Geo-Bio Interfaces (2025), **2**, e7, 1–15
doi:10.1180/gbi.2025.3

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Article

Factors influencing the efficacy of microbial remediation of selenium in groundwater near a coal-fired power plant

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Abstract

Selenium is a widespread contaminant released by industrial activities such as coal combustion. In selenium-contaminated groundwater, native microbial communities commonly have the capability of reducing the toxic oxyanions selenate and selenite to insoluble elemental selenium. The impact of local hydrogeography on microbial selenium reduction was tested by constructing laboratory microcosms using biofilm and groundwater collected from four monitoring wells screened in three distinct stratigraphic units near fly ash disposal ponds in southeastern Montana. Glycerol, methanol and molasses were tested as carbon amendments. Nitrate and selenium concentrations were monitored, and microbial communities were sequenced to examine differences among sites and carbon amendments. All site and carbon combinations resulted in nitrate removal, though molasses had the highest removal rate. Selenium removal was significantly impacted by stratigraphic unit, with microcosms from alluvial wells removing more total selenium than those from coal and interburden wells. Microbial community composition was correlated with site, carbon amendment, and nitrate and selenium removal. Furthermore, two genera from the order *Clostridiales, Desulfosporosinus* and *Gracilibacter*, emerged as potential indicator organisms for selenium reduction in this environment. The site, carbon amendment, and microbial community were all found to potentially impact remediation efficacy.

Keywords: selenium reduction; groundwater remediation; denitrification; microbial community analysis

(Received 30 April 2024; revised 19 September 2024; manuscript accepted: 03 February 2025)

Introduction

Selenium is an essential trace nutrient used for the production of the amino acid selenocysteine and other biologically important molecules. However, selenium can begin to have toxic effects on humans and animals at levels only one order of magnitude higher than essential concentrations (Nancharaiah and Lens, 2015; Lenz and Lens, 2009). Excess selenium intake by humans and other mammals leads to selenosis, which most commonly causes neurological and dermal symptoms (Tan et al., 2016). Recent evidence suggests that selenium can also increase the prevalence of antibiotic resistance genes in microbes, which may lead to difficult-to-treat infections (Shi et al., 2021). Fish, birds and amphibians suffer more severe consequences, including reproductive impairment and embryotoxicity; in extreme cases, chronic exposure has caused the extinction of local fish populations (Chapman et al., 2010).

Anthropogenic sources of selenium in the environment include agriculture, mining and coal combustion (Tan et al., 2016). Fly ash water, one of the waste products of coal combustion generated from the disposal of fly ash in ponds or as a slurry is a major source of selenium contamination from coal-fired power plants (Lemly, 2004). Power plants either recycle fly ash water in plant operations or discharge it in accordance with the Clean Water Act. While the US EPA's 2015 steam electric rule (40 CFR 423) reduced allowable selenium concentrations in wastewater, legacy selenium contamination remains in surface and groundwater near many coal-fired power plants. Both effluent discharge to surface water and leaching from fly ash disposal basins into groundwater have caused selenium contamination. Fly ash basin waters contain, on average, 70 ppb of selenium (Dorman et al., 2010), which exceeds the drinking water maximum contaminant limit of 50 ppb (EPA 2023). Effective remediation technologies are necessary to treat selenium in waters impacted by fly ash leaching.

In situ bioremediation by native microbial communities represents a viable, cost-effective option for treating selenium in contaminated groundwater (Eswayah et al., 2016; Tan et al., 2016). Selenate (Se(VI)) and selenite (Se(IV)) are both soluble and bioavailable, though selenite is 5–10 times more toxic (Fernandez-Martinez and Charlet, 2009; Romero et al., 2019). Elemental selenium (Se(0)) is insoluble and thus precipitates under typical groundwater conditions (Eswayah et al., 2016). Native microbes can typically reduce selenate to selenite and selenite to elemental selenium (Se(0)) (Eswayah et al., 2016). Once selenate reduction begins, selenite reduction typically follows, but selenite accumulation can occur under some conditions. For example, selenate-reducing bioreactors accumulated selenite when retention times

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Cite this article: Koepnick H.R., Peyton B.M., & Lauchnor E.G. (2025). Factors influencing the efficacy of microbial remediation of selenium in groundwater near a coal-fired power plant. *Geo-Bio Interfaces* **2**, e7, 1–15. https://doi.org/10.1180/gbi.2025.3

were low or when the reactors were run in batch mode (Fujita et al., 2002; Yan et al., 2020). More research on these native microbial communities in selenium-impacted groundwater is needed to better understand the environmental factors that impact Se removal *in situ*.

Many selenium-contaminated waters also have elevated nitrate concentrations. For example, pore water in coal mine waste rock dumps typically has elevated levels of both nitrate and selenium (Mahmood et al., 2017). This typically necessitates the removal of nitrate prior to selenium removal, as most microbes capable of selenate reduction do so via nitrate reductases, which have specificity for nitrate and thus will not begin reducing selenate until nitrate is depleted (Basaglia et al., 2007; Gates et al., 2011; Bailey et al., 2012; Hunter 2014). However, studies with mixed consortia sometimes show simultaneous selenate reduction and denitrification, such as in a bioreactor inoculated with sediment from an end-pit lake and in batch experiments using a consortium of enriched mine site bacteria (Luek et al., 2014; Subedi et al., 2017). Nitrite reductases similarly catalyse selenite reduction (DeMoll-Decker and Macy 1993; Basaglia et al., 2007), though several other pathways also exist. Selenite reduction may be catalysed by fumarate, sulfite and glutathione reductases (Hunter, 2014; Li et al., 2015; Huang et al., 2021) or mediated by non-enzymatic mechanisms such as extracellular polymeric substances (EPS) and thiol-containing molecules (Lampis et al., 2017; Zhang et al., 2020).

Successful microbial treatment of selenium-contaminated waters has been demonstrated under field conditions. In situ biotransformation of selenate to Se(0) during injection of acetate occurred in a contaminated aquifer in Colorado (Williams et al., 2013, Fakra et al., 2015). Biofilms containing large amounts of Se(0) formed on the tubing used to circulate groundwater. In another study, field-scale flow reactors treating discharge from an end-pit lake and inoculated with lake sediment achieved > 95% Se reduction (from an initial concentration of 92 ppb) and a complete reduction of nitrate (Luek et al., 2014). Treatment efficacy was not affected by fluctuating water temperatures as low as 2°C.

Further evidence of bioremediation potential has been demonstrated in laboratory experiments using enrichment cultures from a variety of contaminated environments, including coal mining waste and waters impacted by coal mining effluent (Yang et al., 2011; Baldwin and Hodaly 2003; Luek et al., 2014; Nkansah-Boadu et al., 2021), phosphate mining waste and waters impacted by phosphate mining effluent (Knotek-Smith et al., 2006), alluvial aquifers (Nelson et al., 2003), agricultural drainage (Zhang et al., 2008), and paper mill wastewater treatment (Tan et al., 2018). The majority of laboratory experiments described above were incubated at temperatures between room temperature (20°C) and 30°C. The only exception reported is the study using phosphate mining waste, where columns were incubated at 12°C (Knotek-Smith et al., 2006). Microbial selenium remediation potential has not yet been investigated in areas contaminated by coal-fired power production, and only a few studies (Knotek-Smith et al., 2006; Luek et al., 2014; Zhou et al., 2021) have investigated selenium reduction at cold temperatures.

The study presented here investigated the impact of water chemistry, stratigraphy and carbon source on selenium removal efficacy using subsurface microbial communities obtained from the area around the Colstrip coal-fired power plant in southeastern Montana, which, to our knowledge, had not been investigated previously. The groundwater temperature at our study site is approximately 10°C, and unlike many of the laboratory tests described above, enrichments were incubated at the same temperature. Thus, this study provides insight into groundwater microbial communities that reduce selenium at cold temperatures typically encountered in situ in colder climates. Contaminated seepage from fly ash disposal ponds has impacted nearby groundwater (Montana Department of Environmental Quality, 2025). The site's hydrogeology is highly variable, and selenium concentrations in monitoring wells reflect this variability, ranging from < 1 ppb to > 100 ppb total Se (Hydrometrics, 2021). Microbial biofilm samples were obtained from wells screened in different stratigraphic units containing varying concentrations of selenium and nitrate. Three carbon amendments (methanol, glycerol and molasses) were tested in microcosms constructed using the biofilm samples and groundwater from their respective wells. We examined the hypothesis that site conditions – primarily stratigraphy and water chemistry – as well as an added carbon source, affect microbial community composition and selenium removal efficacy, which may ultimately affect the outcome of in situ remediation efforts.

