before and after infiltration (**Table S1**).

3.2 Infiltration.

Sandy soils led to rapid infiltration (**Figure 2A**). For test NS1, bulk infiltration rates were relatively stable between 3.7 and 4.5 m/d. Bulk rates were higher and more variable during tests PRB1 (7.5–19.1 m/d) and PRB2 (14.5–21.1 m/d). It is unlikely that the PRB significantly influenced observed infiltration rates beneath the plots because the woodchips were much larger than soil grains. Infiltration rates were more likely dominated by underlying soil texture, with differences in infiltration rates beneath the plots resulting from soil heterogeneity. Vertical infiltration rates near the plot centers were generally lower than bulk rates: 1.1–3.4 m/d, 1.2–2.6 m/d, and 2.0–6.0 m/d for tests NS1, PRB1, and PRB2, respectively (**Figure 2B**). Vertical flow rates determined from multiple thermal probes in the same plot were similar (**Figure S4**) and were consistent with independent measurements made in the sandiest part of the deeper basin during an earlier study (Racz et al., 2011). The large difference between vertical and total infiltration is to be expected for experimental plots of this size (see **Supporting Information**). The residence time of water within woodchips at the plot center (based on vertical infiltration) was 2.8–6.0 h for test PRB1 and 1.2–3.6 h for test PRB2. Plot walls surrounding the woodchips limited lateral flow within the PRB, though any lateral flow in the PRB would result in even shorter residence times.

We lowered plot water levels midway through each test with the intent of slowing infiltration, but this had limited influence on infiltration rates (**Figure 2A**). Though bulk infiltration rates

slowed immediately after lowering the water level, these rates subsequently increased, ultimately returning to values observed near the start of each test. Vertical infiltration rates did not respond significantly to plot water level changes, indicating that much of the observed dynamics was associated with lateral flow. We surmise that the sustained free-water boundary condition inside the plots resulted in formation of a temporary, inverted shallow water table in adjacent soils. When the water level was abruptly lowered, there was likely a transient period of flow towards the plot, then downward, temporarily reducing bulk infiltration.

3.3 Water chemistry.

Infiltration rates and soil properties determine the depth extent of soil saturation, and pore fluids could be collected only when the piezometers were within saturated zones (**Figure 1**). On most days, inflowing water had consistent composition: 22–25 mg/L NO_3-N, 25–29 mg/L DOC, and little to no nitrite or ammonium. Although inflowing [NO_3-N] was relatively constant, nitrate loads varied as a function of infiltration rate: 40–72 g/day/m^2 NO_3-N for NS1, 30–61 g/day/m^2 NO_3-N for PRB1, and 66–104 g/day/m^2 NO_3-N for test PRB2 (**Figure 2C,D,E**). The water supply occasionally included a fraction of recycled water, readily identified by [NH_4-N] >0.5 mg/L and/or [NO_2-N] >0.25 mg/L (**Tables S2–5**); data from these days were not used for subsequent analyses. All inflowing and subsurface water samples had DOC >20 mg/L. DOC generally showed no trend with depth in native soil, but increased with depth below each PRB (**Figure 3**).

Nitrate removal during test NS1 was inconsistent and modest, ranging from –3.6 g N/day/m^2 (net addition) to 2.7 g N/day/m^2. In total, 1.2 g N/m^2 were removed over 14 days (**Figure 2C**).

During test PRB1, there was nitrate removal on days 5–12, peaking at 7.3 g N/day/m^2 on day 5. Cumulative removal during test PRB1 was 23.1 g N/m^2 over 15 days (**Figure 2D**). Nitrate removal was less consistent during test PRB2, peaking at 3.5 g/day/m^2 and adding to a cumulative removal of 4.9 g N/m^2 over 15 days (**Figure 2E**). On days when nitrate removal was observed, the largest changes occurred below 30 cm-bpb and coincided with small increases in [NO_2-N] (**Figure 3**).

Subsurface $\delta^{15}N$ and $\delta^{18}O$ differed little from surface values on days with no nitrate removal at depth (**Figure 4A**). Residual nitrate was enriched in $\delta^{15}N$ on all four days with measurable nitrate removal and enriched in $\delta^{18}O$ relative to surface water on three of those four days (**Figure 4B**), which is consistent with denitrification (Böhlke et al., 2002; Mariotti et al., 1988). Nitrogen enrichment factors ranged from –1.7 to –21.1‰, overlapping the range of values reported in agricultural regions (–4 to –30‰) (Böhlke et al., 2002; Böttcher et al., 1990). Oxygen enrichment factors ranged from –3.5 to +2.9‰, similar to some reported values (Carrey et al., 2013), but higher (less negative) than others (Böttcher et al., 1990; Fukada et al., 2003). Isotopic enrichment factors often exhibit an inverse relationship with denitrification rate (Mariotti et al., 1988; Vogel et al., 1981), and in the present study, rapid infiltration and denitrification corresponded to relatively low enrichment factors.

3.4 Microbiology.

Soil microbial communities were grouped into four statistically similar sets: native soil before infiltration (NSB), native soil after infiltration (NSA), PRB before infiltration (PRBB), and PRB after infiltration (PRBA). These sets account for two dominant factors explaining community

variance: the presence of a PRB (30%) and sample collection time (20%) (**Figure S5A, Table S7**), while sample depth accounted for 5% of the variance. Compared to other samples, many notable clades were enhanced in PRBA samples.

