ASTROBIOLOGY
Volume 16, Number 4, 2016

DOI: 10.1089/ast.2015.1365

Protection of Nitrate-Reducing Fe(II)-Oxidizing Bacteria from UV Radiation by Biogenic Fe(III) Minerals

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Abstract

Due to the lack of an ozone layer in the Archean, ultraviolet radiation (UVR) reached early Earth's surface almost unattenuated; as a consequence, a terrestrial biosphere in the form of biological soil crusts would have been highly susceptible to lethal doses of irradiation. However, a self-produced external screen in the form of nanoparticular Fe(III) minerals could have effectively protected those early microorganisms. In this study, we use viability studies by quantifying colony-forming units (CFUs), as well as Fe(II) oxidation and nitrate reduction rates, to show that encrustation in biogenic and abiogenic Fe(III) minerals can protect a common soil bacteria such as the nitrate-reducing Fe(II)-oxidizing microorganisms *Acidovorax* sp. strain BoFeN1 and strain 2AN from harmful UVC radiation. Analysis of DNA damage by quantifying cyclobutane pyrimidine dimers (CPD) confirmed the protecting effect by Fe(III) minerals. This study suggests that Fe(II)-oxidizing microorganisms, as would have grown in association with mafic and ultramafic soils/outcrops, would have been able to produce their own UV screen, enabling them to live in terrestrial habitats on early Earth. Key Words: Archean—Biominerals—UVR—Protection. Astrobiology 16, 301–310.

1. Introduction

The timing for the evolution of a terrestrial biosphere remains poorly constrained, but a number of recent studies have pointed to life on land by the Archean. For instance, Stueken *et al.* (2012) suggested that an increase in the total sulfur and molybdenum supply to marginal marine sediments at 2.8 Ga was best explained by the biological oxidation of crustal sulfide minerals, while chromium isotope and uranium enrichment data from a paleosol (ancient soil horizon) of the Pongola Supergroup appear to reflect some oxidative weathering at *ca.* 3.0 Ga (Crowe *et al.*, 2013). Similarly, the occurrence of supermature quartz arenites of fluvial origin in the ~3.0 Ga Superior and Churchill Provinces, Canada (*e.g.*, Donaldson and de Kemp, 1998), and the presence of highly weathered shales in the 3.2 Ga Moodies Group, South Africa (*e.g.*, Hessler and Lowe, 2006), support the notion that the source terranes were subject to intense chemical, and perhaps biological, weathering. It has even been suggested that life must have existed on land, perhaps as early as 3.8 Ga (in the form of thermophilic archaea), such that it facilitated the planet's cooling through enhanced chemical weathering that removes atmospheric pCO_2 (Schwartzman and Volk, 1989).

An Archean terrestrial biosphere may have been in the form of biological soil crusts (BSCs), a cohesive surface layer of soil (millimeters thick) in which mineral particles are held together by various microbial filaments, their sheaths, and extracellular polysaccharides (Belnap *et al.*, 2003). Today, BSCs are restricted to arid and semiarid environments where the restriction of higher plants prevents leaf litter from blocking the surface soil from being illuminated, but prior to plant evolution in the Phanerozoic, BSCs likely colonized much of the exposed land surfaces (Schwartzman and Volk, 1989; Simpson *et al.*, 2013). In the absence of an ozone layer at that time, terrestrial bacteria growing at the surface would have been particularly susceptible to the unattenuated fluxes of incoming ultraviolet radiation (UVR), particularly UVC (wavelengths 100–280 nm), because DNA molecules absorb strongly at 254 nm and proteins at 280 nm. This causes formation of DNA photoproducts, for example, cyclobutane pyrimidine dimers (CPD), and DNA strand breakages (DSB) (Sinha and Häder, 2002) that inhibit DNA transcription and replication, leading to reduced viability or death of the organism (Pfeifer, 1997). Hence, in order to live under such conditions, these early microbes must have used strategies to survive detrimental UVR by either developing UV damage repair systems (Sinha and

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Häder, 2002; Häder and Sinha, 2005), seeking shelter in endolithic habitats (Häder and Sinha, 2005), developing protective pigments (Garcia-Pichel, 1998; Dillon and Castenholz, 1999), or producing an external sunscreen (Olson and Pierson, 1986; Cleaves and Miller, 1998; Garcia-Pichel, 1998; Cockell, 2000; Phoenix *et al.*, 2001; Bishop *et al.*, 2006). Considering the small size of bacteria (on the order of micrometers), such an effective external screen to protect microbial cells could consist of small mineral particles that only need to be available in the close cell environment or even at the cell surface.