Methods

Site description and well selection

The Colstrip Steam Electric Station (SES) consists of four coal-fired steam electric generating units: two 333 megawatt units, which were retired in January 2020, and two 805 megawatt units, which remain active (Neptune and Company, 2017; Hydrometrics, 2021). As of the end of 2020, approximately 80 active coal-fired power plants in the USA were of a similar size (1000–2000 megawatt total nameplate capacity) (United States Energy Information Administration, 2021). Holding ponds in two separate locations receive fly ash scrubber slurry from the plant units. The SES is a zero-discharge facility; water from the ash basins is recycled to the scrubbers (Neptune and Company, 2017). Colstrip is located in southeastern Montana in the northern Powder River Basin (PRB). Geologically, the top stratigraphic layer in the PRB is the Fort Union Formation, which includes sandstone, siltstone and shale strata. Area stratigraphy also includes numerous coal seams, clinker, shallow bedrock, alluvium and colluvium, which makes the site hydrogeology complex and difficult to predict (Neptune and Company, 2017).

The evaporation ponds serving units 1 and 2 of the SES are located northwest of the town of Colstrip, approximately ¼ mile north of Castle Rock Lake, as seen in Fig. 1. The stage I ponds were in operation from 1975 to 1997: these ponds are capped, and a reclamation programme was completed in 2002. The stage II evaporation ponds (STEP), consisting of five lined cells, began receiving scrubber slurry in 1992 and, at this time, are still in operation. The STEP pond area is 110 acres, with an estimated seepage rate of 17 gpm (Hydrometrics, 2017a). Two monitoring wells near the STEP were selected to evaluate the potential impact of stratigraphy on microbial community composition and selenium bioremediation. All sampled wells will be referred to by the well descriptions given in Table 1 in subsequent sections of this paper. The selected wells have the same depth (40 feet) with similar water chemistry but have different selenium concentrations and are screened in different stratigraphic units (NewFields, 2017a). As shown in Fig. 1, well 366S (alluvial) is located approximately 600 feet from Castle Rock Lake and 2600 feet from the STEP and is screened in alluvium. It has an elevated selenium concentration of around 100 ppb, a nitrate-N concentration of 2.0 mg/L and a TDS concentration of 8710 mg/L (Table 1; Talen Montana, unpublished data). Well 368D (interburden) lies approximately 1400 feet northwest of well 366S, approximately 1,500 feet from both the STEP and the lake, and is screened in the interburden. It has a selenium concentration of ~10 ppb, a nitrate concentration of 3.9 mg/L and a TDS of 11,000 mg/L (Table 1).

https://doi.org/10.1180/gbi.2025.3 Published online by Cambridge University Press

Figure 1. Locations of sampled wells (Esri, 2024; Talen Montana, unpublished data).

Table 1. Well descriptions, groundwater Se, NO_3-N and TDS concentrations, and pH. Nitrate concentrations from day 0 of these experiments; all other values from spring 2018 biannual sampling, results provided by Talen Montana LLC (Colstrip, MT, USA)

Well ID	366S	368D	1030A	633M
Well description	Alluvial	Interburden	Background	Coal
Stratigraphic Unit	Alluvium	Interburden	Alluvium	McKay Coal
Well depth, ft	40	40	23	16
Selenium, ppb	101	11	13	111
Nitrate-N, mg/L	2	3.9	1	13.6
TDS, mg/L	8710	11000	8840	9430
pH	7.4	7.4	7.5	7.2
Latitude	45.895	45.898	45.847	45.870
Longitude	–106.649	–106.653	–106.516	–106.534

The effluent holding ponds (EHP) serving units 3 and 4 of the SES began receiving scrubber slurry in 1983 and are located approximately 2.5 miles southeast of the plant. The EHP also includes cells that receive dry bottom ash or coal combustion residuals. The cells were initially lined with native soils amended with bentonite; since 2005, some of the individual cells have been re-lined with a synthetic liner. The capacity of the EHP ponds is 17,000 acre-feet (21,000,000 m^3), with an estimated seepage rate of 243 gpm (Hydrometrics, 2017b). As shown in Fig. 1, in the EHP area, well 1030A (background) is located approximately 1.5 miles southeast of the EHP. It has not been impacted by pond seepage, as a capture system located between the EHP and well 1030A achieves complete capture within the alluvium (NewFields, 2017b). This well was selected as a background reference site because it has nitrate (1.0 mg/L) and TDS (8840 mg/L) concentrations below the background screening levels (BSLs) (Neptune and Company, 2017). It is screened in alluvium at a depth of 23 feet. By contrast, of all of the selected wells, well 633M (coal) has the highest levels of selenium (111 ppb) and nitrate (13 mg/L). The TDS concentration is 9430 mg/L (Table 1). It is approximately 800 feet from the EHP and is screened in McKay coal at a depth of 16 feet (Fig. 1).

Field sampling

Cylindrical stainless steel tea infusers were modified to create downwell microbial samplers similar to those described previously (Peyton and Truex, 1997; Barnhart et al., 2013) by removing the hook and adding a hole for connection to a retrieval wire (Fig. S1). Samplers were filled with coarse sand as it is an inert surface that minimizes resistance to the flow of water and microbes through the sampler. After filling, three samplers were wired together, and the entire sampling apparatus was sterilized by autoclaving (121°C for 20 min). A sampling apparatus consisting of three sand-filled samplers was lowered to the bottom of each selected well and left in place for 35 days to allow colonization of the sand by native microbes. Immediately after the sampling apparatuses were pulled from wells, the sand from each sampler was transferred to a sterile 50 mL Falcon tube. One Falcon tube per well was transported on dry ice and stored in the laboratory at –80°C until DNA extraction. The

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remaining two Falcon tubes per well were transported on ice and stored at 4°C until microcosm construction. Groundwater was also collected from each well using a sterile bailer and stored in sterilized carboys at room temperature.

Microcosm setup and sampling

Groundwater from each well was amended with 1000 ppb selenium from a filter-sterilized stock solution of anhydrous Na_2SeO_4 (95%, Sigma-Aldrich) and ~1 mg/L resazurin (100%, Acros Organics) as a redox indicator. A concentration of 1000 ppb Se was used to allow for the quantification of reduction rates without toxicity to the microbes. For each well, eight sterilized 160 mL clear glass serum bottles were filled with 100 mL amended groundwater and 3.2–3.4 g biofilm-coated sand from the in-well samplers. Each of the three carbon amendments plus a no-carbon control was tested in duplicate for each well. Carbon sources at 0.5 mM carbon concentration were added individually in the form of methanol (99.9%, Fisher Chemical), glycerol (99.5%, Acros Organics), or molasses (B and G Foods Inc.) from filter-sterilized stock solutions. Microcosms for the coal well were amended with an additional 0.5 mM carbon on day 56 of the experiment as nitrate reduction had ceased, potentially indicating that all of the initial carbon amendment had been consumed. The serum bottles were stoppered and crimp sealed. Microcosms were kept in the dark at 10°C without shaking.

Approximately 1.5 mL of water from each microcosm was sampled at regular intervals using sterile 22G needles. Samples were filtered through 0.2 µm syringe filters into 1.5 mL tubes and stored at 4°C for chemical analyses described below. At the end of the experiment, approximately half the sand from each microcosm was removed into sterile Falcon tubes and stored at –80°C until DNA extraction. Microorganisms were removed from the remainder of the sand by agitation with a solution of 5 g/L Tween 80 (Fisher Scientific) in phosphate-buffered saline and preserved as frozen stocks in 20% glycerol at –80°C.

Chemical analyses

Nitrate and nitrite were quantified via ion chromatography (Dionex ICS-1100, Thermo Fisher Scientific) on a Dionex IonPac AS22 4 mm column using a 25 µL loop with 4.5 mM carbonate/1.4 mM bicarbonate eluent (Thermo Fisher Scientific) and a 15 minute elution time. The detection limit for nitrate-N was 0.02 mg/L, and samples were diluted by a factor of two. The standard deviation of multiple measurements was $\leq$ 1.5%. Total selenium was quantified via inductively coupled plasma mass spectrometry (ICP-MS) with a total metals protocol using direct injection in standard mode and with the hydrogen gas collision cell to minimize interferences (Agilent 7500ce). The detection limit for Se via ICP-MS was between 1–5 ppb, and samples were diluted by a factor of ten, giving a detection limit in the samples of 50 ppb Se. Uncertainty of ICP-MS measurements was +/-10%. Selenium speciation was conducted via hydride generation with fluorescence detection using method A3114 C (Baird and Bridgewater 2017) performed by Energy Laboratories (Helena, MT, USA).