Many OTUs enhanced in PRBA samples have the potential to carry out denitrification (**Figure 5**). The OTU with the largest increase in relative abundance below the PRB was genus *Novosphingobium*, present at 14±10% after infiltration and 0.004±0.01% before infiltration, a $\log_2$ fold-change of +11.8. When this genus was proposed, the ability to reduce NO_3^-, the first step in denitrification, was a defining characteristic (Takeuchi et al., 2001). Enhanced OTUs with the potential to reduce NO_3^-, NO_2^-, and NO include *Methylotenera mobilis* (with a $\log_2$ fold-change +4.9) (Kalyuzhnaya et al., 2006); genus *Microbacterium* (+5.6) (Zhou et al., 2016); and family Methylophilaceae (+5.4) (Lapidus et al., 2011). Enhanced OTUs with the potential to reduce NO_3^-, NO_2^-, and N_2O include genera *Polaromonas* (+7.2) (Lycus et al., 2017) and *Microbacterium* and family Comamonadaceae (+4.0) (Khan et al., 2002). These OTUs were present in samples collected before infiltration and were not significantly enhanced in NSA samples. Additionally, many OTUs were inhibited in PRBA samples; one putative denitrifying genus, *Streptomyces*, had a $\log_2$ fold-change of –2.2 (Kumon et al., 2002).

Some OTUs enhanced in PRBA samples are associated with hydrocarbon degradation as well as nitrate reduction, including genera *Novosphingobium* (Liu, 2005), *Microbacterium*, and *Polaromonas*; and families Erythrobacteraceae (Tonon et al., 2014) and Comamonadaceae. Hydrocarbon degradation can occur rapidly under denitrifying conditions (Hutchins et al., 1991); the growth of microbes with the potential for hydrocarbon degradation could signal favorable

349 conditions for denitrification as well. Furthermore, many studies have explored the potential for
350 microbes to co-metabolize micropollutants and other contaminants under denitrifying conditions
351 (Suarez et al., 2010; Tarlera, 2003), and have found that co-metabolic processes can be enhanced
352 with the addition of a carbon source (Li et al., 2013).
353
354 4 DISCUSSION AND IMPLICATIONS
355 *4.1 Infiltration rates and nitrate removal.*
356 To assess the potential benefit of a horizontal PRB during MAR, we compare results from plot-
357 scale experiments to those from an earlier study at the same field site that observed nitrate
358 removal during MAR operations using native soil (without a PRB) (Schmidt et al., 2011). During
359 MAR operations, mean infiltration was slower and initial [NO_3^-] was lower than in the present
360 study. Nitrate removal during infiltration through native soil occurred only when vertical
361 infiltration rates were <0.7±0.2 m/d (**Figure 6A**). At higher infiltration rates, it was inferred that
362 oxic conditions were maintained throughout the saturated soil, limiting redox conditions needed
363 for efficient denitrification (Schmidt et al., 2011). Additionally, for 12 out of 23 measurements
364 when the vertical infiltration rate was <0.9 m/d, there was little to no nitrate removal. Thus,
365 having an infiltration rate through native soil below the identified threshold did not guarantee
366 that nitrate removal would occur during MAR operations. In the present study, vertical
367 infiltration rates in native soil were always >0.9 m/d and limited nitrate removal occurred,
368 consistent with earlier work. However, during tests PRB1 and PRB2, nitrate removal occurred on
369 every day with an infiltration rate <1.9 m/d (**Figure 6B**), a much higher threshold and a more
370 consistent pattern than observed in native soil.
371

No nitrate removal occurred at infiltration rates >1.9 m/d in the present study, even when water passed through a PRB. Although a PRB may extend the range of infiltration rates during which subsurface nitrate removal can occur, the process is still flow-rate limited. We did not observe infiltration rates <1.3 m/d in the present study, but given that a woodchip PRB helped to stimulate denitrification in sandy soils at rapid infiltration rates, it seems likely that the benefit from a PRB would extend to soils with lower infiltration capacities as well. Additional work at lower fluid flow rates would be useful, especially to determine whether the inverse relationship observed between infiltration rate and nitrate removal rate below a PRB (**Figure 6B**) is a consequence of the high infiltration rates near the threshold or a consistent behavior across a typical range of MAR infiltration rates (0.5–2 m/d). Other factors also influence nitrate removal, including carbon/nitrate availability, redox conditions, temperature, and soil properties. Separating these effects will require carefully-controlled experiments with a wide range of fluid and soil conditions and flow rates.

4.2 Linking geochemistry and microbiology.

Isotopic and microbial data provide consistent, strong evidence that nitrate removal occurred via denitrification in soils below a PRB. Pore-fluid nitrate in these soils was enriched in $\delta^{15}N$ and $\delta^{18}O$ relative to surface water on days with nitrate removal, a pattern consistent with denitrification during rapid infiltration (Mariotti et al., 1988). Significantly-enhanced OTUs below the PRB contained putative functionality for complete denitrification, and the abundance of hydrocarbon-degrading bacteria may further indicate that conditions were favorable for denitrification (Hutchins et al., 1991). However, we cannot eliminate the possibility that anammox occurred in these soils as well. On some days during tests NS1, PRB1, and PRB2,

ammonium appeared in the subsurface at ≤6 mg/L, but [NH_4^+] was inconsistent and not correlated with nitrate removal (**Tables S2–S5**). Anammox has also been observed during managed recharge (Fox, 2001) and is often identified (and distinguished from denitrification) by quantification of genes *hzo* or *hzsA* via qPCR or using isotopic tracers in N_2 gas (Jones et al., 2017; Rysgaard, 2004). Ongoing work will use qPCR to quantify nitrogen cycling genes with depth and time below a PRB relative to native soil.