Thick paleosols rarely develop on ultramafic rocks because such rocks are mostly composed of aluminum-poor silicates (olivine, pyroxene) that are easily leached out by rainwater (Watanabe *et al.*, 2000). However, secondary weathering products, such as Fe(III) minerals, silica-iron minerals, as well as mixtures of Fe(III) minerals and clays, have been shown to act as effective UV screens for phototrophic microorganisms (Pierson *et al.*, 1993; Pierson and Parenteau, 2000; Phoenix *et al.*, 2001, 2006; Bishop *et al.*, 2006; Gómez *et al.*, 2007). Based on this, it was proposed that ancient Fe(II)-oxidizing microorganisms would have had the need and possibility to produce their own inorganic UV shield in the form of Fe(III) mineral precipitates (Pierson *et al.*, 1993; Bishop *et al.*, 2006). Whether such a self-protection by Fe(III) mineral–forming bacteria is indeed possible has not been demonstrated so far.

Fe(II)-oxidizing microorganisms are phylogenetically widely distributed in Bacteria and Archaea and have been identified in many different habitats, suggesting that microbial Fe(II) oxidation is an ancient form of microbial energy metabolism (Ilbert and Bonnefoy, 2013). It has been proposed that they arose independently more than once in evolution and evolved convergently (Ilbert and Bonnefoy, 2013). The same authors have suggested that anoxygenic phototrophic Fe(II)-oxidizers arose in surface waters after the Archaea/Bacteria split but before the Great Oxidation Event, while the nitrate-reducing Fe(II)-oxidizers are potentially the most ancient Fe(II)-oxidizers. Such nitrate-reducing Fe(II)-oxidizers, for example, *Acidovorax* sp. strain BoFeN1, are common constituents in soils and sediments and have been shown to become encrusted in Fe(III) minerals (in nanocrystalline goethite) during Fe(II) oxidation in batch cultures (Kappler *et al.*, 2005; Miot *et al.*, 2009; Schädler *et al.*, 2009; Schmid *et al.*, 2014). Similar encrustation patterns were also found in environmental samples from iron-rich hot springs (Konhauser and Ferris, 1996; Konhauser, 1998). The effect of encrustation on these cells is still unclear, but it has been shown that a significant fraction of cells of an encrusted culture is still viable and able to grow (Kappler *et al.*, 2005; Muehe *et al.*, 2009; Klueglein *et al.*, 2014). This suggests that, aside from harmful effects, encrustation might have advantages for populations of nitrate-reducing Fe(II)-oxidizers, for example, screening them from UVR.

To determine potential protective effects of bio(iron)minerals for microorganisms against UVR, the goals of this work were to determine (1) whether biotic Fe(III) mineral formation at the cell surface and cell encrustation by such minerals can protect the *Acidovorax* sp. strains BoFeN1 and 2AN from UVR and (2) whether abiogenically synthesized Fe(III) minerals have the same effect as the biogenic minerals for the nitrate-reducing Fe(II)-oxidizers.

2. Materials and Methods

2.1. Source of microorganisms

Acidovorax sp. strain BoFeN1 was isolated from Lake Constance sediments, Germany. The strain is a chemoorganotrophic, nitrate-reducing and Fe(II)-oxidizing bacterium that belongs to the Betaproteobacteria (Kappler *et al.*, 2005) and has been kept in the authors' laboratory since its isolation. The second nitrate-dependent Fe(II)-oxidizer used in this study, *Acidovorax* sp. strain 2AN, was isolated from an iron-rich river sediment from Dorn Creek, Wisconsin, USA (Chakraborty *et al.*, 2011) and was kindly provided by F. Picardal (Indiana University, USA).