Genomic analysis

DNA was extracted from the initial well sand samples using FastDNA 50 mL spin kit for soil (MPBiomedical), following the manufacturer's protocol for humic acid removal. For post-experiment microcosm sand samples, DNA was extracted from one duplicate of each treatment, except for three treatments where nitrate and selenium reduction differed between duplicates: interburden molasses, coal glycerol and coal methanol. For these treatments, DNA was extracted from both duplicates and sequenced and analysed separately. DNA was extracted from post-experiment microcosm sand samples using a FastDNA spin kit for soil using garnet lysing matrix without ceramic balls (MPBiomedical) instead of lysing matrix E to improve extraction, also following the protocol for humic acid removal. DNA Clean and Concentrator and/or OneStep PCR inhibitor removal kits (Zymo Research) were used as necessary to obtain sufficient concentration and quality of DNA for sequencing. For four post-experiment samples, the above method did not produce sufficient DNA; however, a modified phenol-chloroform-isoamyl alcohol extraction method (Griffiths et al., 2000) was successful in extracting DNA from these samples. The method was modified by extracting from a larger sample mass (2 g instead of 0.5 g) and using sodium acetate and isopropanol instead of polyethylene glycol for the nucleic acid precipitation step.

The V4 region of the 16S ribosomal gene was targeted using the Earth Microbiome primers 515F and 806R (Earth Microbiome Project, 2018; Parada et al., 2016; Apprill et al., 2015) to examine changes in bacteria and archaea populations as relative abundance. Metabarcoding libraries were sequenced using Illumina MiSeq with paired-end 300 bp sequencing that covers the full-length V4 16S region, which allows for accurate identification of sequence differences. SSU sequences were grouped into zero-radius operational taxonomic units (zOTUs) using the UNOISE pipeline (Edgar and Flyvbjerg, 2015), which separates likely biological sequences from sequencing artifacts. ZOTUs were classified against known taxa in the Ribosomal Database Project (Cole et al., 2014).

Statistical analyses

All statistical analyses were performed in *R version 3.6.3* (R Core Team, 2020). The packages *readr* (Wickham et al., 2018) and *dplyr* (Wickham et al., 2020) were used for data manipulation.

Rate constants and order of reaction kinetics for nitrate and selenium reduction were determined using a line of best fit over the time period during which reduction occurred. The R packages *multcomp* (v3.0-3; Hothorn et al., 2008) and *carData* (v1.4-13; Fox et al., 2019) were used to determine statistically significant differences among selenium and nitrate removal rates relative to the independent variables of well and carbon source (Abdi and Williams, 2010). Statistically significant correlations of rate constants and final water chemistry variables with environmental variables were determined using the Kruskal-Wallis rank sum test, as all sets of data for these analyses failed to meet one or more assumptions to perform typical ANOVA tests (Kruskal and Wallis, 1952). Pairwise comparisons were performed using the Wilcoxon rank sum test (Wilcoxon, 1945) with the Benjamini and Hochberg p-value adjustment (Benjamini and Hochberg 1995).

The *labdsv* (v2.0-1; Roberts 2019) and *vegan* (v2.5-6; Oksanen et al., 2019) packages were used for microbial community analyses. A Bray-Curtis distance matrix (Bray and Curtis, 1957) was used as the input into permutational multivariate ANOVA (PerMANOVA) and non-metric multidimensional scaling (NMDS) functions to determine significant correlations between community composition and environmental variables and between community composition and final water chemistry variables (Kruskal, 1964; Roberts, 2019). The *multipatt* function in the R package *indicspecies* (v1.7.9; De Cáceres et al., 2010) was then used to find indicator taxa for the variables found to be significantly correlated with community composition. Only taxa with a relative abundance > 2% at the taxonomic rank for which

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an analysis was performed were included in the indicator species analyses.

Results and discussion

Nitrate

All carbon-amended microcosms from the background and alluvial wells removed nitrate to below quantitative limits (BQL, 0.2 mg/L) by day 49 of the experiment, as shown in Fig. 2a and 2b. All carbon-amended microcosms from the background well (initial nitrate-N = 1 mg/L) and molasses-amended microcosms from the alluvial well (initial nitrate-N = 2 mg/L) removed nitrate to BQL by the second sampling event on day 8. Methanol- and glycerol-amended microcosms from the alluvial well-removed nitrate within 49 days. No nitrate reduction occurred in any of the no-carbon controls.

The interburden and coal wells had somewhat higher initial nitrate concentrations and more variable results for nitrate reduction (Figs 2c and 2d). In the interburden well (initial nitrate-N = 4 mg/L) (Fig. 2d), molasses-amended microcosms removed nitrate within 27 days, while methanol showed 60 to > 99% nitrate removal in 128 days. One glycerol-amended microcosm removed 75% of nitrate-N, while the other removed only 17%, which was not a significant change from the initial concentration (linear regression, $p > 0.05$). For the coal well, which had the highest initial nitrate-N at 13 mg/L, one of the methanol-amended microcosms showed > 99% nitrate removal within 27 days, although all the other microcosms for this well had stopped reducing nitrate by this time, indicating some limitation to microbial nitrate reduction. Additional carbon was added to all coal well microcosms on day 56, and nitrate reduction resumed afterwards; thus, carbon was assumed to be the limitation. The molasses-amended microcosms removed nitrate to below the quantification limit within 7 days of the second carbon addition (by day 63). Conversely, the second methanol-amended microcosm did not remove nitrate to BQL until the end of the experiment (day 128). One glycerol-amended microcosm removed 98% of nitrate-N, while the other removed 72%.

The type of carbon amendment was significantly correlated with percent nitrate removal by day 128 (Kruskal-Wallis, $p < 0.0001$). Pairwise, the comparison showed that removal was significantly higher for molasses than for glycerol (Wilcoxon rank-sum, $p = 0.049$). No significant difference was found between methanol and glycerol or molasses and methanol. Nitrate removal after 128 days was not significantly correlated with the well location or stratigraphic layer ($p > 0.05$).

Zero order nitrate removal rates had a range of 0.034–1.02 mg/L/day across all treatments for the carbon-amended microcosms (Fig. S2). R^2 values ranged from 0.85–1.00, and a minimum of four points were fit to the linear regression except where this was not possible (*e.g.* for the background well) (Table S1). Because more carbon was added to coal well microcosms, the rates from before and after the carbon addition were calculated separately; only rates after carbon addition are shown because some microcosms did not reduce nitrate prior to carbon addition. The rates found here are

