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Authors

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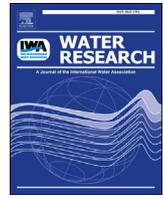
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# A horizontal permeable reactive barrier stimulates nitrate removal and shifts microbial ecology during rapid infiltration for managed recharge

Sarah Beganskas<sup>a,\*</sup>, Galen Gorski<sup>a</sup>, Tess Weathers<sup>a</sup>, Andrew T. Fisher<sup>a</sup>, Calla Schmidt<sup>c</sup>, Chad Saltikov<sup>b</sup>, Kaitlyn Redford<sup>b</sup>, Brendon Stoneburner<sup>b</sup>, Ryan Harmon<sup>a,1</sup>, Walker Weir<sup>a,2</sup>

<sup>a</sup> Earth and Planetary Sciences, University of California Santa Cruz, Santa Cruz, CA, 95064, USA

<sup>b</sup> Microbiology and Environmental Toxicology, University of California Santa Cruz, Santa Cruz, CA, 95064, USA

<sup>c</sup> Environmental Science, University of San Francisco, San Francisco, CA, 94117, USA

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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<sup>2</sup>/day NO<sub>3</sub>-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<sup>2</sup>/day NO<sub>3</sub>-N was removed on average in native soil. Residual nitrate in infiltrating water below the PRB was enriched in δ<sup>15</sup>N and δ<sup>18</sup>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; *Methylobacterium mobilis* and genera *Microbacterium*, *Polaromonas*, and *Novosphingobium* had log<sub>2</sub> 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.

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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 and Fisher, 2017; Bekele et al., 2011; Schmidt et al., 2011).

MAR can impact water quality as well as water supply (Hartog and Stuyfzand, 2017; Ma and 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<sub>3</sub><sup>-</sup>) is the most widespread nonpoint source

**Abbreviations:** MAR, managed aquifer recharge; PRB, permeable reactive barrier; NS, native soil; DOC, dissolved organic carbon; TOC, total organic carbon; TN, total nitrogen; OTU, operational taxonomic unit.

\* Corresponding author.

E-mail address: [sbeganskas@ucsc.edu](mailto:sbeganskas@ucsc.edu) (S. Beganskas).

<sup>1</sup> Present address: Hydrologic Science and Engineering, Colorado School of Mines, Golden, Colorado, 80401, USA.

<sup>2</sup> Present address: Hydrologic Sciences, University of Nevada Reno, Reno, NV, 89557, USA.

groundwater pollutant; elevated nitrate concentrations in streams and groundwater put human health and aquatic ecosystems at risk (Gurdak and Qi, 2012). Denitrification is the most-studied nitrate removal mechanism and involves progressive reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ ,  $\text{NO}$ ,  $\text{N}_2\text{O}$ , and finally  $\text{N}_2$  (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 and 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 and 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 and 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; Thiruvengatathari et al., 2008). Denitrification rates in PRBs and bioreactors vary over time and as a function of inflowing  $[\text{NO}_3^-]$ , 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 woodchip PRB could enhance nitrate removal during rapid infiltration for MAR. Specifically, we seek to address the following questions: (a) Is denitrification during rapid infiltration enhanced by brief passage through a carbon-rich PRB? (b) How does infiltration through a PRB affect microbial ecology in shallow soils? (c) How might using a horizontal PRB improve water quality during MAR? To address these questions, we conducted a novel series of plot-scale field experiments to represent shallow soil conditions during infiltration for MAR, collecting co-located and contemporaneous hydrologic, geochemical, and microbial samples. These interdisciplinary experiments were designed to compare nitrate removal processes in native soils to those within and below a horizontal woodchip PRB, with applications for simultaneously improving groundwater supply and quality via MAR.

## 2. Materials and methods

### 2.1. Field site

Field work was completed within the 2.5-ha Harkins Slough MAR infiltration basin in the Pajaro Valley, central coastal California, USA (Fig. S1). Land use in the Pajaro Valley is a mix of agricultural, urban, residential, and undeveloped, but most

groundwater use supports agricultural activities. Nitrate concentrations in major surface water bodies and groundwater often exceed the U.S. Environmental Protection Agency's Maximum Contaminant Level of 10 mg/L  $\text{NO}_3\text{-N}$  (Los Huertos et al., 2001; Pajaro Valley Water Management Agency, 2016).

The experimental site is located on eolian (dune) and alluvial deposits, and shallow soils are characterized as Baywood loamy sand, typically having ~81% sand, 16% silt, and 2% clay (USDA, 2014). The Harkins Slough MAR system is operated by a local agency that diverts water from a nearby wetland when flows and water quality are sufficiently high. Diverted water passes through a rapid sand filter before entering the infiltration basin. Recharged water is subsequently recovered from a network of shallow wells, mixed with recycled water (tertiary treated and disinfected) and groundwater from farther inland, and distributed to local customers in lieu of pumping from the regional aquifer. Earlier studies of the Harkins Slough MAR system examined infiltration dynamics and water quality during infiltration through native soils, focusing on the central, deeper part of the infiltration basin (Racz et al., 2011; Schmidt et al., 2011); the experiments presented in this study were located at higher elevation near the edge of the infiltration basin and explored how a PRB may stimulate nutrient cycling (Fig. S1).