In soils below a PRB, [NO_3^-] decreased with depth (**Figure 3**); depth was also an important factor in explaining community variance for PRBA samples (**Figure S5B**). Putative nitrate-reducing OTUs ranged from having a total relative abundance in PRBA samples of 16.6% to 58.3%; the relative abundance of putative nitrate reducers was greatest in the shallowest samples (10 cm-bpb) and decreased with depth. This pattern is consistent with the trend of decreasing [NO_3^-] with depth (**Figure 7**).

Most OTUs that were enhanced in PRBA samples was present before infiltration, thus it appears unlikely that woodchips merely transported foreign bacteria to the underlying soil. Rather, woodchips seem to have contributed to more favorable metabolic and growth conditions for native soil microbes that were already present. There are several possible mechanisms by which a PRB might stimulate denitrification. The high porosity and large surface area of woodchips might provide microbial habitat, but that does not account for nitrate removal (and enhanced OTUs) occurring in soils below the PRB. The placement of a PRB could enhance denitrification by thickening the saturated zone (increasing residence time within the saturated zone), leading to lower oxygen and other favorable redox conditions at an equivalent soil depth. The most likely

418 explanation is that the PRB elevated concentrations of biologically available organic carbon in
419 underlying soils (**Figure 3**), promoting more rapid microbially-mediated dissolved oxygen
420 consumption.
421
422 *4.3 Comparing a horizontal PRB to a denitrifying bioreactor.*
423 Results in this study are consistent, in some ways, with previous work using bioreactors;
424 favorable redox and other conditions may stimulate denitrification in many contexts. However,
425 in typical denitrifying bioreactors and beds, most nitrate removal occurs within the reactive
426 material and hydraulic retention times range from several hours to many days (Addy et al., 2016;
427 Warneke et al., 2011). In contrast, in this study we did not observe nitrate removal within the
428 woodchip PRB, where residence times were often 1–2 hr and always <6 hr. Instead, we observed
429 nitrate removal in soils up to 80 cm below the PRB (**Figure 3**), likely due to more favorable
430 conditions for ambient soil microbes capable of nitrate removal (**Figure 5**).
431
432 Denitrifying bioreactors and beds are often used to improve the quality of treated wastewater in
433 which [NO_3^-] and [NH_4^+] (along with other constituents) are elevated far above drinking water
434 standards. These systems can be designed and operated to optimize selected biochemical
435 processes, including nuanced controls on fluid flow rate and associated hydraulic retention time
436 during operation. In contrast, while MAR systems can be designed to achieve specific goals for
437 water supply and quality improvement, they are influenced strongly by ambient, often
438 heterogeneous, soil properties. Rapid infiltration through well-drained soils may result in the
439 formation of a thin (or no) saturated zone below some parts of an infiltration basin, limiting
440 opportunities for establishing redox conditions favorable to denitrification. The flow rate through

441 a denitrifying bioreactor or bed can be reduced to improve nitrate removal rates, but slowing the
442 flow applied to a well-drained infiltration basin is likely to result in development of shallow
443 unsaturated conditions, virtually ending denitrification until saturated soil conditions are
444 restored. This tradeoff emphasizes the importance of considering soil infiltration properties when
445 choosing locations for MAR and/or designing them with specific infiltration targets.
446
447 *4.4 Implications for MAR design and operation.*
448 These results suggest that significant water quality benefit may be achieved by adding a
449 horizontal PRB to an MAR infiltration basin. Nitrate removal rates are sensitive to infiltration
450 rate (**Figure 6A,B**), which varies spatially and temporally during MAR (Mawer et al., 2016;
451 Racz et al., 2011). There is particular potential for a horizontal PRB to facilitate nitrate removal
452 benefit when infiltration rates are above the observed threshold in native soils (0.7±0.2 m/d at
453 this study's location; Schmidt et al., 2011) and below the threshold for soils below a PRB (1.9
454 m/d at this study's location; **Figure 6B**). MAR projects are typically intended to achieve rapid
455 infiltration (0.5–2 m/d) that maximizes water supply benefit. During MAR operations at Harkins
456 Slough for WY2008, mean infiltration rates >0.5 m/d were maintained for about a third of the
457 operating season and >0.9 m/d for 14 days; mean infiltration rates were always <1.9 m/d (**Figure**
458 **6C**). During rapid infiltration for test PRB1, the average nitrate removal rate was 1.5 g/day/m^2
459 NO_3-N. If this rate were representative of average conditions throughout an infiltration basin, it
460 would be equivalent to 15 kg/day/ha. Thus, for a 2.5-ha infiltration basin like Harkins Slough,
461 adding a horizontal PRB could potentially contribute an additional 37.5 kg of NO_3-N removal on
462 each day with infiltration rates >0.7±0.2 m/d and <1.9 m/d. For the 14 days with infiltration >0.9
463 m/d at Harkins Slough in WY2008, this comprises an additional 525 kg of NO_3-N removal; for