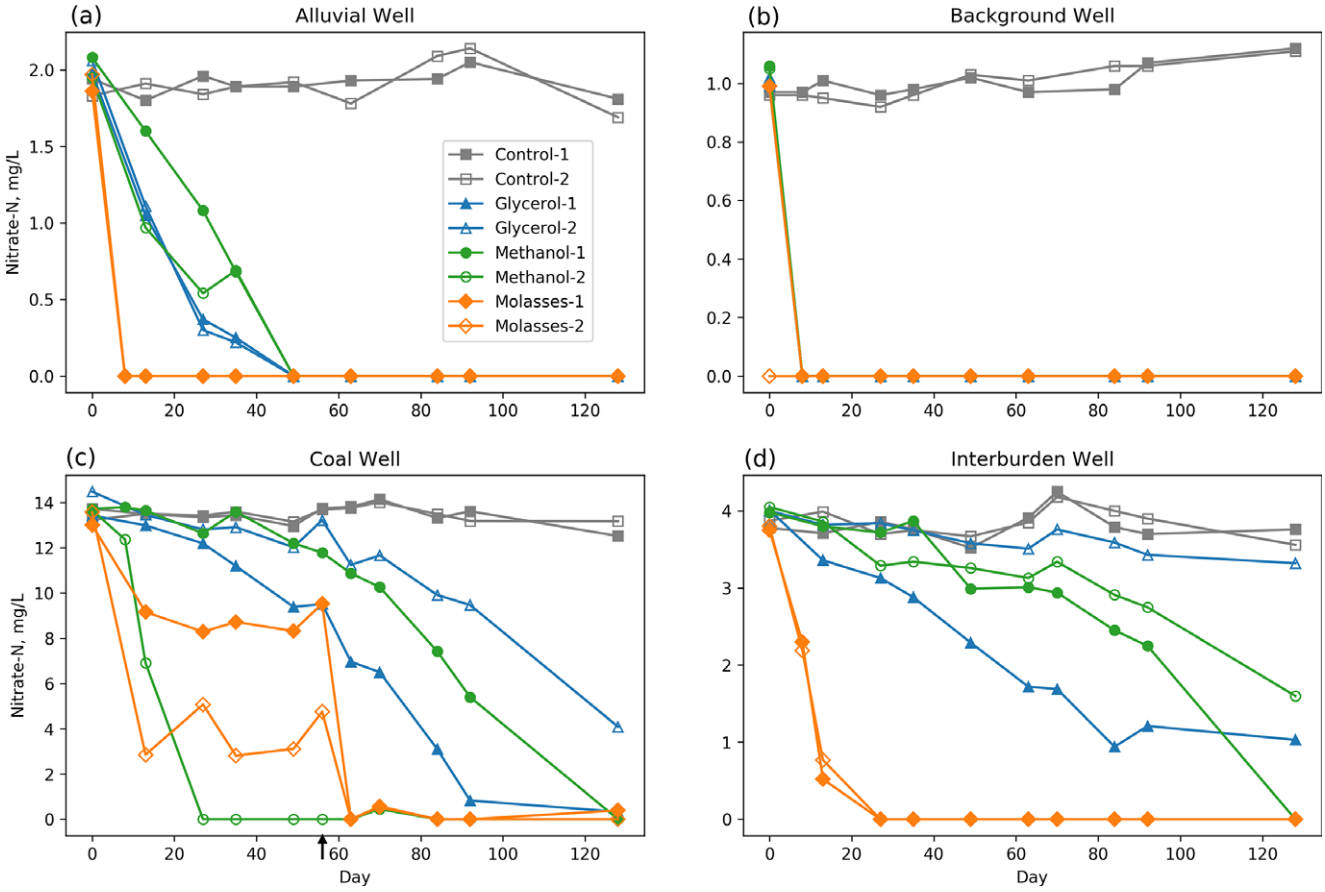

Figure 2. Nitrate-N concentrations over time in microcosm experiments for (a) the alluvial well; (b) the background well; (c) the coal well; and (d) the interburden well. Note the different scales on the y-axes due to different starting concentrations of nitrate. The arrow on 2(c) indicates the day of additional carbon amendment. Duplicate independent replicates are plotted separately rather than averaged.

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similar to rates of 0.09–0.73 mg/L/day estimated for *in situ* denitrification in aquifers with a water temperature of 6–10°C (Korom 1992). Lewandowski (1982) evaluated the temperature dependence of nitrate removal rates in activated sludge batch reactors amended with methanol; for the initial nitrate concentrations in this experiment, Lewandowski's work predicts k values of 0.02232–0.29 mg/L/day at 10°C.

Nitrate removal rates differed significantly among carbon treatments ($p = 0.0001$). In pairwise comparisons, removal rates for molasses were significantly higher than those for either glycerol or methanol ($p = 0.027, 0.028$). There were no significant differences among wells or stratigraphic units ($p > 0.05$).

The type of carbon amendment was the main factor impacting nitrate removal in the microcosms, as it was significantly correlated with both percent nitrate removal and nitrate removal rates. Of the carbon sources tested, molasses was most effective for nitrate removal, as it resulted in significantly higher removal rates than either methanol or glycerol and also in significantly higher extent of nitrate removal than glycerol. Previous studies comparing these carbon sources in wastewater treatment report that molasses has similar denitrification rates when compared to methanol (Hamlin et al., 2008) and glycerol (Horova et al., 2020), with near-complete nitrate removal in all cases. Some comparisons of glycerol and methanol also report near-complete removal, with glycerol having a higher removal rate (Chen et al., 2013; Rocher et al., 2015). However, denitrification with methanol is sometimes reported to be ineffective, probably due to a lack of methylotrophic denitrifiers (Akunna et al., 1993; Horova et al., 2020).

Selenium

As shown in Fig. 3, selenium reduction was observed in microcosms only after the nitrate was depleted, and microcosms without carbon amendment did not reduce selenium. For wells with elevated groundwater selenium concentrations, the alluvial and coal wells, Figs 3b and 3d, show selenium reduction in microcosms began as soon as the nitrate was completely removed. By contrast, there was a lag period between nitrate removal and the beginning of selenium reduction for the wells with low groundwater selenium concentrations. The background microcosms amended with molasses did not have a measurable lag time, but those amended with methanol or glycerol took 4 to 8 weeks to begin selenium reduction after nitrate depletion. The interburden microcosms amended with molasses took approximately 7 weeks to begin selenium reduction, while interburden microcosms amended with methanol or glycerol did not reduce selenium. This suggests that the microbial communities obtained from wells with elevated selenium were better adapted for selenium reduction.

Also shown in Fig. 3, selenium reduction extent varied among wells and carbon treatments. Total dissolved selenium concentrations decreased to BQL (50 ppb) in all of the methanol- and glycerol-amended microcosms for the background well (Fig. 3a). In the molasses-amended microcosms, selenium decreased rapidly after nitrate removal, dropping to 165–300 ppb by day 27; however, concentrations then remained relatively steady for the duration of the experiment. The slight increases in Se concentration between days 27 and 128 (Fig. 3) are not statistically significant ($p > 0.05$).

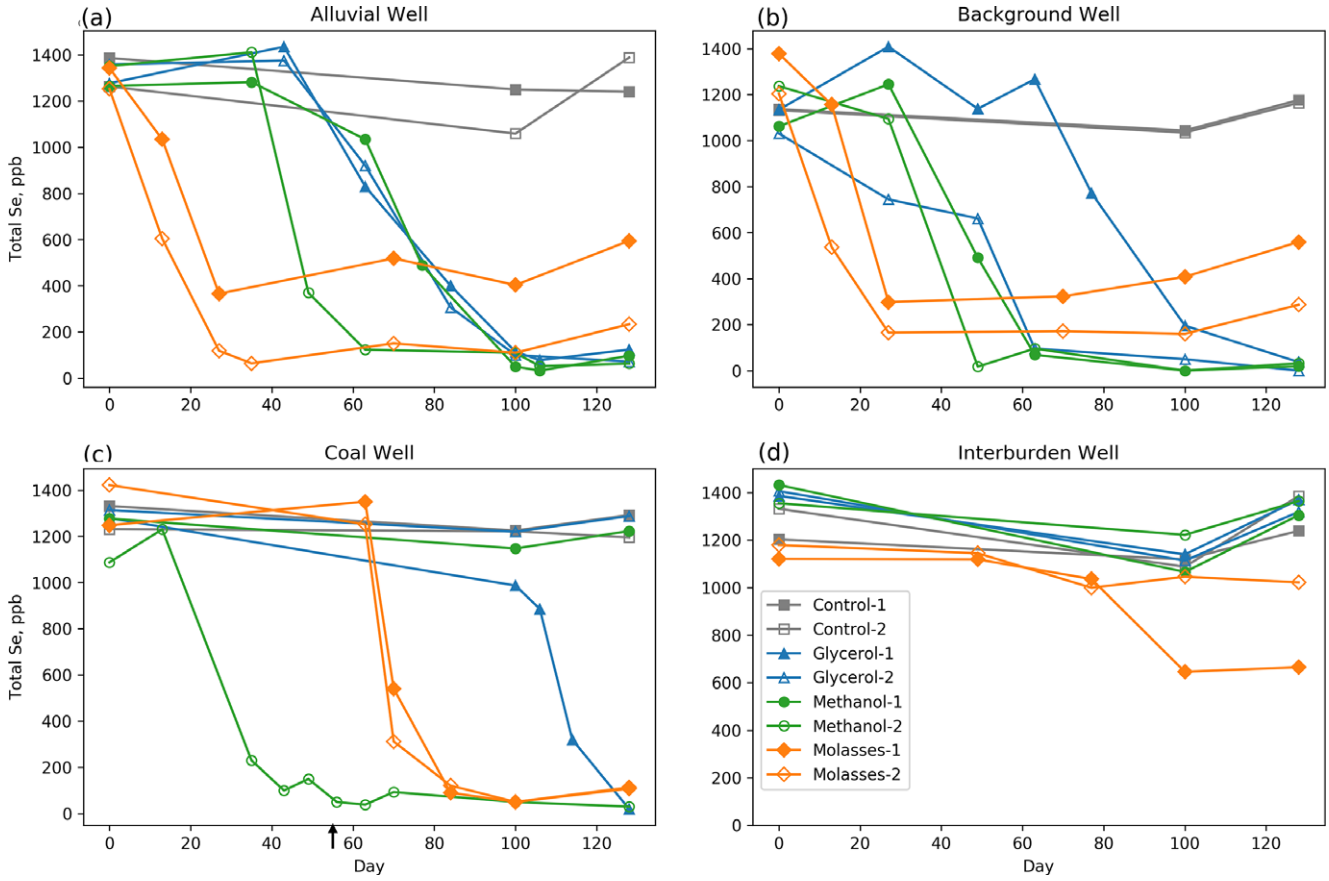

Figure 3. Total selenium concentrations over time in microcosm experiments for (a) the alluvial well; (b) the background well; (c) the coal well; and (d) the interburden well. The arrow on 3(c) indicates the day of additional carbon amendment. Duplicate independent replicates are plotted separately rather than averaged.

