The Impact of Gamma Radiation on Sediment Microbial Processes

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Microbial communities have the potential to control the biogeochemical fate of some radionuclides in contaminated land scenarios or in the vicinity of a geological repository for radioactive waste. However, there have been few studies of ionizing radiation effects on microbial communities in sediment systems. Here, acetate and lactate amended sediment microcosms irradiated with gamma radiation at 0.5 or 30 Gy h\(^{-1}\) for 8 weeks all displayed NO\(_3^-\) and Fe(III) reduction, although the rate of Fe(III) reduction was decreased in 30-Gy h\(^{-1}\) treatments. These systems were dominated by fermentation processes. Pyrosequencing indicated that the 30-Gy h\(^{-1}\) treatment resulted in a community dominated by two Clostridial species. In systems containing no added electron donor, irradiation at either dose rate did not restrict NO\(_3^-\), Fe(III), or SO\(_4^{2-}\) reduction. Rather, Fe(III) reduction was stimulated in the 0.5-Gy h\(^{-1}\)-treated systems. In irradiated systems, there was a relative increase in the proportion of bacteria capable of Fe(III) reduction, with Geothrix fermentans and Geobacter sp. identified in the 0.5-Gy h\(^{-1}\) and 30-Gy h\(^{-1}\) treatments, respectively. These results indicate that biogeochemical processes will likely not be restricted by dose rates in such environments, and electron accepting processes may even be stimulated by radiation.

In many countries, including the United Kingdom, the current policy for the long-term disposal of intermediate-level radioactive waste is to a deep geological disposal facility (GDF). In UK disposal concepts for higher-strength rocks and lower-strength sedimentary rocks, much of the intermediate-level radioactive waste is immobilized with a cementitious grout in stainless steel containers that are then surrounded with a cementitious backfill prior to closure of the facility. The vicinity of a GDF will not be a sterile environment, and microbial activity in the surrounding geosphere could have important implications for the evolution of biogeochemical processes, including microbial gas generation and utilization, microbially induced corrosion of waste containers and contents, and the mobility of radionuclides.

Indeed, there will be elevated concentrations of potential electron donors in and around the repository, including organics from the degradation of cellulose in the waste, and molecular hydrogen from the radioisotopes of water and the anaerobic corrosion of steel drums. The availability of alternative electron acceptors will likely not be limited, since nitrate can be present in nuclear waste materials, and Fe(III) will be present due to aerobic corrosion of waste components and engineered infrastructure during the operational phase of the GDF.

The stimulation of an Fe(III)-reducing community due to an increase in electron donors and acceptors is of particular interest since this may promote the reduction and precipitation of redox-active radionuclides via the production of biogenic Fe(II)-bearing phases. Indeed, many key Fe(III)-reducing species may also possess cytochromes and hydrogenases capable of directly reducing multivalent elements, such as Tc(VII), Np(V), and U(VI), with radionuclides of interest in safety assessments. Because these processes could lower the mobility of these elements, the microbial ecology and potential for Fe(III) reduction in geodisposal environments has been the focus of recent research.

These environments, and the microbiolally driven processes that occur within them, may be subject to significant radiation doses. Firm values for total absorbed doses and dose rates are difficult to predict since they are likely to be highly heterogeneous and dependent on the activity of the waste, the radiation type, decay dynamics, and the absorbing materials in the waste. For example, Canadian researchers predict the maximum dose rate to be 52 GY h\(^{-1}\) at the surface of a waste container. Similarly, Allard and Calas suggest that dose rates in silicate clays used for backfill material may be in the order of 72 GY h\(^{-1}\) over the first 1,000 years of the repository lifetime. For Swedish spent fuel disposal, on the other hand, the maximum estimate of dose rate outside the canister is 0.5 Gy h\(^{-1}\) over the first 1,000 years, followed by significant decay after this.

Significant radiation fluxes may also be associated with near surface sites contaminated by radionuclides; for example, activities up to 0.37 GBq kg\(^{-1}\) have been measured at contaminated U.S. Department of Energy sites. Again, it is difficult to predict how activities such as this relate to dose rates and total absorbed doses; however, as a reference, it has been calculated by particle track calculation and Monte Carlo simulation that activities of 8.1 MBq kg\(^{-1}\) \(^{90}\)Sr and 9.6 MBq kg\(^{-1}\) \(^{137}\)Cs in Chernobyl soils equate to dose rates of 51.7 and 14.8 Gy year\(^{-1}\), respectively.

Irradiation radiation is potentially lethal to organisms since the energies involved are sufficient to cause strand breaks in DNA. Despite this, most bacteria encode conventional enzymatic DNA repair mechanisms, rendering much of the damage repairable. However, cytoplasmic water radiolysis generates quantities of re-
active oxygen species (e.g., HO\textsuperscript{+}, H\textsubscript{2}O\textsubscript{2}, and O\textsubscript{2}\textsuperscript{-})}, which may react indiscriminately with essential biomolecules, such as nucleic acids, proteins, and lipids, causing damage (15–17). Indeed, radiation-induced protein oxidation has been quantifiably related to bacterial viability (17).