464 the 49 days with infiltration <0.5 m/d, this yields 1,840 kg of additional NO_3-N removal. There
465 is considerable spatial heterogeneity and temporal variability in hydrologic, biogeochemical, and
466 microbial conditions during MAR, so these results need to be considered carefully in assessing
467 how a horizontal PRB might best be integrated with MAR for improved water quality.
468
469 An additional consideration is the lifespan and maintenance requirements such a system.
470 Woodchip PRBs can provide years of water quality improvements (Robertson, 2010; Robertson
471 et al., 2008), but aging may reduce denitrification efficiency. The speciation of leached carbon
472 from woodchips (Page et al., 2002), and how that speciation may change or diminish over time
473 during MAR, merits further study. It may be efficacious for MAR to mix the soil amendment
474 into the substrate directly, rather than emplacing it as a distinct layer. This approach has the
475 advantage that adding fresh material can be included as part of regular maintenance, including
476 sediment scraping and disking that "opens up" soil pores after operation. Additional field and lab
477 experiments are underway to evaluate the efficacy of mixing PRB materials in with native soil,
478 rather than creating a separate layer.
479
480 Designing an MAR project that removes contaminants as water infiltrates would provide a
481 measure of safety, which is especially important when non-pristine water sources are collected to
482 augment supplies (e.g., stormwater runoff, recycled wastewater). Installing a PRB horizontally in
483 an MAR basin has advantages over traditional vertical PRBs: the horizontal PRB has relatively
484 low installation and replacement cost, removes nitrate before it reaches an aquifer, does not
485 require the presence or detailed knowledge of a hydraulic gradient, and works within a MAR
486 system that simultaneously increases groundwater supply. Learning more about linked physical,

chemical, and biological mechanisms by which a PRB may enhance the removal of nitrate and other contaminants during MAR will increase understanding of subsurface solute and mineral cycling and facilitate projects that improve both water supply and water quality.

5 CONCLUSIONS

- Controlled field-based experiments representing infiltration for managed recharge demonstrated that a horizontal PRB made of woodchips significantly enhanced nitrate removal during rapid infiltration through shallow soils.
- Compared with nitrate removal observed during managed recharge operations without a PRB, nitrate removal in soils below a PRB occurred more consistently and at greater infiltration rates. When scaled up, this benefit could translate into significant water quality improvement.
- The woodchip PRB appeared to create favorable growing conditions for denitrifying microbes that were already present in the soil by providing bioavailable carbon and/or thickening the saturated zone.
- Using a horizontal PRB and considering soil infiltration properties could facilitate the design of managed recharge systems that address both water supply and water quality goals.

ACKNOWLEDGMENTS and DATA

We gratefully acknowledge field and lab assistance from N. Barshi, F. Fazilat, R. Franks, J. Hernandez, Z. Jones, J. Murray, A. Paytan, K. Redford, D. Runneals, J. Sharp, T. Stewart, E. Teo, C. Trivedi, D. Tran, D. van den Dries, and K. Young. We are grateful to the Pajaro Valley Water Management Agency for their cooperation, including access to the field site and facilities. This project was funded by the UC Water Security and Sustainability Research Initiative

(UCOP Grant # 13941), Gordon and Betty Moore Foundation grant GBMF5595, a P3 award (Grant # 83599501) from the U.S. Environmental Protection Agency, the National Science Foundation Graduate Research Fellowship Program, a USDA-NIFA grant (2017-67026-26315), and The Recharge Initiative (http://www.rechargeinitiative.org/). All data generated/recorded by the authors, including grain size, bulk and vertical infiltration rates, geochemical data and calculations, and microbiology data, is available at https://doi.org/10.7291/D14D4H.