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Selenium reduction in the alluvial well (Fig. 3b) was similar to the background well. In methanol- and glycerol-amended microcosms, selenium reduction began within one week of nitrate removal and was reduced to < 100 ppb. Conversely, while selenium in the molasses-amended microcosms decreased rapidly immediately after nitrate removal, 150–500 ppb selenium persisted until the end of the experiment.

In the interburden well (Fig. 3c), only one microcosm reduced total selenium. The total selenium concentration was approximately 40% lower than the initial concentration in one of the molasses-amended microcosms from day 100 onwards. No change in the selenium concentrations occurred in any of the other microcosms for this well. For the methanol and glycerol treatments, the lack of selenium reduction may be due to the slow nitrate reduction because all but one of those microcosms still contained nitrate at the end of the experiment. The slower nitrate and selenium reduction in this well was probably due at least partly to differences in the initial microbial community composition, discussed in the next section. It is also possible that the initial biomass from this well was lower, or that an inhibitor that was not identified in water quality analyses was present exclusively or at higher concentrations in the water or biofilm samples from the interburden.

For the coal well, shown in Fig. 3d, all microcosms that completely reduced nitrate also removed selenium to BQL by the end of the experiment. The methanol microcosm that removed nitrate by day 27 began reducing selenium between days 13 and 35 and had reduced it to BQL by day 63 (Fig. 3d). Molasses-amended microcosms reduced selenium to BQL by day 100. One glycerol-amended microcosm had 0.33 mg/L nitrate and selenium BQL at day 128.

The percent of total selenium removed by the end of the experiment was significantly correlated with the stratigraphic unit and with the carbon source ($p = 0.03, 0.005$). While the p value of 0.075 for the correlation between the percentage of total selenium removed and the well location was above the cutoff value (0.05), it should be noted that no interburden microcosm removed selenium to below the regulatory limit of 50 ppb, which was not true for any other well. Pairwise comparison among stratigraphic units showed that removal was significantly higher in the alluvial wells than in the interburden well ($p = 0.028$). Pairwise comparison among carbon treatments showed significant differences between all carbon amendments and the controls but found no significant differences between any pair of carbon amendments (*i.e.* glycerol vs. methanol, glycerol vs. molasses and methanol vs. molasses).

For microcosms where selenium was reduced (background – all carbon-amended; alluvial – all carbon-amended; coal – both molasses-amended, one methanol-amended, one glycerol-amended), the reduction of total selenium was approximated by first-order reaction kinetics (average $R^2 = 0.93$; see Table S2 for a full comparison of zero and first-order regressions). The rate constant k was significantly lower for the interburden well compared to the alluvial and background wells ($p = 0.028$); otherwise, k did not differ significantly among either wells or carbon treatments ($p > 0.05$). The average rate constant, excluding the interburden well, was $k = 0.077/\text{day} + 0.019$ (95% confidence interval). This value is near the lower end of those found in other batch experiments using environmental bacteria, such as 0.016–0.333 L/day for five different bacterial strains in molasses-amended artificial drainage water (Zhang et al., 2008) and 0.07–0.82 L/day by enriched mine site bacteria amended with acetate and with varying concentrations of selenate and nitrate (Subedi et al., 2017); both experiments were carried out at 30°C. Selenium removal is strongly temperature dependent (Ma et al., 2007), and the optimum temperature for selenium reduction is consistently reported to be around 30°C or a few degrees higher (*e.g.* Ma et al., 2007; Hageman et al., 2013; Bao et al., 2013; Xia et al., 2013). Reported k values at 30–35°C are approximately 3–15 times higher than those at 10–15°C (Ma et al., 2007; Xia et al., 2013). Thus, the reduction rate observed here is at the low end of the range of reported rates due to the lower temperature (10°C vs. 30°C). At low temperatures, total selenium removal should be unaffected as long as retention times are sufficient; in pilot bioreactors treating end pit lake discharge, the percentage of selenium removal was not affected by temperature decreasing from 17 to 2°C (Luek et al., 2014).

As shown in Figs 4a and 4b, for the background and alluvial wells, the majority of the aqueous phase selenium remaining at the end of the experiment was in the form of selenite. In the interburden well (Fig. 4c), the molasses microcosm that partially reduced total selenium also had mostly selenite remaining; the other molasses microcosm, though it did not reduce total selenium to insoluble forms, did reduce 146 ppb of selenate to selenite. The coal well (Fig. 4d) was the only well for which the majority of the selenium remaining was selenate. The percentage of selenite remaining was significantly correlated with the stratigraphic unit ($p = 0.014$) and with the well ($p = 0.029$). The alluvial wells had a significantly higher percentage of selenite than either the coal or interburden well ($p = 0.041, 0.045$); there were no significant pairwise differences among individual wells. With respect to the stratigraphic unit, alluvial well tests removed a significantly higher percentage of the total selenium than did interburden well tests, but this does not necessarily mean that removal was more effective in the alluvial well tests; they also had a significantly higher percentage of selenite remaining than both the interburden and coal well tests. These findings support the hypothesis that the local microbial community is an important factor in the efficacy of selenium removal. This is probably due in part to how hydrogeology influences (and is influenced by) microbial community composition; in two studies investigating selenate and selenite reduction in selenium-reducing bacterial strains, only one of seven strains reduced selenite as effectively as selenate. The other six strains accumulated 10–40% of the initial total selenium as selenite (Ike et al., 2000; Zhang et al., 2008). Non-enzymatic selenite reduction could also have been limited; when selenite reduction is mediated by EPS, the extent of reduction depends on the thiol concentration (Zhang et al., 2020).

An important limitation of the selenite results is that they do not account for abiotic removal mechanisms. The microcosms did not contain any aquifer sediments, which typically contain materials that sorb selenite, such as iron- and aluminium-bearing minerals, apatites and iron-humus complexes (Dhillon and Dhillon, 2000; Duc et al., 2003; Fernandez-Martinez and Charlet, 2009). Thus, some portion of the selenite would probably be adsorbed during *in situ* remediation, resulting in lower aqueous concentrations than those observed here.

Overall, the stratigraphic unit appeared to have the greatest effect on selenium removal. It correlates significantly with both the percent of total selenium removed and the percent of selenite remaining at the end of the experiment. While carbon was also significantly correlated for both variables, the only significant pairwise differences were between carbon amendments and no-carbon controls. No significant correlation with the end-point variables was found for initial groundwater concentrations of selenium or nitrate.

Microbial communities

To gain a better understanding of the microorganisms responsible for nitrate and selenium reduction, DNA was extracted and the V4 region of the 16S ribosomal gene was used to identify microbial taxa

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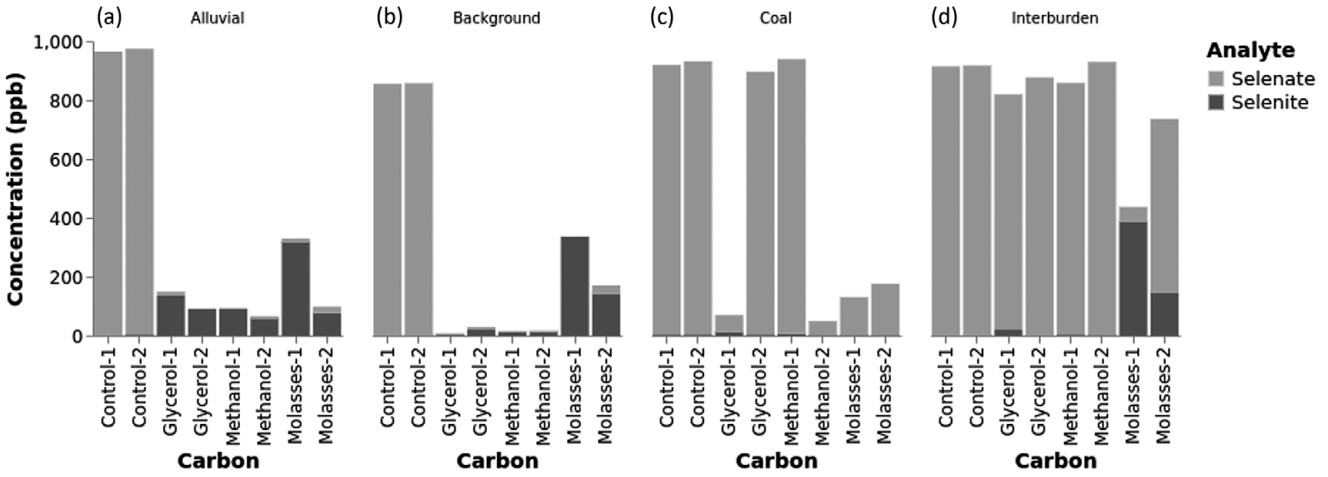

Figure 4. Selenium concentrations and speciation as selenate or selenite in microcosms for each well at the end of the experiment (day 128). Total Se concentrations differ slightly from those in Fig. 3 due to different quantification methods (see methods section). Duplicate independent replicates are plotted separately.

present initially at the sites and after enrichment in the laboratory. After enrichment with the various substrates, 19 samples (two from interburden molasses, coal glycerol and coal methanol; one from each other treatment) were sequenced and analysed to characterize the post-experiment microbial communities. For most treatments, the duplicates performed similarly with respect to nitrate and selenium reduction; only one duplicate was sequenced for these treatments under the assumption that microbial communities would also be similar. For the three treatments listed above, nitrate and selenium reduction differed and so both duplicates were sequenced to investigate whether there were also microbial community differences. These communities were compared to the microbial communities initially present in each well and evaluated for potential physiological capabilities that correlated with laboratory observations.