When the generation of reactive oxygen species exceeds the scavenging capacity of the cell, oxidative stress is incurred. This, when combined with the inability of a cell’s metabolism to replenish damaged molecules as a result of radiation stress, likely results in fatality. The dose at which this occurs in a specific species is very variable and, as such, there has long been a focus on determining radiation sensitivity in environmentally important species. For example, the extreme radiation resistance of Deinococcus radio-
durans and the sensitivity of subsurface bacterial species such as Fe(III)-reducing Shewanella sp. have been assessed (15, 18). However, many of these studies were conducted with pure cultures at high acute doses, and while acute-dose laboratory studies may predict canister vicinities to be sterile (9, 19), survival may actually be possible under dose rates more relevant to nuclear environments. For example, under a chronic dose rate of ~2 Gy h\textsuperscript{-1}, microorganisms isolated from a spent nuclear fuel pond were capable of surviving total absorbed doses five times greater than tolerated in acute-dose experiments (>426 Gy h\textsuperscript{-1}) (20, 21). Furthermore, microbes from the indigenous endolithic community of a proposed repository were capable of surviving low gamma doses in a viable-but-nonculturable state (22), such that resuscitation may be possible when environmental conditions become more favorable (23). This highlights the importance of gathering low dose rate data, particularly since lower dose rates may allow species to respond via upregulating repair mechanisms (24) or even adapting over geological timescales, relevant to radiwaste disposal scenarios. Similarly, the survival data from pure culture studies may not be applicable to relatively nutrient limited sediments, where there is competition from different species of the community, and where radiation is perhaps not the only selective stress. Indeed, Bruhn et al. (21) showed that the survival of the usually radioresistant D. radio
durans in a mixed culture was somewhat limited, probably as a result of competition with Pseudomo
nas spp. However, this study was conducted in a rich tryptic soy broth medium that is far from representative of in situ GDF conditions.

Although it is important to examine the radiation tolerance of microbial community members, radiation may also impact upon the extracellular environment, which may consequently influence the capacity for microbial processes. For instance, the radiolysis of water generates molecular hydrogen, which may be used as an electron donor for a range of microbial electron accepting processes (2, 25–27). Furthermore, radiation has been shown to break down natural organic matter in soils, resulting in increases in dissolved organic carbon (DOC) (28, 29). This radiolytic degradation of organic matter may enhance the bioavailability of organic carbon for microbial metabolism.

The oxidation state of potential electron acceptors may also be altered by ionizing radiation. For instance, irradiation led to Fe(III) reduction in a range of materials, including clays and go
ethite (28, 30–32). On the other hand, irradiation induced oxidation of Fe in steel and aqueous Fe(II) solutions led to the generation of the Fe(II)/(III) oxides lepidocrocite, maghemite, and magnetite (33, 34). Such changes to the oxidation state of Fe have important implications for the bioavailability of Fe(III) for microbial respiration.

Even when no radiation-induced oxidation or reduction is observed, Fe(III) in both ferrihydrite and hematite may be made more available for microbial reduction via alteration to the crystalline structure (35). Since Fe is likely to be a significant component of waste packaging and repository infrastructure, such radiation effects could have important implications to deep subsurface microbial communities. With regard to other electron acceptors, many studies have shown a decrease in the concentration of nitrate in irradiated soils (36). On the other hand, sulfate concentrations increased in a soil by 17% after 30 kGy of gamma irradiation, although this was attributed to releases from lysed cells (37).

It is therefore evident that radiation may impact upon both cellular physiology and the bioavailability of growth substrates, i.e., electron donors, acceptors, and presumably nutrients (36). However, despite the potential consequences to the evolution of biogeochemical processes in nuclear environments, there is a lack of information on the combined effect of all of these radiological processes on microbial metabolism at low dose rates. Here, we address the impact of low-dose chronic gamma radiation upon a sediment microbial community and the biogeochemical processes controlled by this community both during irradiation and throughout a subsequent recovery stage. In addition, Fe(II) concentrations were probed to assess the ability of an irradiated community to carry out Fe(III) reduction. To our knowledge, this was conducted using the lowest dose rate over the longest irradiation period of any comparable study to date. Two dose rates were used: 30 Gy h\textsuperscript{-1}, representative of dose rates at radwaste canister surfaces, and 0.5 Gy h\textsuperscript{-1}, simulating dose rates further afield, or after decay of radiation levels and microbial repopulation of the repository vicinity. This is in sharp contrast to the acute radiation levels used in other pure culture studies.

MATERIALS AND METHODS
Sediment collection. Sediment samples were taken from a location representative of the Quaternary, unconsolidated alluvial flood-plain deposits in the vicinity of the UK Sellafield reprocessing site. This site was selected because our group has extensive experience studying the biogeochemistry of sediment from this area. Samples were collected from the shallow subsurface at a locality ~2 km from the Sellafield site, in the Calder Valley, Cumbria (38–40). Samples were transferred to sterile containers, sealed, and stored in the dark at 4°C prior to use.