ACCEPTED MANUSCRIPT

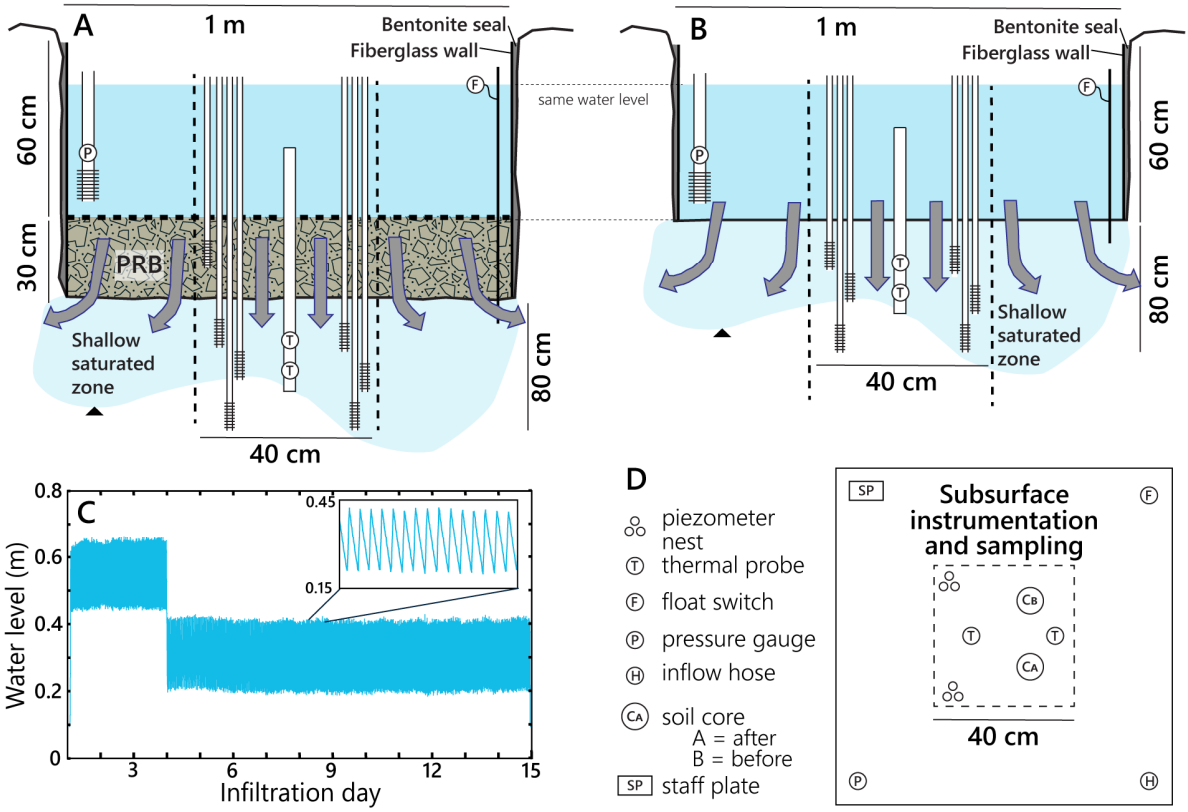

Figure 1. Experimental design. A. Cross section of PRB experimental plot, showing float valve (*F*), thermal sensors (*T*), pressure gauge (*P*), and piezometers. Black triangle shows the inverted water table created by infiltration. B. Cross section of native soil plot. C. Water level over time during test PRB1; inset highlights three hours of infiltration. D. Plan view of instrumented experimental plots, with central instrumented area delineated.

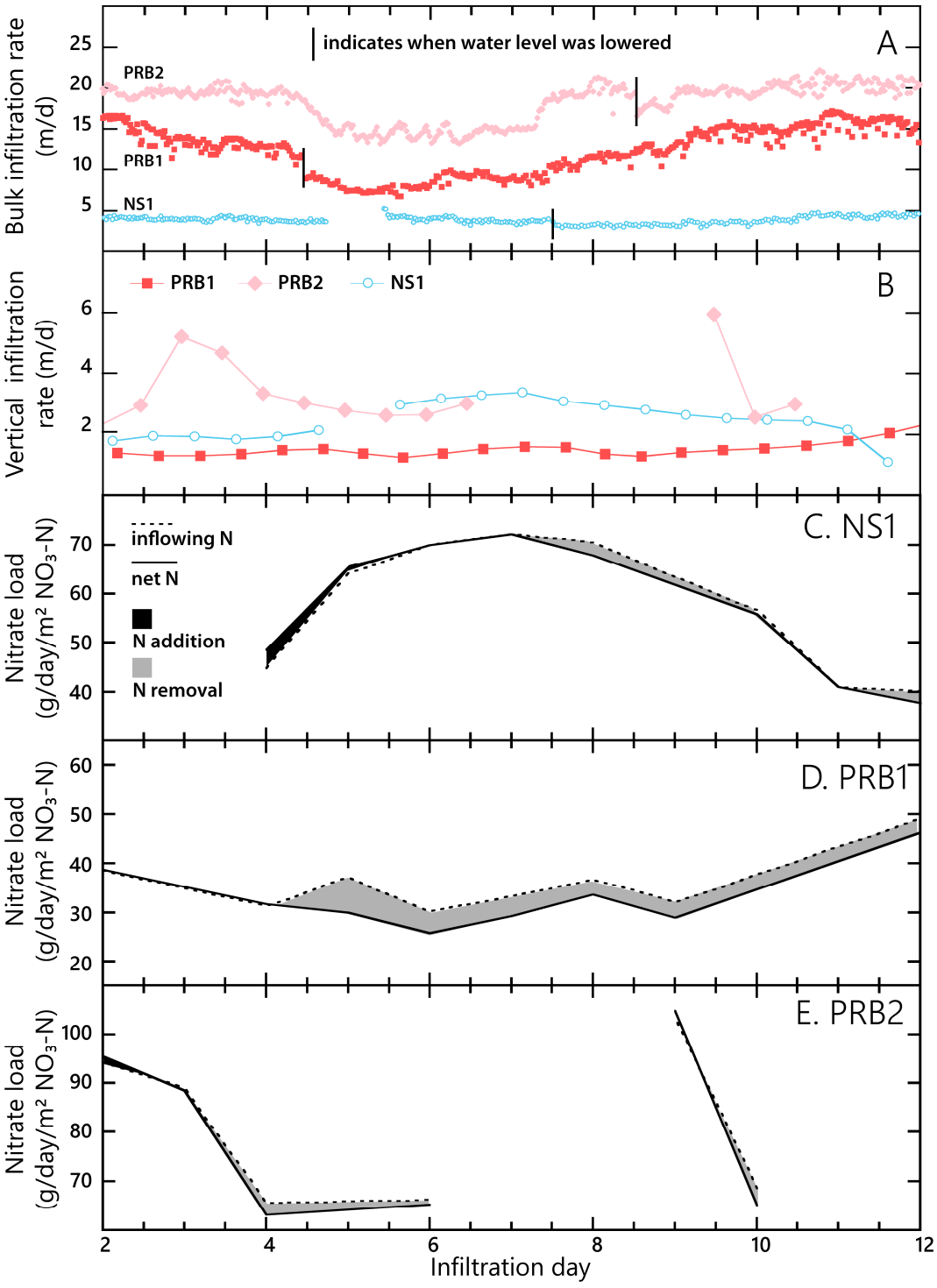

Figure 2. More nitrate was removed below a PRB than in native soil. A. Bulk infiltration rates. B. Vertical infiltration rates. C,D,E. Inflowing nitrate load (*dashed lines*) and net nitrate