Initial well communities

All microbial communities recovered from the sampled wells contained taxa reported to be capable of reducing nitrate. As shown in Fig. 5, the alluvial and coal well communities were very similar to each other. The most abundant taxa in the well samples were two zOTUs of the family *Aerococcaceae*, which could not be identified at the genus level. Together these zOTUs comprised 60% of the initial alluvial well community and 53% of the coal well community. *Lactobacillus* was present in the alluvial and coal well communities at 18% and 25%, respectively. None of these zOTUs were observed in the other well communities. *Aerococcaceae* are facultative organisms, but do not reduce nitrate; they are found in air, soil and marine environments as well as in mammalian microbiomes (Hlazapfel and Wood, 2014). *Lactobacillus* occurs in soils (Chen and Yanagida, 2006; Kim et al., 2018) and industrially-contaminated groundwater (Gao et al., 2010; Ukah et al., 2018), and some strains can reduce nitrate (Rogosa 1961; Rzepkowska et al., 2017). In the background well, genera present at $\geq$ 5% relative abundance included *Janthinobacterium* (29%), *Nitrincola* (21%) and *Ralstonia* (7%). *Ralstonia* also comprised the majority (52%) of the interburden well community, 2% of the alluvial well community, and < 0.1% of the coal well community. *Janthinobacterium* also comprised 1% of the alluvial community and < 1% of the coal community. *Nitrincola* was present at < 0.1% in the alluvial and coal well communities and was not present in the interburden community. *Janthinobacteria* are facultative, psychrotolerant organisms that are widespread in soil and freshwater and can reduce nitrate both aerobically and anaerobically (Yang et al., 2018; Chernogor et al., 2022). *Nitrincola* bacteria are chemoorganotrophic, alkaliphilic facultative anaerobes that reduce nitrate to ammonia (Borsodi et al., 2017). *Ralstonia* were previously included in the genus *Pseudomonas* and may be aerobic or facultative; some species reduce nitrate (Ryan et al., 2007; Tiemeyer et al., 2007; Shariati et al., 2022). The interburden well community also contained 29% *Limnospira*, an alkaliphilic, halotolerant genus of cyanobacteria (Nowicka-Krawczyk et al., 2019). One species of *Limnospira* has been cultivated heterotrophically (Marchão et al., 2021). *Limnospira* was present at < 0.1% in all other well samples.

Of the most abundant taxa initially detected, a few have putative selenium-reducing abilities but none have as yet been shown to reduce selenate. Some strains of *Lactobacillus* and *Ralstonia* have been shown to reduce selenite (Rajasree and Gayathri, 2015; Sarret et al., 2005). However, because selenate can be reduced to selenite via nitrate reductase, it is probable that these communities contain some taxa with the cometabolic capacity to reduce selenate.

Enriched microbial community composition

Dominant taxa observed after enrichment appeared to depend more on the carbon amendment than on the initial community or groundwater chemistry. All of the carbon-enriched communities had a high relative abundance of taxa capable of nitrate removal, as well as at least one genus that has been reported to reduce selenium and/or was a high-abundance member of a community shown to reduce selenium. Combined with direct selenium concentration measurements (Fig. 3), this demonstrates that microbes capable of selenium reduction were enriched from both high- and low-selenium sites.

As shown in Fig. 5, the genus *Ralstonia* had the highest relative abundance in all no-carbon control tests: 23% in background, 75% in alluvial, 53% in coal and 37% in interburden. Not surprisingly, methylotrophic genera dominated the methanol-amended tests (Fig. 5). *Methylophilus* comprised 30% of the sequenced community for the background well, 13% for alluvial, 51% and 30% for coal 1 and 2 and 10% for interburden. *Methyloversatilis* had the highest relative abundance for the interburden well at 36%, and *Methylophaga* for the alluvial well at 24%; however, these two genera each

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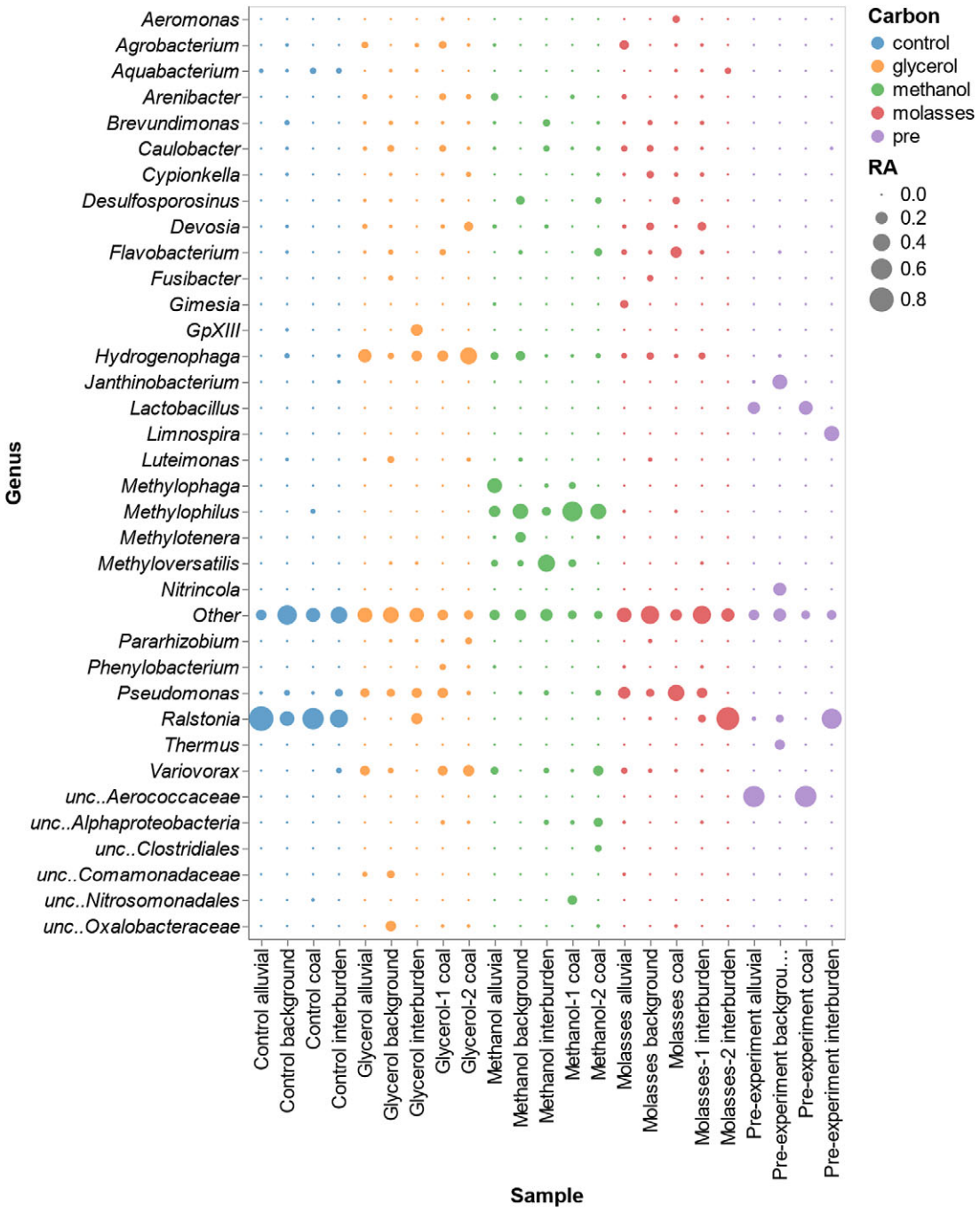

Figure 5. Relative abundance (RA) of microbial genera in enrichment communities. Includes all genera ≥ 5% relative abundance in at least one community. Unclassified (unc.) taxa represent single zOTUs that comprise ≥ 5% of one or more communities but could not be identified at the genus level. Genera < 5% relative abundance in all communities are grouped together as "Other".

comprised 7% or less of the other methanol-amended communities. Only the background and coal well methanol-amended communities contained non-methylotrophic genera at > 10% relative abundance (Fig. 5). The background well comprised 10% *Hydrogenophaga*. The first coal well duplicate contained 11% an unclassified zOTU of *Nitrosomonadales* and the second duplicate contained 13% *Variovorax* and 10% an unclassified zOTU of *Alphaproteobacteria*.