Sediment microcosms. To assess the impact of gamma radiation on the indigenous microorganisms of the sediment, microcosms were prepared in sterile 100-ml serum bottles by the addition of a sterile synthetic groundwater representative of the region (41) to samples of sediment (10 ± 0.1 g of sediment, 100 ± 1 ml of groundwater buffered at pH 7 using NaHCO\textsubscript{3}, at 0.24 g l\textsuperscript{-1}). After addition of the buffered groundwater, the pH of the microcosms was ~6.4. Sodium lactate and sodium acetate were added as electron donors, where necessary, to give final added concentrations of 7 mM for each. Thus, a range of microcosm conditions were produced, as shown in Table 1. Triplicates of each of the different microcosms were then sealed with butyl rubber stoppers prior to irradiation.

Microcosms containing no added electron donor were also irradiated prior to the addition of an active Geobacter sulfurreducens culture to investigate the effect of radiation on Fe(III) reduction in the sediments, while evaluating the impact of radiation toxicity on the indigenous microorganisms. After irradiation, microcosms were purged with an N\textsubscript{2}/CO\textsubscript{2} (80:20) gas mixture to render the sediments anoxic to support microbial Fe(III) reduction. Suspensions of G. sulfurreducens (100 μl) were
added, where necessary, to give a final cell density of \(-10^7\) cells ml\(^{-1}\). Cultures were initially prepared by growing \(G.\ sulfurreducens\) at 30°C in a fully defined anaerobic medium, as described previously (42). Sodium acetate (20 mM) and fumarate (40 mM) were added as electron donor and acceptor, respectively. After 24 h, late-log/early-stationary-phase cultures were harvested anaerobically by centrifugation at 4,920 \(g\) and washed twice with sterile nitrogen-purged 30 mM sodium bicarbonate (pH 7.2).

**Irradiation**. Microcosm irradiations were carried out in the dark at Cell 5, AMEC, Harwell, United Kingdom. Co-60 gamma (1.25 MeV) was co-irradiated with the sediment slurry (2 ml) from each replicate microcosm was centrifuged at 4,920 \(g\) for 3 min. The supernatant was used for analysis by ion chromatography (44). Low-quality reads (mean quality score of <25) and short sequences (<390 bp) were discarded, and both forward and reverse primers were removed from further analysis. Denoising and chimera removal were performed during operational taxonomic unit (OTU) picking (at 97% sequence similarity) using "usearch" (48) in Qiime, and a representative sequence for each OTU was identified. The taxonomic classification of all reads was performed in Qiime using the Ribosomal Database Project (RDP) at a 80% confidence threshold (48), while the closest GenBank match for the OTU that contained the highest number of reads (the representative sequence for each OTU was used) was identified by BLASTN nucleotide search.

**RESULTS AND DISCUSSION**

Biogeochemistry of irradiated microcosms containing added electron donor. To assess the impact of chronic gamma irradiation on the biogeochemical processes in the sediment, a series of microcosms were prepared with or without added electron donor and irradiated for 56 days at 0.5 and 30 Gy h\(^{-1}\). In control and irradiated microcosms spiked with lactate and acetate (final added concentrations of 7 mM each), electron acceptor usage progressed in the order nitrate > Fe(III) > sulfate during the irradiation period (Fig. 1). Treatment with gamma radiation at 30 Gy h\(^{-1}\) did not appear to affect the reduction of nitrate, which was removed completely from porewaters after 28 days in both treated and control microcosms. However, 0.5 N HCl extractable Fe(II) concentrations in microcosms after treatment with 30 Gy h\(^{-1}\) for 56 days were ~0.5 mM compared to ~2 mM in nonirradiated controls. This limited Fe(III) reduction was likely due to decreased viability of Fe(III)-reducing microorganisms arising from a total absorbed dose of 38.6 kGy. After completion of the irradiation, the levels of 0.5 N HCl extractable Fe(II) increased gradually over 176 days to levels comparable to those in nonirradiated microcosms, suggesting that significant Fe(III) reduction was still possible, albeit at a slower rate, even after the maximum radiation dose was applied.
Reducing bacteria survived irradiation, followed by their gradual regrowth after the irradiation was terminated.

Treatment with gamma radiation at 0.5 Gy h\(^{-1}\) did not have a significant impact on the amount of nitrate, Fe(III) or sulfate reduction noted (Fig. 1). Indeed, the extent of nitrate and Fe(III) reduction after irradiation of the microcosms for 58 days was the same as in the nonirradiated controls. These data suggest that irradiation at this lower dose rate did not have a significant effect on the microbial communities which control electron acceptor turnover.

Lactate concentrations in all spiked systems decreased throughout the irradiation period, resulting in the complete removal from solution after 56 days for both 0.5- and 30-Gy h\(^{-1}\)-treated systems and in nonirradiated control microcosms (Fig. 2). This suggests that lactate was likely used as a carbon source or as an electron donor for the electron accepting processes described earlier. Lactate removal was not as rapid in 30-Gy h\(^{-1}\)-treated systems, and this may be related to a reduction in microbial activity or viability associated with radiation toxicity at this higher dose rate.