### 2.2. Plot construction and operation

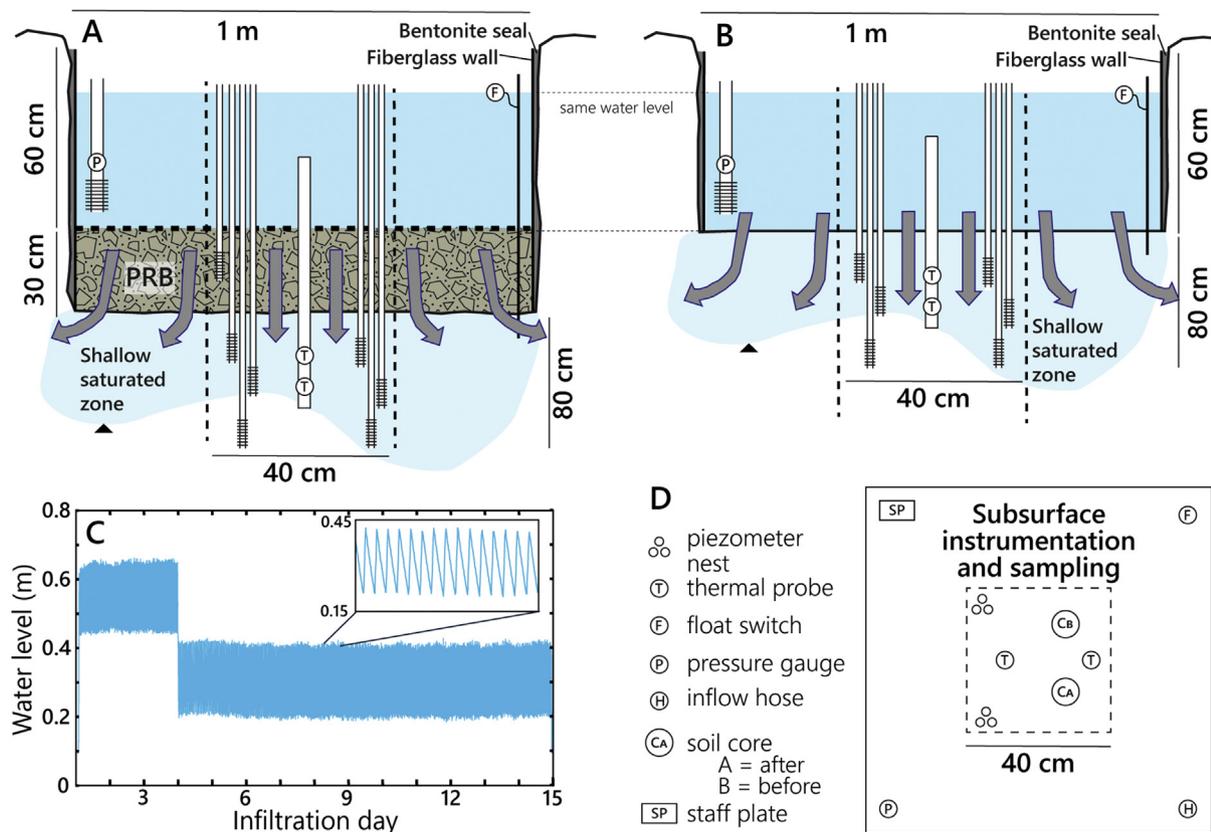
We constructed four hand-excavated infiltration plots, each 1 m<sup>2</sup> in area. Each plot was lined with fiberglass walls, caulked at the corners, and backed by bentonite (Fig. 1A and B). Two plots (NS1 and NS2) contained only native soil. In plots PRB1 and PRB2, a horizontal PRB consisting of a 30-cm-thick layer of redwood chips (0.5–2 cm in dimension and rough in shape) was installed above native soils. Woodchips were added to the plots by hand and gently consolidated, then covered with a coarse nylon screen held down by washed, rounded river rocks to prevent floatation. A hose delivered groundwater with elevated  $[\text{NO}_3^-]$  to the plots from a nearby well. A float switch and solenoid valve controlled water delivery, keeping the water level within a limited range (Fig. 1C). For each test, water infiltrated continuously for 14–15 days; we ran four tests in series over a 10-week period.

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<sup>2</sup> plots, especially near the edges (see Supporting Information). Accordingly, all instruments for subsurface sampling and measurements were installed within the central 0.16 m<sup>2</sup> of each plot, where flow is dominantly vertical (Fig. 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 (Fig. 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



**Fig. 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.

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 through a 0.45  $\mu\text{m}$  cellulose acetate filter into acid-washed, pre-rinsed polyethylene and glass bottles. Samples were put on ice immediately and stored at  $-20^\circ\text{C}$  until analysis.

#### 2.4. Infiltration rates

We converted absolute pressure at the plot base to water level, correcting for local barometric pressure. During periods when the solenoid valve was closed (every 10–20 min), preventing water from flowing into the plot, we calculated the bulk infiltration rate as change in water level over time. We independently calculated the vertical component of infiltration in the center of the plot with thermal probes installed in the soil. This technique uses heat as a tracer to quantify the vertical infiltration rate from the amplitude reduction with depth of periodic temperature fluctuations (Hatch et al., 2006). Thermal probes do not provide reliable infiltration data during the first and last few days of each test due to edge effects equivalent to the order of the filter applied to the thermal data (2–3 days); thus we focused our analysis on days 4–12 of each test.