2.2. Microbial growth medium and growth conditions

For routine cultivation, strains were grown in anoxic, 22 m*M* bicarbonate-buffered low-phosphate mineral medium (pH 7), which was prepared anoxically as described previously (Hegler *et al.*, 2008; Hohmann *et al.*, 2010). A Fe(II)-containing medium was prepared by adding a 1 *M* $FeCl_2$ stock solution to a concentration of 10 m*M* and filtering the medium after storage for 48 h at 4°C to remove Fe(II) precipitates, leading to a final concentration of $\sim$8 m*M*. Strains BoFeN1 and 2AN were cultivated with 10 m*M* sodium nitrate as the electron acceptor and 5 m*M* sodium acetate as the carbon source in the dark at 28°C in either Fe(II)-free or Fe(II)-containing medium.

2.3. UV treatment

Two different experimental setups for UV treatments were used. The first setup used a UVC water disinfection lamp (254 nm, 12 W; Type GPH 265 T5L/4, LP 254; Deconta Tauchrohr System DTS 25; UV Consulting Peschl, Germany) under oxic conditions. The second setup was carried out in an anoxic chamber where a UV analysis lamp (8 W, S/L; Herolab GmbH Laborgeräte, Germany) was used to irradiate the bacterial cultures with UVC at 254 nm. Cultures were poured into sterile Petri dishes to a liquid layer thickness of <0.3 cm, and UVC radiation (254 nm) was applied from the top at a distance of 20 or 13 cm, respectively. After testing different time periods (data not shown), we decided to use 1 min of irradiation for viability experiments using the oxic setup with a stronger UV lamp and 30 min for molecular assays using the anoxic setup. Non-irradiated controls were treated the same way but covered with aluminum foil to avoid UVR. Subsequently, the irradiated and non-irradiated cultures were used either to inoculate new Fe(II)-free or Fe(II)-containing medium and perform growth experiments, or taken for protein or DNA extractions to perform protein and DNA damage analysis as described later.

2.4. Viability methods

For colony-forming unit (CFU) analysis, LB agar plates (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 15 g/L agar) were prepared. Briefly, samples were diluted, and

100 μL of sample was applied to the plates. Inoculated plates were incubated in the dark at 28°C under oxic conditions. The influence of UVR on nitrate reduction and Fe(II) oxidation rates was also determined. To this end, non-irradiated and irradiated samples were used to inoculate fresh medium containing either nitrate/acetate or nitrate/Fe(II)/acetate for nitrate-reducing Fe(II)-oxidizers, and samples were taken over the time course of quantification of nitrate reduction or Fe(II) oxidation rates, respectively.

2.5. Analytical methods

Iron quantification was carried out in microtiter plates with a modified spectrophotometric ferrozine assay (Stookey, 1970), as described previously by Klueglein and Kappler (2013). Samples were acidified with 40 m*M* sulfamic acid to stabilize Fe(II) and avoid Fe(II) oxidation by nitrite present in the samples. Ferrozine measurements were performed in triplicate and measured with a spectrophotometric plate reader (FlashScan 550; Analytik Jena, Germany, or Multiskan GO, Thermo Fisher Scientific). For photometric quantification of nitrate and nitrite, an automated continuous-flow analyzer system was used that contains a dialysis membrane to remove iron and prevent side reactions during analysis (Seal Analytical, Norderstedt, Germany). Hydrazine sulfate was used to reduce nitrate to nitrite and quantify it photometrically with *N*-1-naphthylethylenediamine at 550 nm (Klueglein and Kappler, 2013). Sampling was carried out in an anoxic chamber, and samples were stored anoxically at 4°C for up to 7 days before analysis.