Acetate concentrations did not change significantly in any of the microcosms during the irradiation period; however, acetate was completely removed from solution in control systems and in the 0.5-Gy h\(^{-1}\)-treated microcosms after a 48-day recovery period. This is consistent with the use of acetate as an electron donor, as observed in previous studies with this sediment type (39), albeit after more thermodynamically favorable processes had consumed other electron donors, such as lactate. However, in microcosms treated with 30 Gy h\(^{-1}\), acetate concentrations increased significantly to \(~11\) mM after 147 days, followed by its complete removal from solution after 280 days. The increase in acetate levels is likely a result of fermentation reactions catalyzed by more radiation resistant members of the community (see below). Indeed, the delayed removal from solution may suggest an initial decrease in viability of members of the microbial community capable of respiring acetate as a result of irradiation, although these processes appear able to recover after a period of removal from the radiation source.

Propionate appeared in both control systems and systems irradiated with 0.5 Gy h\(^{-1}\) and increased throughout the irradiation period to a concentration of \(~3.5\) mM. After 56 days, when the experimental microcosms were removed from the radiation source, propionate concentrations decreased throughout the recovery period at approximately the same rate as in the nonirradiated microcosm controls, resulting in complete removal from solution after 205 days. This generation and removal of propionate is consistent with its production via fermentation of lactate (52) and subsequent use as an electron donor. Propionate was not detected in the 30-Gy h\(^{-1}\)-treated systems at any sampling point, suggesting that other metabolic pathways were more dominant for this treatment.

A slight increase in formate concentrations to \(~50\) \(\mu\)M was observed in the nonirradiated microcosms and 0.5-Gy h\(^{-1}\) treatments during the recovery period after 147 days. In the 30-Gy h\(^{-1}\) treatments, on the other hand, formate appeared during the latter half of irradiation to a concentration of \(~120\) \(\mu\)M. In addition to formate, a large increase in malate was also observed in the 30-Gy h\(^{-1}\)-treated microcosms only, with a significant increase during the recovery period to \(~12\) mM after 105 days. Although DOC has previously been observed to increase as a result of sediment
gamma irradiation (29), the significant production of malate during the recovery period and formate during the latter half of the irradiation period suggests that they are likely fermentation products. Furthermore, their subsequent removal from solution is consistent with their use as electron donors.

Microbial community changes in irradiated microcosms containing added electron donor. Analysis of the bacterial community in the oxic starting sediment revealed a relatively diverse community with 16 phyla detected through pyrosequencing of 16S rRNA gene amplicons. Communities were dominated by species representing the acidobacteria (47%) and proteobacteria (32%), a finding consistent with previous studies conducted on Sellafield-type sediments (38, 39). Of the most dominant individual species, an uncharacterized acidobacterium and a bacterium of the Bradyrhizobiaceae family (proteobacteria) represented 5 and 4% of the complex microbial community, respectively.

After 147 days, the microbial community of nonirradiated sediment microcosms containing added lactate and acetate showed a decrease in the relative contributions of acidobacteria (21%) and proteobacteria (17%) (Fig. 3). However, the most marked shift was an increase in organisms affiliated with the Bacteroidetes (29% of the community) and Firmicutes (22%; of which, 97% were affiliated with clostridia). The Bacteroidetes included uncultured Prolixibacter spp. (7% of the total microbial community), two uncultured Bacteroidetes bacteria (4% and 3%) and an organism affiliated with Paludibacter propionicigenes (2%). The Prolixibacter genus comprises facultative anaerobes capable of sugar fermentation (53), with P. propionicigenes an anaerobic propionate producing strain which can utilize a range of sugars to produce acetate and propionate as major fermentation products (54). In addition, organisms affiliated with the clostridial group (Firmicutes) catalyze a mixed acid fermentation under anoxic conditions (55). Thus, the relative increase in clostridia and Paludibacter species is likely related to the significant production of propionate observed during the first 56 days (Fig. 2) (52). Furthermore, clostridia, such as Pelotomaculum spp. (10% of the community), includes species capable of oxidizing propionate (56). The increase in such species may be related to the decrease in propionate observed after 56 days (Fig. 2).

Species of the known Fe(III)-reducing genus Geobacter showed a slight increase to represent 1% of the community in control systems. This correlates with the increase in Fe(III) reduction observed during the first 56 days (Fig. 1). Although Fe(III) reduction is clearly a significant electron-accepting process in these sediments, Geobacter spp. or other known Fe(III)-reducing bacteria were not dominant components of this community, probably due to the dominance of fermentative processes as a result of the addition of significant organic carbon concentrations.

Similar community shifts were also observed in 0.5 Gy h\(^{-1}\) treatments. An organism affiliated with the Bradyrhizobiaceae was also present in the irradiated microcosm community at a proportion similar to that in the nonirradiated microcosm community (4%). In contrast to control systems, bacteria of the Firmicutes phylum were not as well represented in the microcosm community irradiated with 0.5 Gy h\(^{-1}\) (13%). However, an organism closely related to a member of the genus Pelotomaculum (97% match) was again the main representative of this class (3%), and this may be related to the similar levels of propionate observed in these two treatments. The proteobacteria appeared slightly enriched in this treatment (24%) compared to the control sample (17%), with Geobacter spp. comprising 8% of this group. Thus, significant Fe(III) reduction by this genus was likely more important in these systems. A betaproteobacterium closely related to species of the genus Jahnthinobacterium also represented a significant proportion of the community at 4%. Species of this genus
were well represented in a previous study using similar sediments containing added nitrate, and it was suggested that Betaproteobacteria such as this may be involved in the reduction of nitrate (57). Thus, the appearance of this genus in sediments treated with 0.5 Gy h \(^{-1}\) may be related to nitrate reduction observed early on in the irradiation period.