load at deepest subsurface sample (*solid lines*). *Grey shading* indicates nitrate removal; *black shading* indicates nitrate addition. Data gaps during test PRB2 are times when vertical infiltration was too rapid to resolve accurately (see **Supporting Information**).

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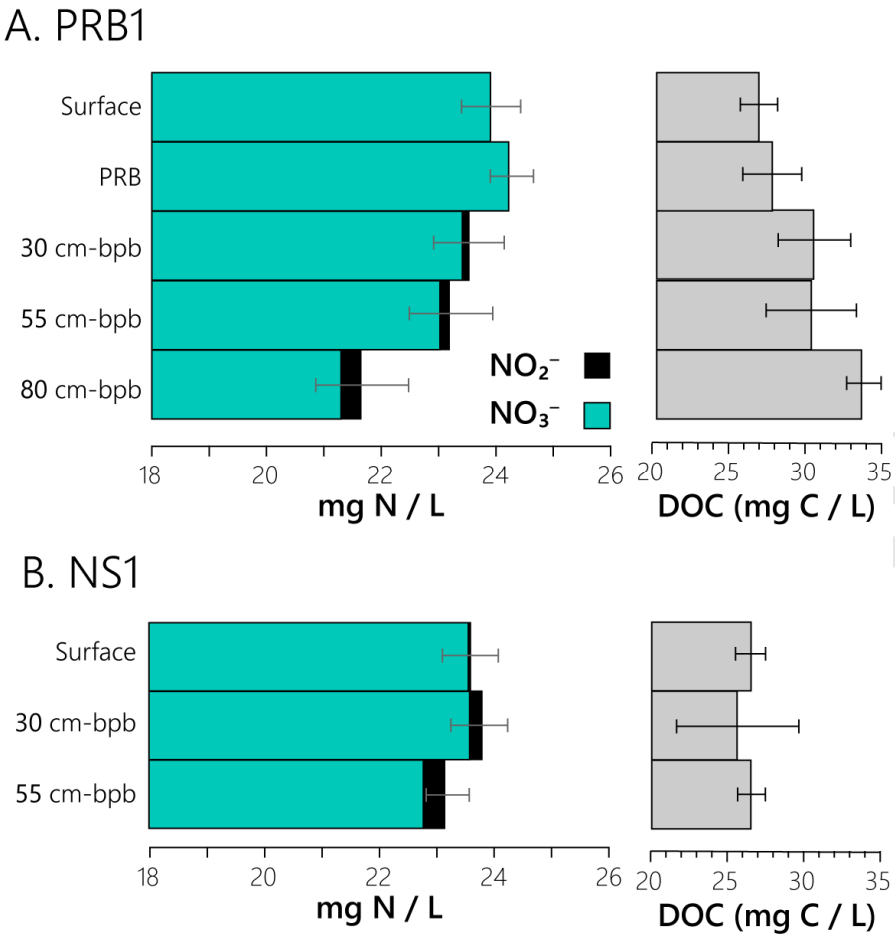

Figure 3. [NO_3-N] decreased and [DOC] increased with depth below the PRB; changes were less consistent in native soil. Average [NO_3-N] and [NO_2-N] (*left*) and [DOC] (*right*) with depth over days 5–12 from tests PRB1 (A) and NS1 (B). Error bars show one standard deviation for all samples.