2.6. Scanning electron microscopy (SEM)

Samples of BoFeN1 were taken under anoxic conditions, centrifuged to achieve higher cell densities, and fixed in 2.5% glutaraldehyde on ice for 1 h. Samples were dehydrated on polylysine-coated glass slides with a series of increasing ethanol concentrations (30, 70, 95, and three times 100% on molecular sieve for 10 min each) and critical point dried with a POLARON E3000 (now Quorum Technologies Ltd. East Grinstead, UK) critical point dryer. In case of 2AN, samples were centrifuged for higher cell densities, washed with acetone, and dried in an anoxic glove box on a platinum-coated plate fixed on an aluminum stub using conductive carbon pads. Dried samples were coated with Pt by using a Balzers Union MED 010 and a BAL-TEC SCD 005 (BAL-TEC, Liechtenstein) sputter coater. SEM imaging was conducted with a Zeiss Crossbeam 1540XB at the Natural and Medical Sciences Institute at the University of Tübingen (Reutlingen, Germany) at an acceleration voltage of 1.2 kV using an InLens detector and a LEO 1450 VP at 7 kV using an Everhard-Thornley detector at the Center for Applied Geoscience at the University of Tübingen (Tübingen, Germany).

2.7. UV-induced changes in protein carbonyl levels

To assess protein damage, the level of carbonyl groups was quantified by using the 2,4-dinitrophenylhydrazine (DNPH) assay (Santos *et al.*, 2013). A 20 mL portion of sample was washed with 1× PBS and used for protein extraction. To remove any Fe(III) precipitates in the Fe-containing samples, these samples were incubated with 6 *M* HCl until all Fe precipitates were dissolved. HCl was used since we found that a similar treatment with oxalate affected carbonyl levels in the protein extracts (data not shown). Trichloroacetic acid (TCA) was added to a final concentration of 10%, and the samples were incubated on ice for 1 h to precipitate the cells. The pellet was subsequently resuspended in 0.1 *N* NaOH, heated to 100°C for 20 min to lyse the cells, and then cooled on ice. Protein concentrations in these crude extracts were determined with a Bradford assay Kit (Roti-Nanoquant, Carl Roth GmbH, Karlsruhe, Germany) using bovine serum albumin (BSA) as a standard. To quantify carbonyl levels in these bacterial samples, DNPH was used. DNPH binds carbonyl groups by forming a hydrazone bond, which can be quantified spectrophotometrically. The absorbance at 360 nm was determined (SPEKOL 1300; Analytik Jena, Germany), and concentrations were calculated by using a molar absorption coefficient of 22 mM cm^{-1}. Values were normalized to the protein content.

2.8. UV-induced DNA damage

To assess DNA damage, the relative production of CPD was determined with an enzyme-linked immunosorbent assay (ELISA), as described by Wang *et al.* (2013). Samples for DNA extraction were stored directly at −20°C until further processing. DNA extractions were performed with an UltraClean DNA isolation kit (MO BIO Laboratories, Inc., USA), and concentrations and quality were determined with a NanoDrop spectrophotometer (ND 1000, Thermo Fisher Scientific, USA). DNA extracts were diluted to an end concentration of 1 μg/mL before use in the assay.

Polyvinyl chloride (PVC) flat-bottomed microtiter plates were coated with 0.04% protamine sulfate for 2 h at 37°C, and subsequently 50 μL of DNA were pipetted into plate wells in triplicate and incubated at 90°C for 1.5 h to bind the DNA. The dry plate was then washed five times with phosphate-buffered saline with Tween (PBS-T). To block free binding sites, 3% BSA was added to each well, incubated for 1 h at 37°C, and washed again five times with PBS-T. A 100 μL portion of the first antibody, an anti-thymine dimer antibody [H3] (Sigma-Aldrich) specific to thymine dimers, was then added, and binding was enabled at 37°C for 1 h. After washing, 100 μL of the second antibody (HRP affinipure goat anti-mouse IgG; Sigma-Aldrich) was added and the plate incubated at 37°C for 1 h. The plate was then washed with PBS-T three times and two times with a citrate-phosphate buffer (pH 5). As substrate for the horse radish peroxidase (HRP), 100 μL of *o*-phenylenediamine (OPD) and 0.007% H_2O_2 in citrate-phosphate buffer were added to each well and incubated for 30 min at 37°C. To stop the reaction, 50 μL of 2 *M* H_2SO_4 was added to each well after 30 min. The absorbance at 490 nm was measured with a microplate reader (Multiskan GO, Thermo Fisher Scientific, USA) to determine the relative CPD photoproduct. Although identical concentrations of DNA (50 ng) were used for each sample and experiment, the absolute absorbance values differed between independent assays due to substrate variations. Therefore, only relative values for CPD production could be determined.