In the microcosms irradiated at 30 Gy h \(^{-1}\), a marked loss in diversity occurred after 147 days, with a strong shift toward species of the Firmicutes phylum (91%) (Fig. 3). Two close relatives of known clostridial species were the main components of this phylum. The first, an uncultured Clostridiaceae bacterium, represented 83% of the total community. This species is most closely related to an organism isolated from a sulfate-reducing enrich-ment of sediments from an acid mine lake (95% match) (58). The second, an organism most closely related to a novel Clostridium bowmanii species (98% match) originally isolated from a microbial mat in the McMurdo Dry Valley region of Antarctica (59), represented 8% of the total community. Members of the clostridial family catalyze a mixed acid fermentation, with C. bowmanii capable of generating butyrate, acetate, formate, ethanol, and lactate (59). As such, it is possible that these species may have been involved in fermentation processes, including acetate and formate production, observed throughout the incubation period (Fig. 2).

In addition, most of the species within this family are able to form endospores, which may allow cells to survive a range of environmental stresses (60). As such, it is likely that both these species represent radioresistant members of the sediment community. Thus, in environments with significant radiation fluxes, some non-spore-formers may be able to recover to become dominant members of a sediment microbial community.

Despite Clostridiaceae species dominating the bacterial community after 147 days in the 30-Gy h \(^{-1}\) treatment, subsequent incubation to 280 days in the absence of radiation led to a significant reduction in the contribution of these organisms to the total microbial community. Firmicutes comprised \(\sim 13\%\) of the community, with Bacillus spp. the main component (\(\sim 10\%\) of the total community), whereas relatives of Clostridiaceae only comprised \(\sim 1\%\) of the total community. Of the remaining clostridia, species of the Desulfovosporinus genus comprised 1.5% of the total microbial community. This genus contains known spore formers capable of reducing Fe(III) and sulfate (61), and thus the increase in relative abundance of species of this genus may indicate radiation tolerance (via endospore formation) and subsequent contribution to significant Fe(III) and sulfate reduction observed throughout the incubation period to 280 days.

In addition, there was a significant increase in members of the Bacteroides phylum (\(\sim 71\%\) of the total community), of which members of the order Bacteroidales comprised \(\sim 57\%\) of the total community. Deeper phylogenetic classification of these organisms was not possible in this analysis; however, the emergence of members of this class after the 30 Gy h \(^{-1}\) treatment is somewhat surprising since this taxon comprises non-spore-forming, Gram-negative anaerobes. This result therefore suggests that even after exposure to high dose rates of gamma radiation, some non-spore-forming microbial species may be able to recover to become dominant members of a sediment microbial community.

Although Fe(II) concentrations in the 30-Gy h \(^{-1}\) treatment returned to the same level as noted in nonirradiated controls during the period prior to phylogenetic analysis after 280 days, relatives of known Fe(III)-reducing species were not well represented in the microbial community. A close relative of Geotheix fermen-tans (acidobacteria; 97% match) comprised 1.9% of the total microbial community, while Geobacter spp. contributed 0.8% to the community. Despite this, these results highlight the potential for electron accepting processes to recover in sediments subject to significant radiation fluxes with available organic carbon substrates present.

**Biogeochmistry in microcosms containing no added electron donor.** In addition to systems containing added carbon, the impact of gamma radiation on sediment biogeochemistry and microbial communities was also assessed with microcosms containing no added electron donor (lactate or acetate). Radiation had no
significant effect on the generation or reduction of sulfate throughout the irradiation period. However, after irradiation, significant Fe(III) reduction was observed in microcosms treated with gamma radiation at 0.5 Gy h\(^{-1}\), whereas Fe(III) reduction in control and 30-Gy h\(^{-1}\)-treated microcosms was not observed until day 105. Indeed, Fe(III) reduction in the 0.5-Gy h\(^{-1}\)-treated systems continued throughout the incubation period at an enhanced rate. No increase in 0.5 N HCl extractable Fe(II) was observed in control or irradiated microcosms during the 56-day irradiation period since the absence of added electron donor likely precluded microbial Fe(III) reduction during this initial period. In contrast to systems containing added lactate and acetate, nitrate removal in the unamended microcosm controls and in those irradiated with 30 Gy h\(^{-1}\) was slower. However, nitrate concentrations in the 0.5-Gy h\(^{-1}\)-treated microcosms were slightly lower (\(\sim 70 \mu M\)) than in nonirradiated microcosms (\(\sim 120 \mu M\)) (Fig. 4). General removal of nitrate in both systems is likely related to the activity of denitrifying bacteria. However, the increased removal in the 30-Gy h\(^{-1}\)-treated microcosms is consistent with the abiotic removal of nitrate in previous studies of gamma sterilization of sediments (36). The reasons for this are unclear; however, radiolysis studies have shown that abiotic decomposition of nitrate to nitrite is possible (62). It is not possible to say whether this process also occurred in the 0.5-Gy h\(^{-1}\) treatments because nitrate concentrations were not determined during the irradiation of these microcosms. However, these results suggest that radiolysis of nitrate may promote the removal of nitrate in irradiated sediments. In turn, this may have resulted in the early onset of Fe(III) reduction, followed by enhanced sulfate reduction (after 150 days) observed in the 0.5-Gy h\(^{-1}\)-treated microcosms, due to a decreased competition for the alternative electron acceptor. It is not clear why this enhanced Fe(III) reduction was not observed in the 30-Gy h\(^{-1}\)-treated microcosms; however, this may be precluded by increased radiation toxicity associated with a higher dose.