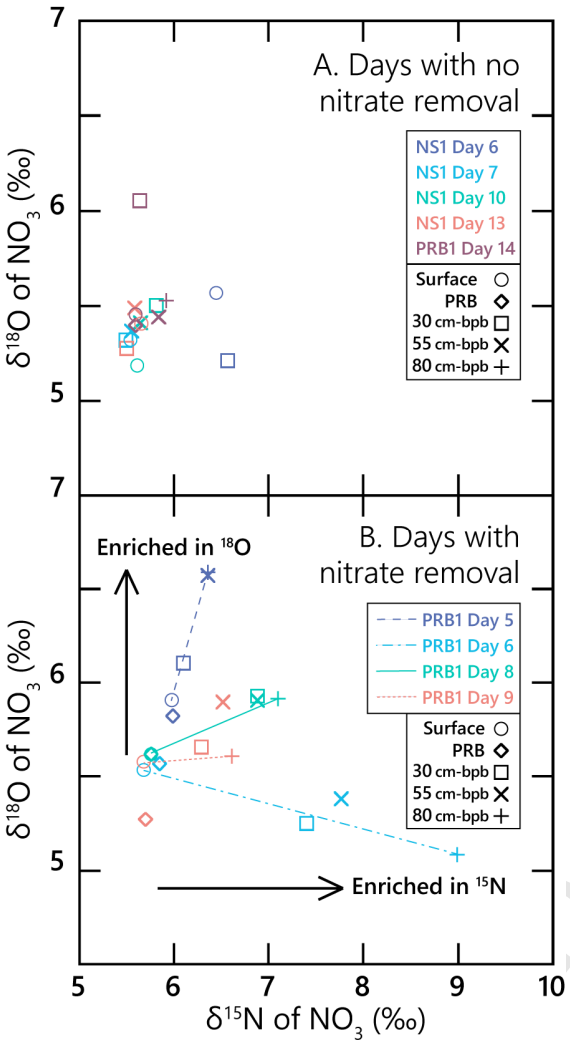

Figure 4. On days with nitrate removal, residual subsurface nitrate is enriched in $\delta^{15}N$ and $\delta^{18}O$, consistent with denitrification. A. $\delta^{15}N$ and $\delta^{18}O$ for five days with no nitrate removal. B. $\delta^{15}N$ and $\delta^{18}O$ for four days with nitrate removal; *dashed lines* connect surface and deepest samples.

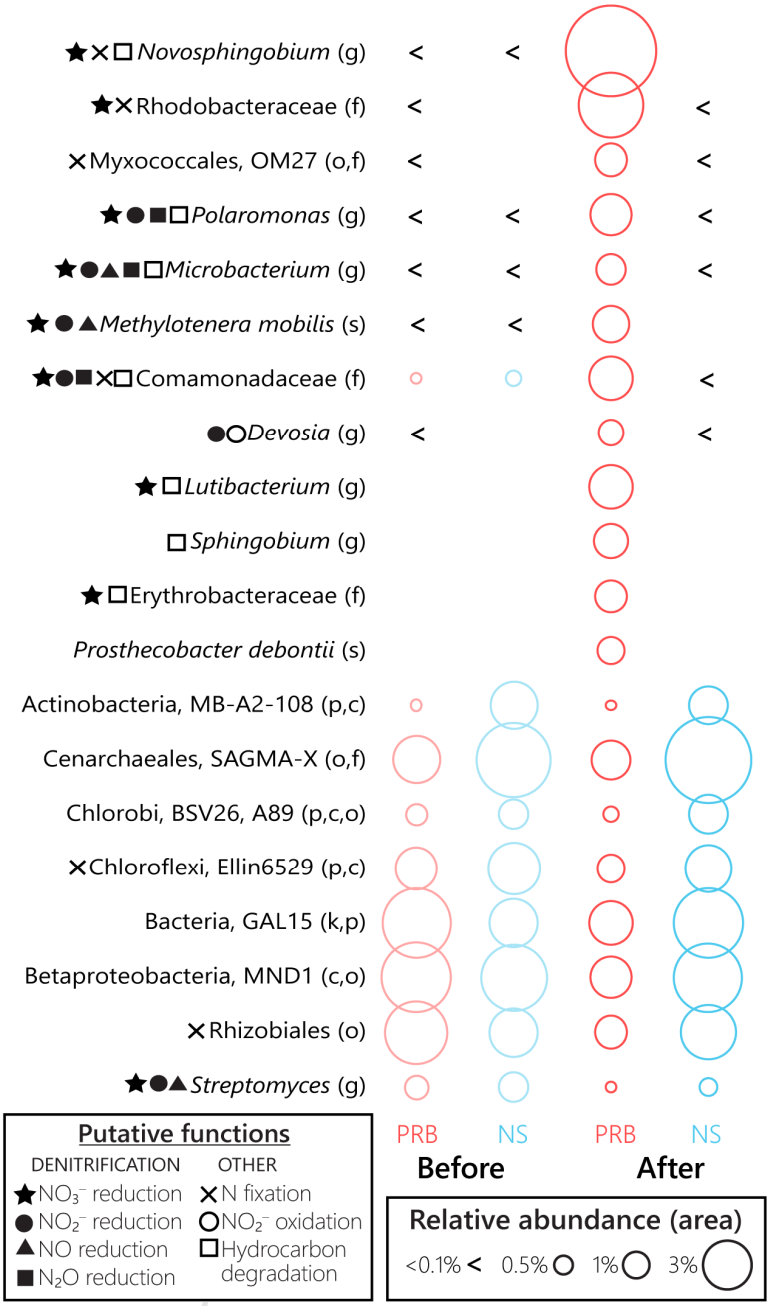

Figure 5. Infiltration through the PRB was associated with microbial community shifts, especially enhanced putative denitrifiers. OTUs with significant differences in relative abundance as determined by ANCOM (Mandal et al., 2015) between PRBA and NSA samples are shown at the lowest identified taxonomic level (see **Table S8** for complete taxonomy). Circle area represents relative abundance; relevant putative functions are labeled.