#### 2.5. Soils characterization

To determine soil texture, splits from pre-infiltration samples were digested with 30% hydrogen peroxide (to remove organic

carbon), freeze dried, suspended in an eluent with 4 g/mL of sodium hexametaphosphate,  $(\text{NaPO}_3)_6$ , as a deflocculant, and analyzed in a Beckman Coulter LS 13320 Particle Size Analyzer. Running commercial and internal lab standards and repeat analyses of field samples indicated repeatability and precision of 3–5% (relative deviation) for each of 92 grain-size bins across a range of  $\leq 0.4 \mu\text{m}$  to 2 mm. Total organic carbon (TOC) and total nitrogen (TN) were analyzed in pre-infiltration samples using a Thermo Fisher Flash 2000. Samples were homogenized, oven-dried, vapor acidified for 24 h, and oven-dried again. They were packed into tin capsules, crushed into cubes, and analyzed. A certified soil reference material was analyzed every ten samples, giving a relative standard deviation of  $<3\%$ .

#### 2.6. Water chemistry

$[\text{NO}_3^-]$ ,  $[\text{NO}_2^-]$ , and  $[\text{NH}_4^+]$  were determined by colorimetric flow injection analysis on a Lachat Instrument QuickChem 800. Dissolved organic carbon (DOC) was measured by combustion catalytic oxidation using a Shimadzu TOC-VCSH total organic carbon analyzer. Regular analyses of sample splits, blanks, and laboratory standards indicate accuracy for both instruments of 3–5%. Concentrations of chloride, sulfate, bromide, and phosphate were analyzed using a Dionex ICS 2100. Standards were run every ten samples and all had errors  $\leq 10\%$ , with most  $\leq 5\%$ . We calculated daily nitrate load reduction ( $\text{g N/m}^2/\text{day}$ ) as:

$$[\text{NO}_3^-]_{\text{surface}} IR_V - [\text{NO}_3^-]_{\text{deepest}} IR_V$$

where  $IR_V$  is the vertical infiltration rate measured at the center of the plot. Normalized nitrate removal rates ( $\text{day}^{-1}$ ) were calculated as:

$$\frac{[\text{NO}_3^-]_{\text{surface}} - [\text{NO}_3^-]_{\text{deepest}}}{T_R [\text{NO}_3^-]_{\text{surface}}}$$

where  $T_R$  is the residence time of water between the surface and the deepest subsurface sample, calculated using  $IR_V$ .

A subset of 36 water samples from NS1 and PRB1 were analyzed for  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of nitrate (relative to air and Vienna Standard Mean Ocean Water, respectively) using bacterial denitrification at University of California, Davis's Stable Isotope Facility (Casciotti et al., 2002). This facility uses a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer. Twelve standards were run at regular intervals and showed no discernible drift, with standard deviations of 0.06‰ for  $\delta^{15}\text{N}$  and 0.15‰ for  $\delta^{18}\text{O}$ . For each day, nitrogen and oxygen enrichment factors were calculated using an approximation of the Rayleigh equation.

### 2.7. Phylogenetic sequencing

Soil DNA was extracted with the PowerSoil DNA Isolation Kit (QIAGEN) and quantified with a Qubit 4 Fluorometer (Invitrogen). Partial 16S rRNA genes (V4 and V5 variable regions) were amplified using primers modified to contain 5' sequencing adapters for barcoding and sequencing using the Illumina MiSeq platform. Samples were analyzed by agarose gel electrophoresis to confirm the presence of ~550 bp amplicons. An amplicon sequencing pipeline was adapted from the Illumina MiSeq platform protocol for 16S metagenomic libraries (Illumina Inc, 2013). The overall pipeline included the primary PCR using 16S rRNA gene primers (Parada et al., 2016), PCR clean-up, library preparation (adding unique sequencing indices [barcodes] to each PCR amplicon), normalizing DNA concentrations of each library, and library pooling. The pooled library was sequenced on the Illumina MiSeq (600 cycles v3 PE300 flow cell kit) at the University of California, Davis Genome Center. See Supporting Information for primer specifications, amplification protocols, and additional method details.

Paired-end sequence post-processing was performed with QIIME version 2018.2 (Caporaso et al., 2010) based on the analytical pipeline steps specified in Weathers et al. (2016) and using the QIIME2 plugins described below. Demultiplexing was summarized using demux (<https://github.com/qiime2/q2-demux>) and DADA2 (Callahan et al., 2016) was used for truncating and denoising. Truncation thresholds were calculated as in Parada et al. (2016) ensuring the average quality score for a 50 bp sliding window remained above 33. The QIIME2 plugin feature-table (McDonald et al., 2012b) was used to create visual summaries of sequences per sample. We trained a Naive Bayes classifier to our specific primers and assigned taxonomy with Greengenes reference database version 13.8 with 99% OTUs (McDonald et al., 2012a) using the feature-classifier plugin (<https://github.com/qiime2/q2-feature-classifier>). An additional comparison with SILVA 128 99% OTUs did not yield significant taxonomic differences after filtering, therefore the results presented herein were generated with the Greengenes reference database. See Supporting Information for details regarding plugins for taxonomy visualization, alignment, tree generation, diversity, and differential abundance.