3. Results

3.1. Cell-Fe(III) mineral aggregate formation

Both strains of nitrate-reducing Fe(II)-oxidizing bacteria investigated, *Acidovorax* sp. strain BoFeN1 and strain 2AN, show a close association with Fe(III) minerals, often in the form of Fe(III) minerals encrusting the cell walls at the end of Fe(II) oxidation (Fig. 1). Structures of the minerals at the biomineralized cells appear either as spiky needles or broccoli-like forms, similar to that previously shown (Kappler *et al.*, 2005; Miot *et al.*, 2009; Schädler *et al.*, 2009; Schmid *et al.*, 2014). However, not all cells are completely encrusted in Fe(III) minerals; some cells remain free from an Fe(III) mineral crust and are only loosely associated with Fe(III) minerals (Fig. 1). Identification of the minerals showed that the Fe(III) phases produced by these nitrate-reducing Fe(II)-oxidizing bacterial strains during Fe(II) oxidation were poorly crystalline and related to nanoparticular goethite, lepidocrocite, or even ferrihydrite with primary particle sizes in the nanometer size range (Kappler *et al.*, 2005, 2010; Larese-Casanova *et al.*, 2010; Dippon *et al.*, 2012; Pantke *et al.*, 2012).

3.2. Viability after UV treatment and protection by Fe(III) minerals

To assess protection from UVR, different approaches to quantify cell viability were used. Firstly, the nitrate-reducing Fe(II)-oxidizing strains *Acidovorax* sp. BoFeN1 and *Acidovorax* sp. 2AN were grown either with (i) acetate/nitrate, but without Fe(II), to obtain cultures with non-encrusted cells; (ii) acetate/nitrate plus Fe(II) to get cultures with cells encrusted in biogenic Fe(III) minerals (encrusted culture); or (iii) acetate/nitrate without Fe(II) but amended with abiogenic Fe(III) minerals (ferrihydrite) before UV treatment. These cultures were either UV treated, or not, to determine the effect of UV treatment on their viability.

Analysis of subsequent cultures that were inoculated with UV-treated, non-encrusted cells showed a delay of 48 h for strain BoFeN1 in both Fe(II) oxidation and nitrate reduction compared to non-UV-treated controls (Fig. 2). This demonstrates damage to the cells by the UVR, with a concomitant decrease in the number of viable cells. In contrast, cultures inoculated with UV-treated inoculum stemming from cultures with encrusted cells, as well as from cultures with non-encrusted cells but with added ferrihydrite, showed similar Fe(II) oxidation and nitrate reduction behavior as cultures inoculated with inoculum without UV treatment. These results suggest a protective effect by the Fe(III) minerals. The second nitrate-reducing Fe(II)-oxidizer used in our study, *Acidovorax* sp. strain 2AN, showed an even stronger delay of 72 h in Fe(II) oxidation when no Fe(III) minerals were present during UV treatment, but a similar rescued Fe(II) oxidation pattern in the presence of biogenic or abiogenic iron minerals (Fig. 3).

Colony-forming units (CFUs) were quantified before and after UV treatment of strain BoFeN1 in the presence and absence of Fe minerals to evaluate the protection of the cells by Fe(III) minerals from UVR. We found that non-encrusted cells of BoFeN1 showed a survival rate of $2 \pm 3\%$ after UV treatment, while Fe(II)-grown and thus Fe(III)-mineral-encrusted cells showed a survival rate of $42 \pm 3\%$. Cells of BoFeN1 to which ferrihydrite was added before UV treatment showed a similar survival rate as the Fe(III)-mineral-encrusted cells of $41 \pm 11\%$ (Fig. 4).