For glycerol-amended tests, unclassified zOTUs from *Oxalobacteraceae* (11%) and *Comamonadaceae* (6%) were most abundant in the background well community. *Pseudomonas* was present at 5%. Other glycerol-amended communities contained *Pseudomonas* at abundances of 9% in alluvial, 12% in interburden, 13% in coal-1 and 3% in coal-2. *Hydrogenophaga* had the highest relative abundance for the alluvial and both duplicates from the coal well, at 21%, 14% and 33% (Fig. 5). It comprised 13% of the community from the interburden and 7% from the background. Glycerol-amended communities from the coal well contained *Variovorax* at 12% and 15% for replicates 1 and 2. *Variovorax* was also present at 11% in the alluvial community. *Acidobacteria* GpXIII comprised 16% of the glycerol-amended interburden community.

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Pseudomonas had the highest relative abundance in all but one of the molasses-amended tests: 7% in background, 15% in alluvial, 35% in coal and 11% in interburden-1. The exception was duplicate 2 for the interburden well, which reduced little selenium despite reducing nitrate at the same rate as interburden duplicate 1. This community, similar to the controls, was predominantly *Ralstonia* (63%). *Ralstonia* also comprised 7% of the interburden-1 community. Other abundant genera for molasses-amended tests were *Devosia* in the background (6%) and interburden-1 (8%) communities, *Cypionkella* (6%) and *Variovorax* (5%) in the background community, *Agrobacterium* (10%) and *Gimesia* (8%) in the alluvial community and *Flavobacterium* (16%) and *Aeromonas* (7%) in the coal community.

NMDS was used to visualise differences among the enriched communities (Fig. 6), and PerMANOVA was used to analyse significant correlations between community composition and environmental and experimental variables. While there were some differences in community composition between sequenced duplicates, in NMDS, these duplicates still visually clustered together, apart from the interburden molasses community that was predominantly *Ralstonia*. Additionally, PerMANOVA showed that microbial community composition was strongly correlated with carbon amendment and with well ($p = 0.0005$, $p = 0.0099$). Thus, differences between duplicates were small compared to differences between treatments. There were no significant correlations with other environmental variables (stratigraphic unit and groundwater chemistry). There were also significant correlations of community composition with the percent nitrate removed ($p = 0.002$) and percent total selenium removed ($p = 0.007$). There was no significant correlation with other experimental variables (nitrate removal rate, initial selenium concentration, percent selenite). For variables significantly correlated with community composition, indicator species analyses were performed to find taxa contributing to differences among communities.

Indicator taxa for carbon amendments

In indicator species analyses, when an indicator taxon is identified for a group, the taxon is found significantly more frequently (*i.e.* at a higher relative abundance) in that group than in any of the groups to which it is being compared. The results of these analyses, while context-dependent, can be used to predict which taxa an environmental variable (*e.g.* well site) may select for or what taxa may be involved in an environmental process (*e.g.* nitrate reduction) (De Cáceres and Legendre, 2009). Indicator species analyses were also performed on sets of groups, indicated on the heat maps (Fig. 7, Figs S3–S5) by dashed boxes. For example, *Pseudomonas* was significantly more abundant in glycerol- and molasses-amended communities than in no-carbon control and methanol-amended communities (Fig. 7).

Indicator species analysis found 10 genera significantly associated with specific carbon amendments (Fig. 7). The genus *Ralstonia* was a significant taxon observed for the no-carbon controls and also had the highest relative abundance in all no-carbon control tests (Fig. 5). All no-carbon control tests had oxygen remaining at the end of the experiment, as indicated by resazurin colour. *Ralstonia* may be aerobic or facultative; some strains are extremely metal-resistant, and they may enter a viable but non-culturable state under environmental stress, which may explain why they became dominant community members under low-carbon conditions (Mergeay et al., 2003; Ryan et al., 2007; Kong et al., 2014). *Nitrososphaera*, while also a genus-level indicator taxon, comprised ≤ 4% of each control community.

All of the methanol-associated indicator genera (Fig. 7) are methylotrophs; *Methylophilus* and *Methyloversatilis* belong to the order Nitrosomondales, while *Methylophaga* belongs to Thiotrichales. While ammonia oxidation is a defining feature of Nitrosomonadales, some members of this order also contain nitrate reductases; there is evidence of nitrate removal for strains belonging to both of the indicator genera from this order (Lu et al., 2012; Mauffrey et al., 2015). *Methylophilus* removes nitrate via reduction to ammonia (DNRA) but not via denitrification (Lin et al., 2021). Nitrate reductase was expressed when *Methyloversatilis universalis* was grown on methanol (Lu et al., 2012). Only one species of *Methylophaga* has been reported capable of denitrification; its genome contains reductases for nitrate, nitric oxide and nitrous oxide, but not for nitrite (Mauffrey et al., 2015). Based on known metabolic pathways, communities with high abundances of *Methylophilus* may have reduced nitrate primarily via DNRA rather than denitrification to N_2; however, because ammonia was not measured, this cannot be determined. *Methyloversatilis* is the only one of these genera previously connected to selenium removal; it was

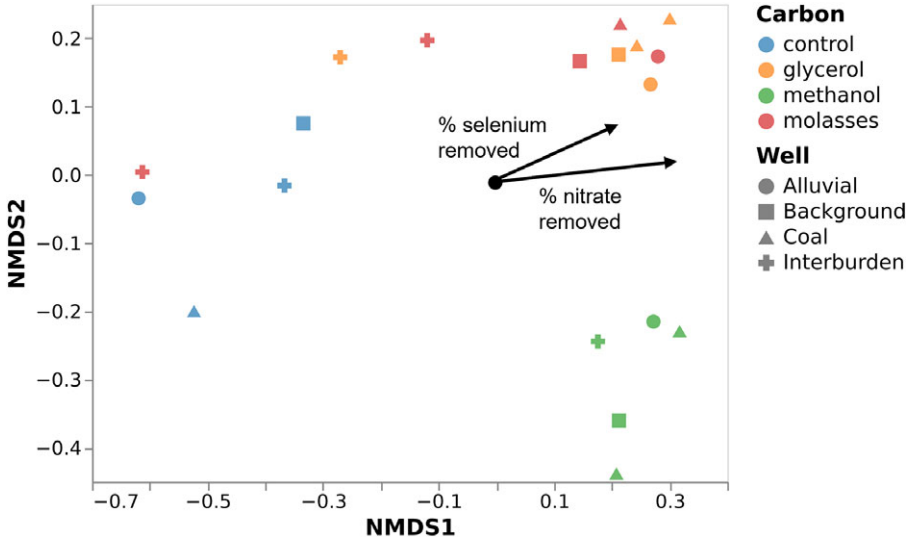

Figure 6. NMDS results for microbial enrichment communities.

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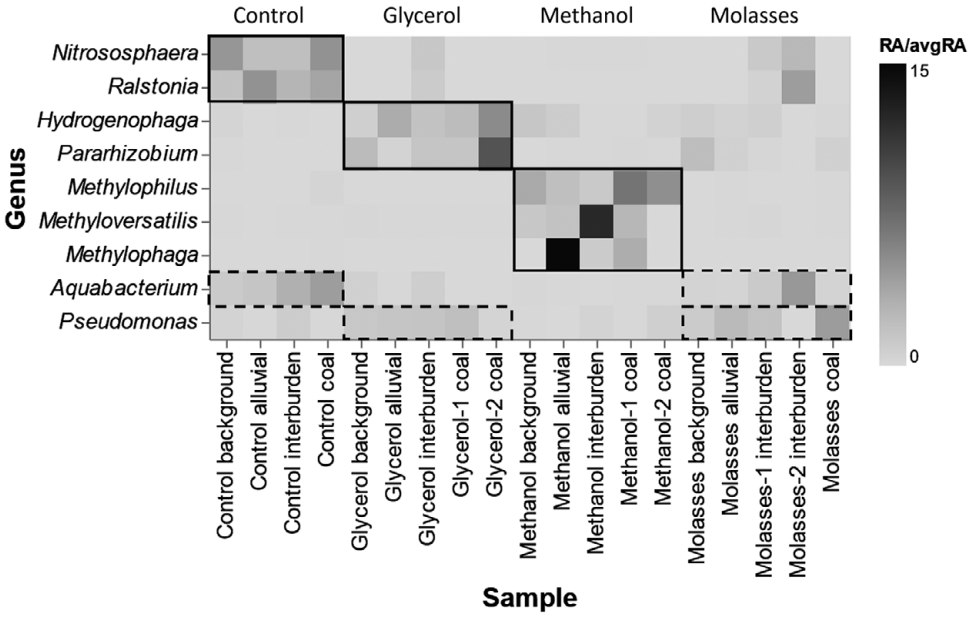

Figure 7. Indicator genera for carbon sources. Boxes enclose indicators for each carbon group; zOTUs within solid boxes are indicators for one group, and zOTUs within dashed boxes are indicators for two groups.

among the most abundant genera in hydrogen-fed membrane biofilm reactors treating nitrate and selenate (Esquivel-Hernandez et al., 2021).