All samples were filtered so the minimum total observed percent per OTU summed across all samples was 0.1%. OTUs are reported at the lowest identified taxonomic level. Log<sub>2</sub> fold-

changes were calculated per treatment as log<sub>2</sub> (average abundance after/average abundance before) to quantify each OTU's enhancement or inhibition during infiltration (Love et al., 2014). Sequence data have been submitted to the National Center for Biotechnology Information Sequence Read Archive database (SRA accession: SRP151895).

## 3. Results

### 3.1. Soils

Grain size data revealed predominantly sandy soils in all plots (Fig. S3), consistent with regional geology and mapped soil units. For all soil samples,  $d_{10}$  (10% finer) was >100  $\mu\text{m}$  and  $d_{50}$  > 245  $\mu\text{m}$ . 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).

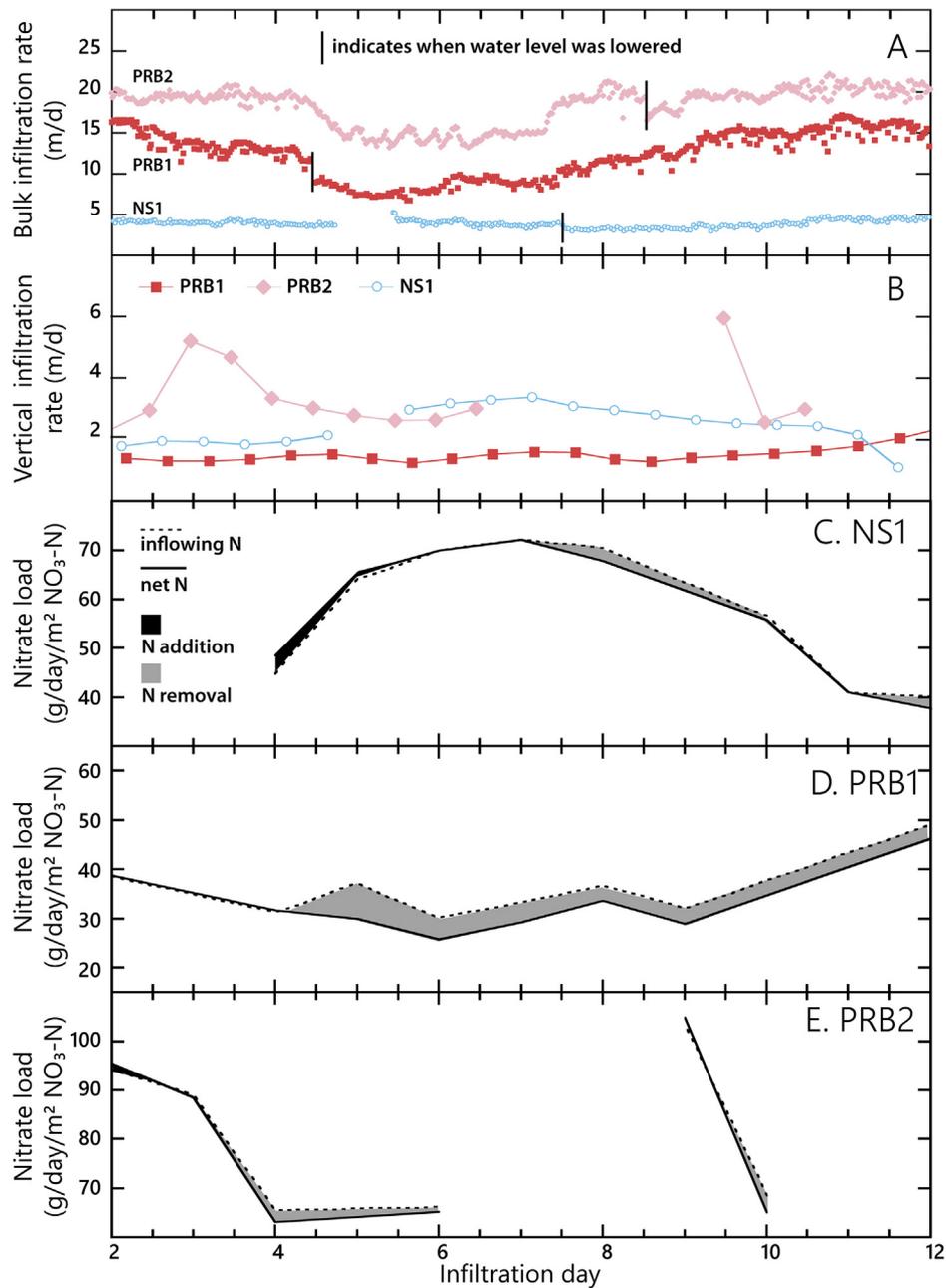
### 3.2. Infiltration

Sandy soils led to rapid infiltration (Fig. 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 (Fig. 2B). Vertical flow rates determined from multiple thermal probes in the same plot were similar (Fig. 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 (Fig. 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 (Fig. 1). On most days, inflowing water had consistent composition: 22–25 mg/L  $\text{NO}_3^-$ -N, 25–29 mg/L DOC, and little to no nitrite or ammonium. Although inflowing  $[\text{NO}_3^-]$  was relatively constant, nitrate load varied as a function of infiltration rate: 40–72 g/day/m<sup>2</sup>  $\text{NO}_3^-$ -N for NS1,