3.3. Damage of DNA and proteins caused by UV treatment

In addition to assessing UV damage by following cell viability, the direct effect of UVR on two biomolecules, DNA and protein, and the protection of these biomolecules by Fe(III) minerals, was quantified. UV-induced damage on DNA was assessed by determination of the relative production of CPD in DNA extracts. CPD is one of the main UV-induced photoproducts that can block DNA replication and transcription. Absorption values (at 490 nm) of DNA extracts in the ELISA from non-encrusted cultures of strain BoFeN1 were 0.043 ± 0.000 for a non-UV-treated control and 0.189 ± 0.005 for DNA extracts obtained after 30 min

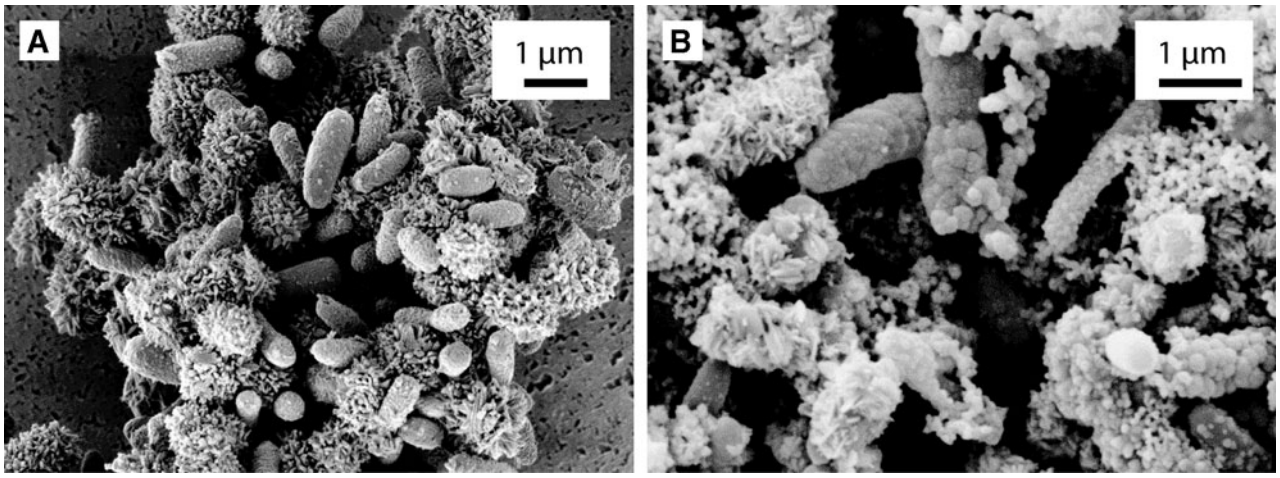


FIG. 1. Scanning electron micrographs of the nitrate-reducing Fe(II)-oxidizing bacteria *Acidovorax* sp. strain BoFeN1 (**A**) and 2AN (**B**) at the end of oxidation of *ca.* 8 m*M* Fe(II). The biomineralized cells show spiky needle or broccoli-like biomineral structures on the cell surfaces. Cells that are not fully encrusted are also present in both cultures.