Pseudomonas, a genus containing numerous environmental denitrifiers (*e.g.* Spain and Krumholz, 2012), was an indicator for both glycerol and molasses amendment (Fig. 7). Multiple isolates of *Pseudomonas*, from a variety of environments, have been shown to reduce selenate (*e.g.* Ike et al., 2000; Morita et al., 2007; Hunter and Manter, 2009; Kuroda et al., 2011). Two *Pseudomonas* strains have been reported to anaerobically reduce selenite (Maltman et al., 2015; Javed et al., 2015); however, most strains that reduce selenate accumulate selenite under anaerobic conditions (Ike et al., 2000; Hunter and Manter, 2009; Kuroda et al., 2011). Several strains of *Pseudomonas* have been found to reduce selenite only under aerobic conditions (Hunter and Manter, 2009; Kuroda et al., 2011; Avendaño et al., 2016; Wang et al., 2019), with glutathione reductase responsible for selenite reduction in at least some of these strains (Hunter 2014; Wang et al., 2019). The possible requirement of aerobic conditions to reduce selenite may partly explain the high concentrations of selenite remaining in some glycerol- and molasses-amended microcosms, though, as methanol-amended alluvial microcosms also had high concentrations of selenite with low relative abundance of *Pseudomonas*, there are probably other factors involved as well.

For glycerol amendments, indicator taxa other than *Pseudomonas* included *Hydrogenophaga*, along with the family *Comamonadaceae* to which it belongs, and *Pararhizobium* (Fig. 7). While hydrogen oxidation is a defining feature of *Hydrogenophaga*, at least one strain is known to grow on glycerol (Nedwell and Rutter, 1994). *Hydrogenophaga* has been shown to reduce nitrate and selenate in hydrogen-fed membrane biofilm reactors (Zhou et al., 2018; Xia et al., 2019; Esquivel-Hernandez et al., 2021). *Pararhizobium* comprised 7% of the community from coal-2 but was present at < 2% for all other glycerol-amended communities. *Pararhizobia* are aerobic, though they have been found in abundance (0.3–19.7%) in nitrate-reducing bioreactors (Mousavi et al., 2015; He et al., 2021).

The genus with the highest abundance for glycerol-amended interburden enrichments was not determined to be an indicator taxon: *Acidobacteria* GpXIII comprised 16% of this community. Group 13 is one of the many subdivisions of *Acidobacteria* containing no taxonomically described members, and there is little other research on group 13. The phylum Acidobacteriota is ubiquitous and frequently abundant in terrestrial environments but is difficult to study as members are difficult to cultivate (Kalam et al., 2020; Kielak et al., 2016). Metagenomic analysis of one genome from group 13 found evidence of nitrite reduction potential, and members of other subdivisions of the *Acidobacteria* are capable of denitrification, but very little is known about Acidobacteria group 13 (Wegner and Liesack, 2017; Dedysh and Yilmaz, 2018; Kalam et al., 2020).

The similarities in community composition and indicator species for glycerol and molasses suggest that they might perform similarly for nitrate and selenium removal; however, the nitrate removal rate was significantly slower for glycerol than for molasses. This may be influenced by the indicator taxa, which are not shared with molasses; for example, while *Hydrogenophaga* reduces nitrate, it is also an indicator taxon for incomplete nitrate removal, as discussed in the next section.

To summarise the impacts of carbon amendments on indicator species, carbon amendment type was the experimental condition most strongly correlated with differences in the resulting community composition. *Ralstonia* was an indicator taxon for the no-carbon controls as well as the most abundant genus in all the control communities, possibly due to their ability to remain viable under environmental stresses. Methanol amendment enriched for methylotrophic taxa, resulting in communities predominantly composed of organisms capable of metabolising methanol. As glycerol and molasses can be utilised in a broader range of microbial metabolisms than can methanol, indicator taxa for these carbon sources were facultative anaerobes from a variety of taxa commonly found in soil and groundwater. All carbon sources promoted enrichment of organisms capable of reducing selenium and nitrate. However, carbon amendment may have impacted nitrate removal efficacy, as discussed below.

Indicator taxa for post-enrichment communities by well

There were no commonalities among the most abundant taxa for each well. Indicator species analysis showed 11 taxa significantly

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associated with individual wells (Fig. S3); of these, eight were indicator organisms for the background well. The coal well had no indicator taxa, while the alluvial well had one, and the interburden well had two. None of the indicator taxa were among the most abundant taxa in any of the sequenced communities; furthermore, none of the indicator taxa were present at > 2% relative abundance in all sequenced communities from the well it was associated with in the analysis. Thus, although community composition is significantly associated with well, it appears that differences among the well communities are largely driven by overall community composition rather than associated with specific indicator taxa.

Indicator taxa for nitrate removal

Nine taxa were indicators for percentage of nitrate removed (six at the genus level), all of which were associated with low (0%) or medium (72–74%) removal (Fig. S4). No indicator species were found for high (98–100%) nitrate removal. It appears that important differences in the percentage of nitrate removed may be driven by the type of carbon amendment, as indicator taxa for zero and medium (72–74%) nitrate removal were also indicator organisms for control or glycerol carbon treatments.

Indicator taxa for selenium removal

Eight genera were indicators for the percentage of selenium removed. As shown in Fig. S5, *Ralstonia* was the sole indicator genus for low and no removal. Five genera were indicators for moderate removal (approximately 50% of total Se removed), and two genera from the order *Clostridiales*, *Gracilibacter* and *Desulfosporosinus* were indicators for high removal (90–100%). Members of *Clostridiales* have been found to be capable of selenate reduction in waters impacted by coal mine seepage (Nkansah-Boadu et al., 2021) and in bioreactors treating synthetic mine water (Cheng et al., 2017). Additionally, *Desulfosporosinus* was the dominant community member in Se-reducing enrichments from tunnel excavation waste rock (Aoyagi et al., 2021) and was included among the highest abundance OTUs in Se-reducing enrichments from saline mining wastewater (Liu et al., 2018). The latter *Desulfosporosinus* contained the putative selenate reductase YgfK (Liu et al., 2018); other members of *Clostridiales* also probably contain putative selenate reductases (Nkansah-Boadu et al., 2021). No literature to date has associated *Gracilibacter* with selenium or nitrate reduction. Given the data on *Desulfosporosinus* and *Clostridiales*, it is probable that the indicator taxa for high selenium removal did, in fact, contribute to selenium reduction.

Neither indicator taxon for high selenium removal was also an indicator for any specific well or carbon source. This is unsurprising when examining enrichments grouped into the 'high' category, which included glycerol and methanol amendments for the background, alluvial and coal wells, as well as molasses amendment for the coal well. Clostridiales was also not an indicator taxon for any of the environmental variables that were not significantly correlated with community composition (stratigraphic unit, groundwater selenium and groundwater nitrate), or for any combination of environmental variables. Thus, while members of Clostridiales might have contributed to selenium removal, the environmental variable(s) that may be selected for their presence are not clear.

Conclusions

Both carbon amendment and site conditions influenced the efficacy of nitrate and selenium removal. Molasses amendment resulted in faster nitrate removal than either glycerol or methanol, while the alluvial wells removed more total selenium but also had a higher percentage of selenite remaining than the coal and interburden wells. In situ, selenite is likely to be removed via adsorption; however, under the constraints of this experiment, the stratigraphic unit significantly impacted selenite accumulation. The site geochemistry impacted the microbial community and is also an important consideration for the sorption of aqueous selenium species. Microbial community composition was also significantly correlated with carbon amendment, as well as with the removal of nitrate and selenium. Because carbon amendment, microbial community and local site conditions can interact in ways that impact contaminant removal efficacy, running treatability tests with site-specific water and solids is highly recommended before selecting a carbon source for nitrate and selenium bioremediation.

Supplementary material. To view supplementary material for this article, please visit http://doi.org/10.1180/gbi.2025.3.

Acknowledgements. This research was supported by a Montana Water Center fellowship. Kordelle Stephenson, Cody Cole and Gordon Criswell at Talen Montana provided assistance with sample collection and site data. Dr Rebecca Mueller and Dr Hannah Goemann provided support for the sequencing and analysis of microbial community data.

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