Formate was generated in all treated and untreated microcosms (up to \(\sim 50 \mu M\)) throughout the recovery period and was likely a product of fermentation (Fig. 5). Acetate, on the other hand, was not observed in control microcosms or in the microcosms irradiated at 0.5 Gy h\(^{-1}\); however, \(\sim 0.2 \mu M\) acetate was produced in the 30-Gy h\(^{-1}\)-treated microcosms during the latter half of the irradiation period. Acetate generation in this irradiated system continued throughout the recovery period until 105 days,
but by the end of the incubation period acetate had largely been removed from the solution. The production of acetate (during the latter part of irradiation only) and its subsequent removal suggests its production by microbial fermentation, followed by its oxidation as an electron donor. It is possible that these processes occurred in the nonirradiated systems and in the 0.5-Gy h\(^{-1}\)-treated systems; however, acetate may have been metabolized as quickly as it was formed. Thus, the detection of acetate in the 30-Gy h\(^{-1}\)-treated systems may be a result of radiation toxicity in acetate-oxidizing species.

It is unclear from these data whether the enhanced reduction of Fe(III) in the 0.5-Gy h\(^{-1}\)-treated microcosms was related to an increase in the availability of organic electron donors, increases in the bioavailability of Fe(III), or a decrease in electron acceptor competition arising from enhanced nitrate removal.

**Microbial community changes in the absence of added electron donor.** To assess potential changes to the microbial community which may have led to the enhanced Fe(III) reduction observed in 0.5-Gy h\(^{-1}\)-treated microcosms, bacterial phylogenetic diversity was assessed in samples taken immediately after irradiation (\(T = 57\), where \(T\) represents time in days; Fig. 6). Both the nonirradiated and the 0.5-Gy h\(^{-1}\)-treated microcosms showed slight enrichment of proteobacterial species, including a representative of the *Bradyrhizobiaceae* (*Alphaproteobacteria*) (6% in controls and 8% in 0.5-Gy h\(^{-1}\) treatments). Controls were also enriched in a relative of a known *Janthinobacterium* sp. (*Betaproteobacteria*; 5%), but this was not observed in the treated systems. However, the 0.5-Gy h\(^{-1}\)-treated microcosms did show a slight increase in an organism affiliated with *Rhodoferax* spp. (99% sequence similarity; 2% in treated versus <1% in controls). The closest known relative was originally identified in Arctic glacier melt water and has 98% sequence similarity to a *Rhodoferax furri-reducens* strain that exhibits dissimilatory Fe(III) reduction (63, 64). Although the increase in abundance of relatives of this organism may be consistent with the enhanced levels of Fe(III) reduction which was observed in the 0.5-Gy h\(^{-1}\)-treated system after the removal of experiments from the radiation source, this organism was not detected in the microbial community analyzed at 147 days, after significant Fe(III) reduction had been observed in these microcosms.

As with electron donor spiked systems, community analysis after 147 days of incubation of the nonirradiated system containing no added carbon revealed a relative increase in the *Bacteroidetes* (9%) and *Firmicutes* (3%) phyla (Fig. 6). As with electron donor spiked systems, the *Bacteroidetes* phylum was strongly represented by *Proluxibacter*-related species (6%). A significant increase in abundance of a relative of *Geothrix fermentans* (acido-bacteria) was also observed (from <1% in the \(T = 57\) sample to 5% in the \(T = 147\) sample) and, since this is a known Fe(III)-reducing species (65), this increase is likely related to the Fe(III) reduction observed in this system after 105 days.

Community analysis of the 0.5-Gy h\(^{-1}\) treatment after 147 days displayed a further relative increase in representatives of the *Bacteroidetes* (19%) and *Firmicutes* (7%) phyla. Unclassified species of the *Bacteroidales* order showed a significant increase, representing 17% of the total microbial community, with respect to the control sample (6%). Uncultured *Proluxibacter* spp. also showed an increase with respect to control samples and were well represented in this treatment at 10% of the total community. The increase in representatives of the *Firmicutes* phylum mainly arose from a general increase in clostridial species, which may indicate an increase in fermentation activity or is perhaps related to spore formation and enhanced survival.