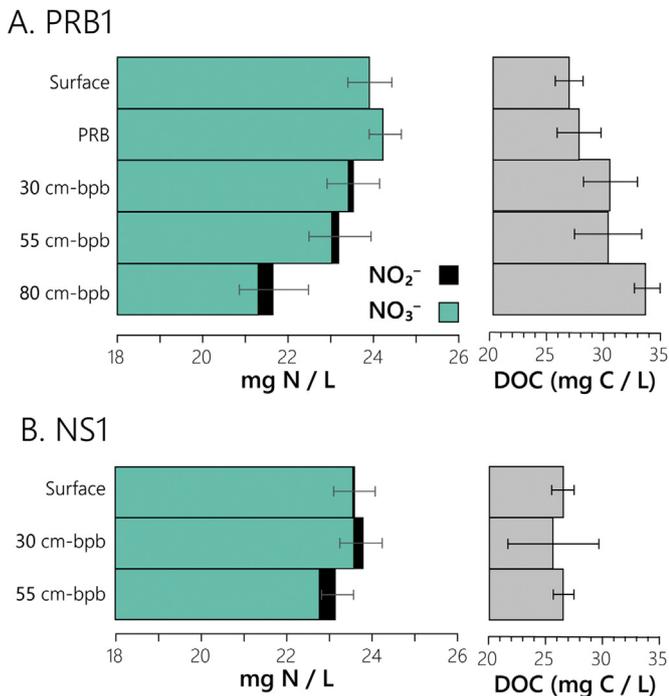
**Fig. 2.** More nitrate was removed below a PRB than in native soil. A. Bulk infiltration rates. B. Vertical infiltration rates. C,D,E. Inflowing nitrate load (dashed lines) and net nitrate load at deepest subsurface sample (solid lines). Grey shading indicates nitrate removal; black shading indicates nitrate addition. Data gaps during test PRB2 are times when vertical infiltration was too rapid to resolve accurately (see Supporting Information).

30–61 g/day/m<sup>2</sup> NO<sub>3</sub>-N for PRB1, and 66–104 g/day/m<sup>2</sup> NO<sub>3</sub>-N for test PRB2 (Fig. 2C,D,E). The water supply occasionally included a fraction of recycled water, readily identified by [NH<sub>4</sub>-N] >0.5 mg/L and/or [NO<sub>2</sub>-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 (Fig. 3).

Nitrate removal during test NS1 was inconsistent and modest, ranging from –3.6 gN/day/m<sup>2</sup> (net addition) to 2.7 gN/day/m<sup>2</sup>. In total, 1.2 gN/m<sup>2</sup> was removed over 14 days (Fig. 2C). During test PRB1, there was nitrate removal on days 5–12, peaking at 7.3 gN/day/m<sup>2</sup> on day 5. Cumulative removal during test PRB1

was 23.1 gN/m<sup>2</sup> over 15 days (Fig. 2D). Nitrate removal was less consistent during test PRB2, peaking at 3.5 g/day/m<sup>2</sup> and adding to a cumulative removal of 4.9 gN/m<sup>2</sup> over 15 days (Fig. 2E). On days when nitrate removal was observed, the largest changes occurred below 30 cm-bpb and coincided with small increases in [NO<sub>2</sub>-N] (Fig. 3).

Subsurface δ<sup>15</sup>N and δ<sup>18</sup>O differed little from surface values on days with no nitrate removal at depth (Fig. 4A). Residual nitrate was enriched in δ<sup>15</sup>N on all four days with measurable nitrate removal and enriched in δ<sup>18</sup>O relative to surface water on three of those four days (Fig. 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