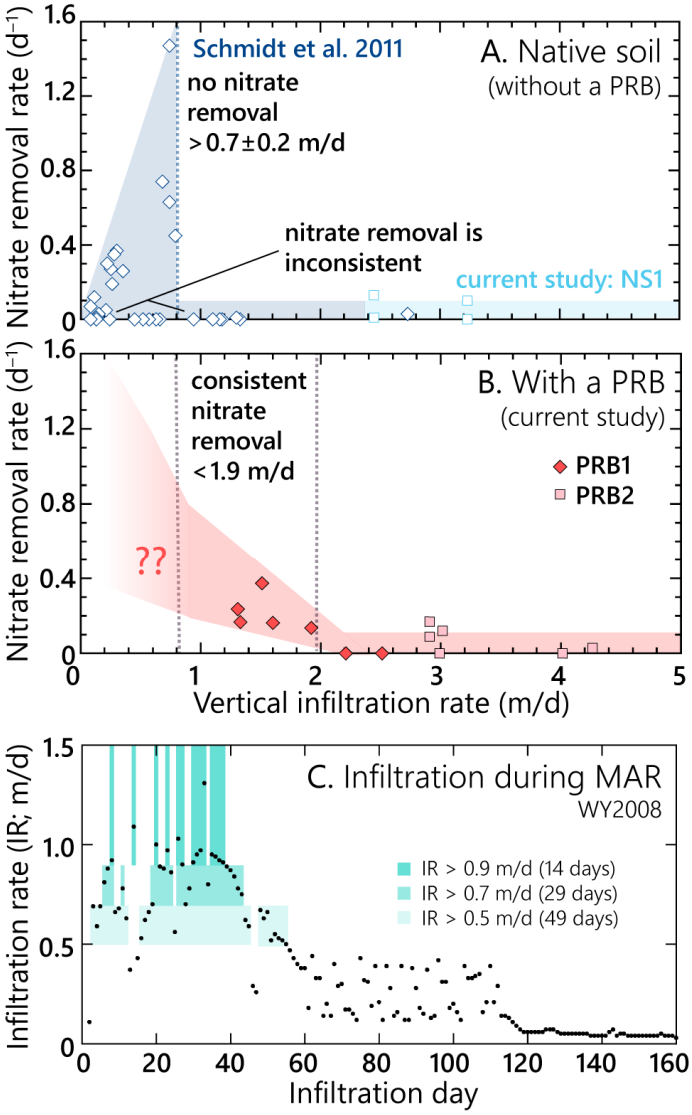

Figure 6. Below the PRB, nitrate removal was more consistent and occurred at higher infiltration rates than in native soil. A. Nitrate removal rate in native soil plotted against infiltration rate, using data from Schmidt et al. (2011) and the current study. B. Nitrate removal rate below a PRB plotted against infiltration rate. C. Infiltration rates during WY2008 at the Harkins Slough MAR project (Racz et al. 2011). Color shading indicates periods of rapid infiltration used to calculate additional nitrate removal that might have occurred with a PRB.

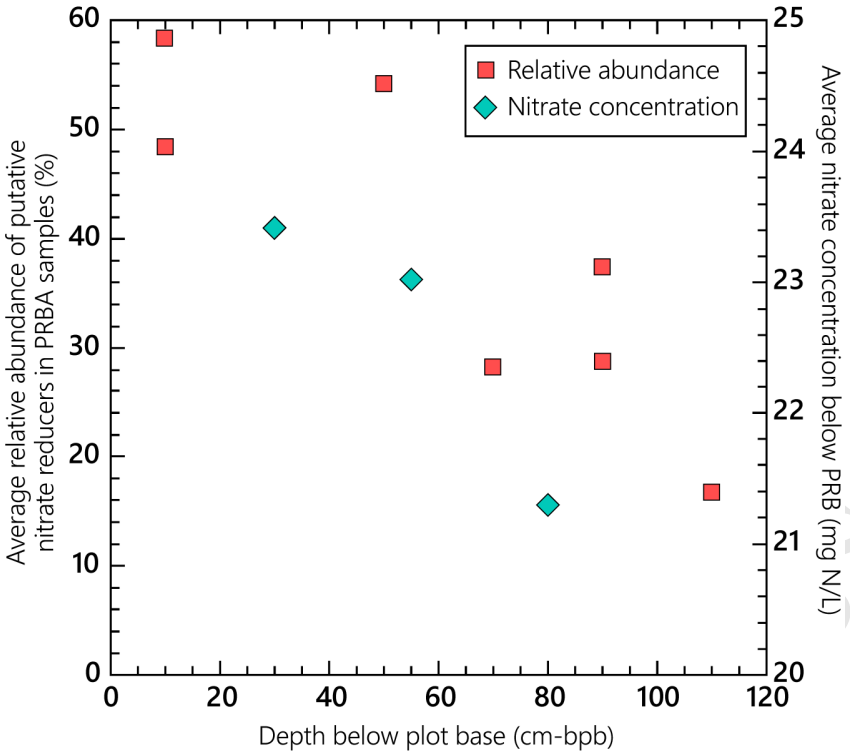

Figure 7. The relative abundance of putative nitrate reducers correlated with average nitrate concentrations in the soil below a PRB; both decreased with depth. *Red squares* show the average relative abundance of all putative nitrate reducers in PRBA samples up to 110 cm-bpb. *Teal diamonds* show the average concentration of nitrate in samples collected from 30, 55, and 80 cm-bpb during test PRB1.

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- A horizontal permeable reactive barrier (PRB) was tested for impacts on N-cycling
- The PRB was associated with enhanced nitrate removal during rapid infiltration
- Putative denitrifying microbial clades were enhanced in soils below a PRB
- Geochemical, isotopic, and microbial data are consistent with denitrification
- Using a horizontal woodchip PRB could improve water quality during managed recharge

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