In addition, an organism most closely related to *Geothrix fermentans* (acidobacteria; ~98% sequence similarity) showed a significant increase with respect to the control sample, representing 22% of the total microbial community. *Geobacter* spp. were increasingly represented with respect to controls, with an organism most closely related to *G. chapellei* comprising 3% of the community, compared to the most populous in control samples: a *G. bremenensis* relative (0.2% of the total community). This relative increase in *Geothrix* and *Geobacter* spp. after 147 days, rather than the emergence of the *Rhodoferax* relative described earlier (after 57 days), is more consistent with the enhanced level of Fe(III) reduction in the 0.5-Gy h\(^{-1}\)-treated microcosms.

In response to the 30-Gy h\(^{-1}\) treatment, further relative in-
increases were observed in the Bacteroidetes and Firmicutes phyla (Fig. 6). Unclassified species from the order Bacteroidales represented 37% of the total microbial community. This comprised two dominant species: the first (12% of the community) was most closely related to an uncultured bacterium isolated from moss pillars at an Antarctic lake (66) and the second was an uncultured Pseudoxanthomonas sp. (11%). Paludibacter spp., also of the order Bacteroidales, represented 5% of the total community and species of the family Chitinophagaceae (Bacteroidetes phylum) comprised 7% of the total community. Two uncultured spirochetes (Bacteroidetes phylum) represented 14% of the community. Interestingly, these observations are similar to those from microcosms amended with acetate and lactate and irradiated at 30 Gy h\(^{-1}\), in which members of the Bacteroidales represented ~57% of the total microbial community after 280 days. These results suggest that members of the Bacteroidetes phylum may exhibit high levels of radiation resistance and potentially represent a group of respiratory generalists capable of dominating a community after significant radiation stress.

Of the key Fe(III)-reducing species in the microcosms irradiated at 30 Gy h\(^{-1}\), 18% of the total community was affiliated with known Geobacter species. However, unlike in the microcosms irradiated at 0.5 Gy h\(^{-1}\), Geothrix species were not well represented, comprising <0.1% of the total microbial community. In addition, a close relative of the Herbaspirillum frisingense (Betaproteobacteria) comprised 5% of the total community (98% sequence similarity). This species is capable of nitrate reduction and nitrogen fixation and can oxidize a broad range of sugars and alcohols (67). As in the microcosms irradiated at 0.5 Gy h\(^{-1}\), the increase in Firmicutes mainly arose from a general increase in clostridial species.

These results indicate that despite sediments receiving a total absorbed dose of nearly 40 kGy, Fe(III) reduction was still possible in sediments without added electron donor. Furthermore, irradiation of these sediments resulted in significant increases in abundance of Fe(III)-reducing species compared to nonirradiated systems. This suggests that, although Fe(III) reduction was not enhanced in the 30-Gy h\(^{-1}\)-treated systems, these sediments may be poised for Fe(III) reduction.

Fe(III) reduction in irradiated microcosms inoculated with Geobacter sulfurreducens. To assess the potential for enhanced Fe(III) reduction in the microcosms irradiated at 30 Gy h\(^{-1}\), irradiated and control microcosms were inoculated with cultures of G. sulfurreducens, and 0.5 N HCl extractable Fe(II) was monitored (Fig. 7). Both 0.5- and 30-Gy h\(^{-1}\)-treated microcosms showed enhanced Fe(III) reduction with respect to the control systems, 21 days after inoculation. Fe(III) reduction was observed in inoculated nonirradiated microcosms after 35 days. Fe(II) concentrations approached those in the irradiated microcosms after 92 days, albeit at a slower rate. These results suggest that, as in the microcosms irradiated at 0.5 Gy h\(^{-1}\), a potential for enhanced Fe(III) reduction existed in the microcosms irradiated at 30 Gy h\(^{-1}\) (Fig. 4), but reduced viability of Fe(III)-reducing species at this radiation dose precluded it.

Radiation has been previously shown to release significant quantities of DOC into solution in a range of soils exposed to 25 to 60 kGy (29, 68, 69). This could potentially increase the availability of carbon for use as a carbon source or electron donor. Irradiation at 30 Gy h\(^{-1}\) did lead to increased concentrations of organic acids representative of the bioavailable organic fraction in sediments (Table 2). However, such micromolar increases were probably not sufficient to account for the observed Fe(III) reduction. Moreover, no significant increases in organic acids were observed in microcosms irradiated at 0.5 Gy h\(^{-1}\), nor were there significant radiation induced increases during the irradiation period of the noninoculated microcosms.

Previous experiments indicated that gamma radiation may lead to an increase in the availability of Fe(III) oxides for microbial Fe(III) reduction (35). It is possible that the enhanced Fe(III) reduction observed here may be related to this phenomenon. Although the previous study observed this effect after acute irradiation to 1 MGy, our results may suggest that a similar, more subtle process may also occur at lower doses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bioavailable Fe (mM)</th>
<th>NO(_3) (mM)</th>
<th>NO(_2) (mM)</th>
<th>SO(_4^{2-}) (mM)</th>
<th>Lactate ((\mu)M)</th>
<th>Acetate ((\mu)M)</th>
<th>Propionate ((\mu)M)</th>
<th>Butyrate ((\mu)M)</th>
<th>Formate ((\mu)M)</th>
<th>Fumarate ((\mu)M)</th>
<th>Oxalate ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonirradiated</td>
<td>0.74 ± 0.02</td>
<td>0.53 ± 0.02</td>
<td>ND</td>
<td>0.40 ± 0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.5 Gy h(^{-1})</td>
<td>0.70 ± 0.02</td>
<td>0.29 ± 0.03</td>
<td>0.01 ± 0.02</td>
<td>0.40 ± 0.00</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>30 Gy h(^{-1})</td>
<td>0.81 ± 0.03</td>
<td>0.13 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>3.5 ± 3.0</td>
<td>57.2 ± 70.0</td>
<td>3.9 ± 0.4</td>
<td>1.1 ± 0.9</td>
<td>32.7 ± 18.1</td>
<td>45.5 ± 13.7</td>
<td>59.5 ± 17.9</td>
</tr>
</tbody>
</table>