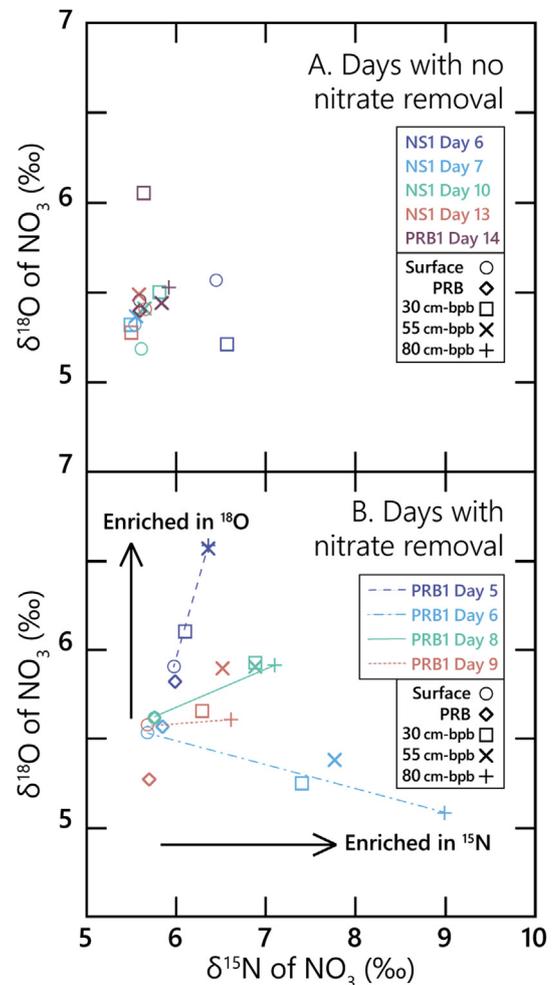
**Fig. 3.** [NO<sub>3</sub>-N] decreased and [DOC] increased with depth below the PRB; changes were less consistent in native soil. Average [NO<sub>3</sub>-N] and [NO<sub>2</sub>-N] (left) and [DOC] (right) with depth over days 5–12 from tests PRB1 (A) and NS1 (B). Error bars show one standard deviation for all samples.

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; Fukuda 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%) (Fig. 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 (Fig. 5). The OTU with the largest increase in relative abundance below the PRB was genus *Novosphingobium*, present at  $14 \pm 10\%$  after infiltration and  $0.004 \pm 0.01\%$  before infiltration, a log<sub>2</sub> fold-change of +11.8. When this genus was proposed, the ability to reduce NO<sub>3</sub><sup>−</sup>, the first step in denitrification, was a defining characteristic (Takeuchi et al., 2001). Enhanced OTUs with the potential to reduce NO<sub>3</sub><sup>−</sup>, NO<sub>2</sub><sup>−</sup>, and NO include *Methylotenera mobilis* (with a log<sub>2</sub> 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<sub>3</sub><sup>−</sup>, NO<sub>2</sub><sup>−</sup>, and N<sub>2</sub>O 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



**Fig. 4.** On days with nitrate removal, residual subsurface nitrate is enriched in δ<sup>15</sup>N and δ<sup>18</sup>O, consistent with denitrification. A. δ<sup>15</sup>N and δ<sup>18</sup>O for five days with no nitrate removal. B. δ<sup>15</sup>N and δ<sup>18</sup>O for four days with nitrate removal; dashed lines connect surface and deepest samples.

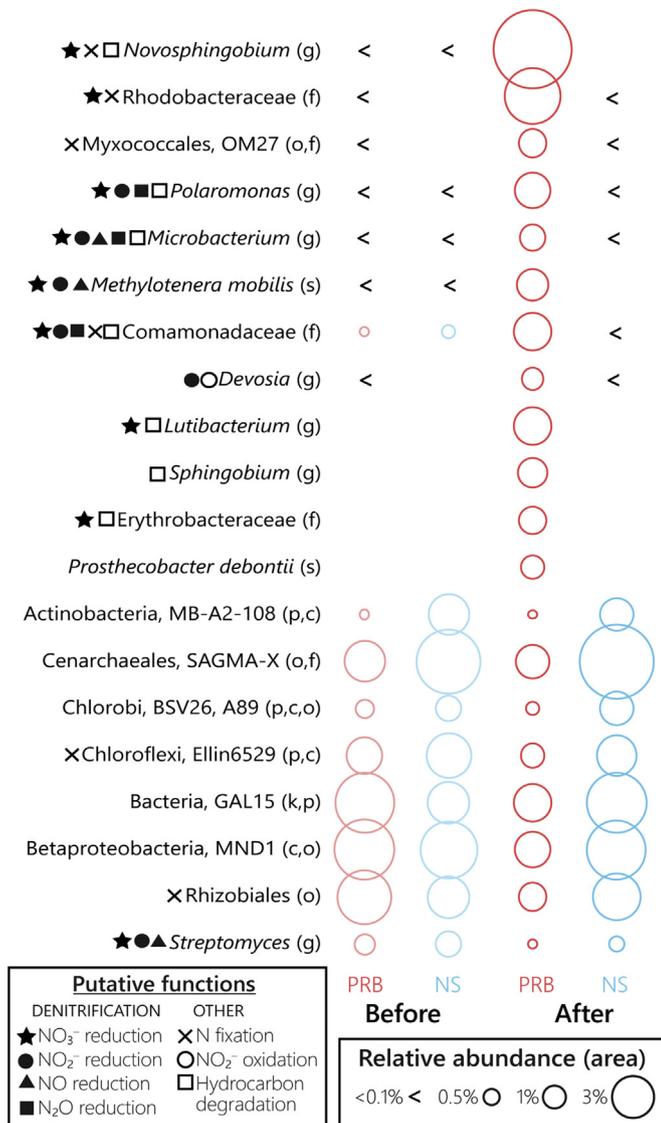
significantly enhanced in NSA samples. Additionally, many OTUs were inhibited in PRBA samples; one putative denitrifying genus, *Streptomyces*, had a log<sub>2</sub> fold-change of −2.2 (Kumon et al., 2002).