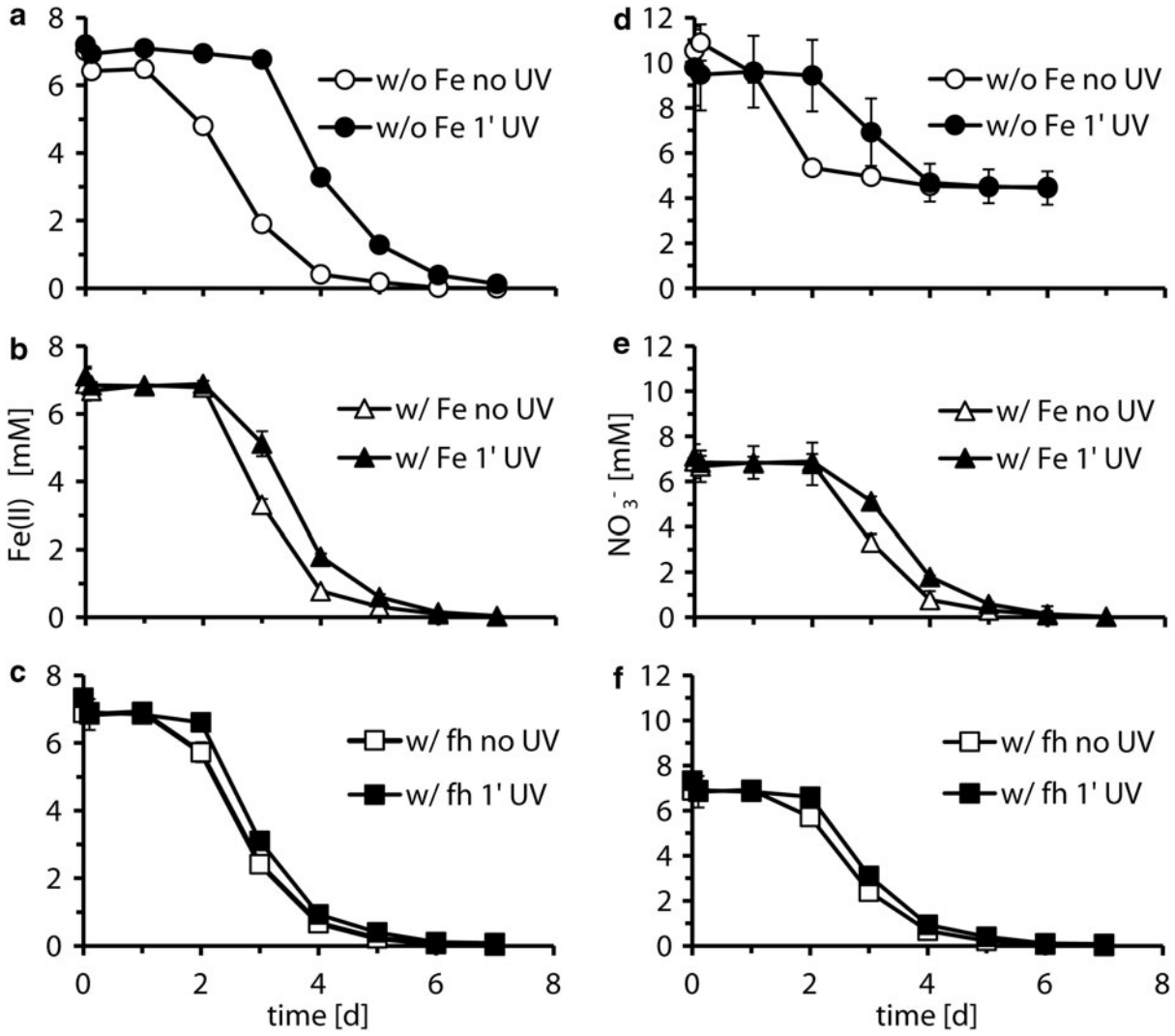


FIG. 2. Fe(II) oxidation (left) and NO_3^- reduction (right) over time by the nitrate-reducing Fe(II)-oxidizer *Acidovorax* sp. strain BoFeN1 in cultures with acetate/nitrate/Fe(II) inoculated with inoculum that was either UV-treated (1′ UV) or non-UV-treated (no UV). The inoculum stemmed from cultures that were grown with acetate/nitrate in the absence of Fe(II) (non-encrusted) (**a**, **d**); grown with acetate/nitrate/Fe(II) (encrusted) (**b**, **e**); or grown with acetate/nitrate without Fe(II) but with added abiogenic ferrihydrite (fh) (**c**, **f**). Open symbols show data for non-UV-treated inoculum, filled symbols for 1 min UV-treated inoculum. Error bars indicate standard deviation calculated from three parallels.