\(a\) Errors indicate the standard errors of triplicate measurements. ND, not detected.
\(b\) Fumarate and oxalate have identical retention times in the chromatography system used. The concentrations have therefore been determined for each based on their respective molecular masses.

FIG 7 0.5 N HCl extractable Fe(II) concentrations in control and irradiated microcosms inoculated with G. sulfurreducens. Microcosms were removed from the irradiation cell and inoculated at \(T = 0\). Error bars represent the standard errors of the mean of triplicate experiments and, where not visible, error bars are within the symbol size.
On the other hand, the enhanced Fe(III) reduction may also be related to the removal of nitrate by radiolysis, as in the irradiation of sediments containing no added \textit{G. sulfurreducens} cells or electron donors. Nitrate concentrations in irradiated microcosms (0.13 mM in 30-Gy h^{-1} treatments and -0.3 mM in 0.5-Gy h^{-1} treatments) were significantly lower immediately after irradiation than in nonirradiated systems (-0.5 mM). Again, these results are consistent with radiation enhanced removal of nitrate and the early onset of Fe(III) reduction, as discussed previously.

\textbf{Implications for the geodisposal of radioactive waste.} In the present study, we have highlighted microbial activities under dose rates representative of gamma radiation emitted from radioactive waste canister surfaces in the near field of a geological disposal facility. We then assessed microbial activities under a simulated recovery period that would exist after significant radioactive decay had occurred.

Previous studies suggested that microbial activity will be suppressed in these environments. For instance, studies of survival of microorganisms from clay buffer material have suggested that typically only 10% of the population survives after doses of -1.6 kGy (9) and that the dose rate may not have a significant impact on the viability of microbial populations (19). On the other hand, indigenous members of an endolithic microbial community from a proposed high-level radioactive waste repository may have been able to survive in a nonculturable state after irradiation (9.34 kGy at 1.63 Gy min^{-1}), to be rejuvenated when conditions become favorable (22, 23).

In contrast, the results presented here indicate that a sediment community can survive long-term gamma irradiation and that components of these communities can remain active and catalyze biogeochemical processes, including Fe(III) reduction. We have shown this to be the case for doses of up to ~38 kGy using a lower, environmentally relevant dose rate of 30 Gy h^{-1}. Indeed, the dose rate had a strong influence on the community structure in systems with or without added carbon. This demonstrates the importance of acquiring low dose rate data, particularly since lower dose rates may allow species to respond via upregulating repair mechanisms (24) or adapting over the geological timescales involved.

Radiation led to significant changes in the microbial communities, with fermentative bacteria, such as clostridia, dominant in systems with added carbon. Such changes may be important in environments where there is an excess of carbon substrates, such as in cellulosic wastes (3). Despite this loss of diversity, these results suggest that Fe(III) reduction can still be an important electron accepting process in such sediments. Furthermore, in environments with lower electron donor concentrations, an Fe(III)-reducing community may be selected by radiation. This may occur both directly, by making Fe(III) more bioavailable through radiation-induced changes to the mineralogy, or indirectly, by radiation-induced removal of other electron acceptors, such as nitrate, which may lead to the early onset of microbial Fe(III) reduction. Regardless, a relative increase in Fe(III)-reducing species was also observed in irradiated systems that did not display enhanced Fe(III) reduction. These results have positive implications for the geodisposal of radioactive waste, where the stimulation of an Fe(III)-reducing community by radiation may enhance the reduction and subsequent precipitation of radionuclides by direct enzymatic or indirect [e.g., biogenic Fe(II)]-mediated] mechanisms. Furthermore, the oxidation of molecular hydrogen by the radiolysis of water coupled to the enhanced reduction of alternative electron acceptors by low-dose gamma radiation could provide the basis of a novel ecosystem in the deep biosphere. Future studies will focus on the radiolysis of recalcitrant organic matter and the potential for enhanced carbon mineralization by subsurface microbial communities. Further work would be required to assess how these altered communities may affect the mobility of key radionuclides.

\textbf{ACKNOWLEDGMENTS}

This study was funded by a BBSRC studentship awarded to A.R.B. and CASE funding from Radioactive Waste Management, Ltd. Irradiations were carried out by AMEC, Harwell, Oxfordshire, United Kingdom, and we are grateful for the assistance of Victoria Smith and Alan Hollinrake. The work of Clare Thorpe in sediment collection and Alastair Bewsher in IC analysis is also greatly appreciated.

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