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 conditions for denitrification as well. Furthermore, many studies have explored the potential for microbes to co-metabolize micropollutants and other contaminants under denitrifying conditions (Suarez et al., 2010; Tarlera, 2003), and have found that co-metabolic processes can be enhanced with the addition of a carbon source (Li et al., 2013).

## 4. Discussion and implications

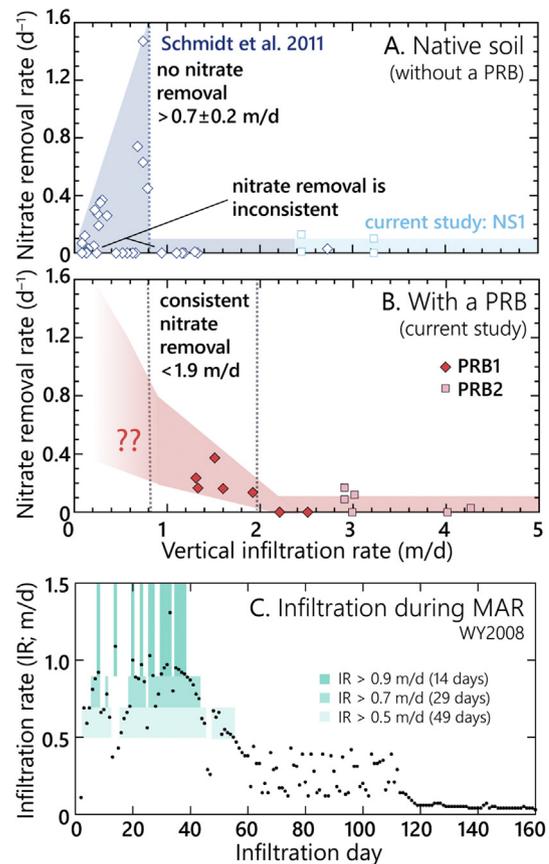
### 4.1. Infiltration rates and nitrate removal

To assess the potential benefit of a horizontal PRB during MAR, we compare results from plot-scale experiments to those from an



**Fig. 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.

earlier study at the same field site that observed nitrate removal during MAR operations using native soil (without a PRB) (Schmidt et al., 2011). During MAR operations, mean infiltration was slower and initial [NO<sub>3</sub><sup>-</sup>] was lower than in the present study. Nitrate removal during infiltration through native soil occurred only when vertical infiltration rates were  $<0.7 \pm 0.2$  m/d (Fig. 6A). At higher infiltration rates, it was inferred that oxic conditions were maintained throughout the saturated soil, limiting redox conditions needed for efficient denitrification (Schmidt et al., 2011). Additionally, for 12 out of 23 measurements when the vertical infiltration rate was  $<0.9$  m/d, there was little to no nitrate removal. Thus, having an infiltration rate through native soil below the identified threshold did not guarantee that nitrate removal would occur during MAR operations. In the present study, vertical infiltration rates in native soil were always  $>0.9$  m/d and limited nitrate removal occurred, consistent with earlier work. However, during tests PRB1 and PRB2, nitrate removal occurred on every day with an



**Fig. 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). Shading indicates periods of rapid infiltration used to calculate additional nitrate removal that might have occurred with a PRB.

infiltration rate  $<1.9$  m/d (Fig. 6B), a much higher threshold and a more consistent pattern than observed in native soil.

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 (Fig. 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  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  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  $\leq 6$  mg/L, but  $[\text{NH}_4^+]$  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  $\text{N}_2$  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,  $[\text{NO}_3^-]$  decreased with depth (Fig. 3); depth was also an important factor in explaining community variance for PRBA samples (Fig. S5B). Putative nitrate-reducing OTUs ranged from having a total relative abundance in PRBA samples of 16.6%–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  $[\text{NO}_3^-]$  with depth (Fig. 7).

Most OTUs that were enhanced in PRBA samples were 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 explanation is that the PRB elevated concentrations of biologically available organic carbon in underlying soils, consistent with higher observed [DOC] (Fig. 3), promoting more rapid microbially-mediated dissolved oxygen consumption.

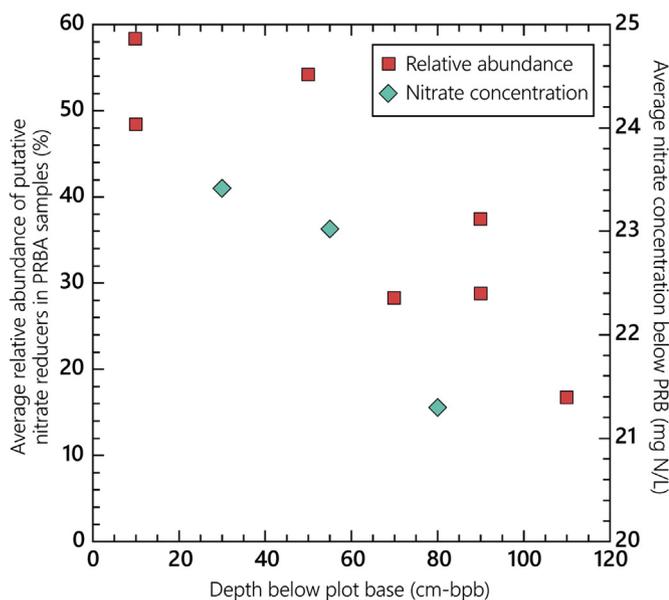
#### 4.3. Comparing a horizontal PRB to a denitrifying bioreactor

Results in this study are consistent, in some ways, with previous work using bioreactors; favorable redox and other conditions may stimulate denitrification in many contexts. However, in typical denitrifying bioreactors and beds, most nitrate removal occurs within the reactive material and hydraulic retention times range from several hours to many days (Addy et al., 2016; Warneke et al., 2011). In contrast, in this study we did not observe nitrate removal within the woodchip PRB, where residence times were often 1–2 h and always  $< 6$  h. Instead, we observed nitrate removal in soils up to 80 cm below the PRB (Fig. 3), likely due to more favorable conditions for ambient soil microbes capable of nitrate removal (Fig. 5).

Denitrifying bioreactors and beds are often used to improve the quality of treated wastewater in which  $[\text{NO}_3^-]$  and  $[\text{NH}_4^+]$  (along with other constituents) are elevated far above drinking water standards. These systems can be designed and operated to optimize selected biochemical processes, including nuanced controls on fluid flow rate and associated hydraulic retention time during operation. In contrast, while MAR systems can be designed to achieve specific goals for water supply and quality improvement, they are influenced strongly by ambient, often heterogeneous, soil properties. Rapid infiltration through well-drained soils may result in the formation of a thin (or no) saturated zone below some parts of an infiltration basin, limiting opportunities for establishing redox conditions favorable to denitrification. The flow rate through a denitrifying bioreactor or bed can be reduced to improve nitrate removal rates, but slowing the flow applied to a well-drained infiltration basin is likely to result in development of shallow unsaturated conditions, virtually ending denitrification until saturated soil conditions are restored. This tradeoff emphasizes the importance of considering soil infiltration properties when choosing locations for MAR and/or designing them with specific infiltration targets.

#### 4.4. Implications for MAR design and operation

These results suggest that significant water quality benefit may be achieved by adding a horizontal PRB to an MAR infiltration basin. Nitrate removal rates are sensitive to infiltration rate (Fig. 6A and B), which varies spatially and temporally during MAR (Mawer et al., 2016; Racz et al., 2011). There is particular potential for a horizontal PRB to facilitate nitrate removal benefit when infiltration rates are above the observed threshold in native soils ( $0.7 \pm 0.2$  m/d at this study's location; Schmidt et al., 2011) and below the threshold for soils below a PRB (1.9 m/d at this study's location; Fig. 6B). MAR projects are typically intended to achieve rapid infiltration (0.5–2 m/d) that maximizes water supply benefit. During MAR operations at Harkins Slough for WY2008, mean infiltration rates  $> 0.5$  m/d were maintained for about a third of the operating season and  $> 0.9$  m/d for 14 days; mean infiltration rates were always  $< 1.9$  m/d (Fig. 6C). During rapid infiltration for test PRB1, the average nitrate removal rate was  $1.5$  g/day/m<sup>2</sup>  $\text{NO}_3\text{-N}$ . If this rate were representative of average conditions throughout an infiltration basin, it would be equivalent to  $15$  kg/day/ha. Thus, for a 2.5-ha infiltration basin like Harkins Slough, adding a horizontal PRB could potentially contribute an additional  $37.5$  kg of  $\text{NO}_3\text{-N}$  removal on each day with infiltration rates  $> 0.7 \pm 0.2$  m/d and  $< 1.9$  m/d. For the 14 days with infiltration  $> 0.9$  m/d at Harkins Slough in WY2008,



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

this comprises an additional 525 kg of NO<sub>3</sub>-N removal; for the 49 days with infiltration <0.5 m/d, this yields 1840 kg of additional NO<sub>3</sub>-N removal. There is considerable spatial heterogeneity and temporal variability in hydrologic, biogeochemical, and microbial conditions during MAR, so these results need to be considered carefully in assessing how a horizontal PRB might best be integrated with MAR for improved water quality.

An additional consideration is the lifespan and maintenance requirements such a system. Woodchip PRBs can provide years of water quality improvements (Robertson, 2010; Robertson et al., 2008), but aging may reduce denitrification efficiency. The speciation of leached carbon from woodchips (Page et al., 2002), and how that speciation may change or diminish over time during MAR, merits further study. It may be efficacious for MAR to mix the soil amendment into the substrate directly, rather than emplacing it as a distinct layer. This approach has the advantage that adding fresh material can be included as part of regular maintenance, including sediment scraping and disking that “opens up” soil pores after operation. Additional field and lab experiments are underway to evaluate the efficacy of mixing PRB materials in with native soil, rather than creating a separate layer.

Designing an MAR project that removes contaminants as water infiltrates would provide a measure of safety, which is especially important when non-pristine water sources are collected to augment supplies (e.g., stormwater runoff, recycled wastewater). Installing a PRB horizontally in an MAR basin has advantages over traditional vertical PRBs: the horizontal PRB has relatively low installation and replacement cost, removes nitrate before it reaches an aquifer, does not require the presence or detailed knowledge of a hydraulic gradient, and works within an MAR 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.

## Declarations of interest

None.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.watres.2018.07.039>.

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