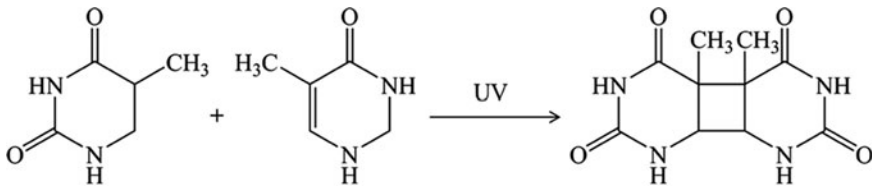
of UVC treatment, suggesting an almost 5-fold increase in CPD by the UV treatment. For comparison, DNA of Fe(III)-mineral-encrusted cultures showed values of 0.043 ± 0.000 without UV treatment and 0.092 ± 0.003 with UV treatment (Fig. 5A), clearly indicating a protective effect of the Fe(III) minerals on the DNA.

Ultraviolet-induced protein oxidation was assessed by a spectrophotometric assay quantifying carbonyl (CO) groups in protein extracts of UV-treated cultures compared to non-UV-treated cultures. These were then further compared to a positive control where protein oxidation was induced by addition of the strong oxidant hydrogen peroxide (H_2O_2). In both the presence and absence of Fe(III) minerals, a UV-induced protein oxidation was not observed in any samples of BoFeN1 under anoxic conditions, although positive controls with H_2O_2 showed an increase in CO levels from 18.50 ± 0.19 pmol per μg protein to 37.81 ± 2.36 pmol per μg protein (Fig. 5B). This suggests that the presence of molecular oxygen is required to cause protein oxidation in the presence of UVC radiation.

4. Discussion

4.1. Effect of Fe(III) minerals on DNA damage during UV exposure

In our experiments without an Fe(III) mineral shield, we observed an increase in UV-induced photoproducts in the DNA of the nitrate-reducing Fe(II)-oxidizing *Acidovorax* sp. strain BoFeN1 in the form of CPD. In the presence of biogenic Fe(III) minerals, this damaging UV effect was significantly lowered (Fig. 5A). UV-induced damage of DNA is due to DNA's property to absorb UVR strongly at 254 nm, which causes DSB but also formation of a variety of photoproducts, for example, CPD. CPDs can form between two adjacent pyrimidines, for example, thymines (Patrick and Rahn, 1976; Häder and Sinha, 2005; Bolton and Cotton, 2008):



These DNA lesions can either inhibit transcription and replication of the chromosome by blocking enzyme binding sites or cause mutations (Pfeifer, 1997). As a cellular response to the exposure to harmful UVR, CPD burden can be lowered by repair mechanisms that are common in a wide variety of organisms, for example, photoreactivation by the enzyme photolyase or nucleotide excision repair (Sinha and Häder, 2002; Häder and Sinha, 2005).

Fe(III) minerals are known to absorb light particularly in the low UV wavelength range (<400 nm), but visible light (~390 to 700 nm) is still transmitted (Bishop *et al.*, 2006), in particular by small, nanoparticular Fe(III) mineral phases, such as ferrihydrite, and to some extent also by the poorly crystalline nanoparticular goethite/lepidocrocite, which is produced by nitrate-reducing Fe(II)-oxidizing bacteria (Kappler and Newman, 2004; Larese-Casanova *et al.*, 2010). Hence, the Fe(III) minerals produced by nitrate-reducing Fe(II)-oxidizing bacteria, but also abiogenic Fe(III) minerals (*e.g.*, ferrihydrite), absorb radiation exactly in those wavelength ranges that cause damage to DNA (<320 nm, see Supplementary Fig. S1, available online at www.liebertonline.com/ast). This supports our finding that Fe(III) minerals are an effective UV shield for chemotrophic microorganisms by reducing UVR that induces DNA damage in the form of CPDs.

We did not observe protein damage in the form of CO groups after UVC treatment in our experiments with protein extracts of *Acidovorax* sp. strain BoFeN1. UVR-induced protein damage can be caused either directly by absorption peaks of the proteins below 210 nm and at 280 nm or indirectly by the production of reactive oxygen species (ROS) that then oxidize biomolecules in the cells. When proteins are oxidized, CO groups are produced at amino acid side chains (Dalle-Donne *et al.*, 2003), and UVR damage can be

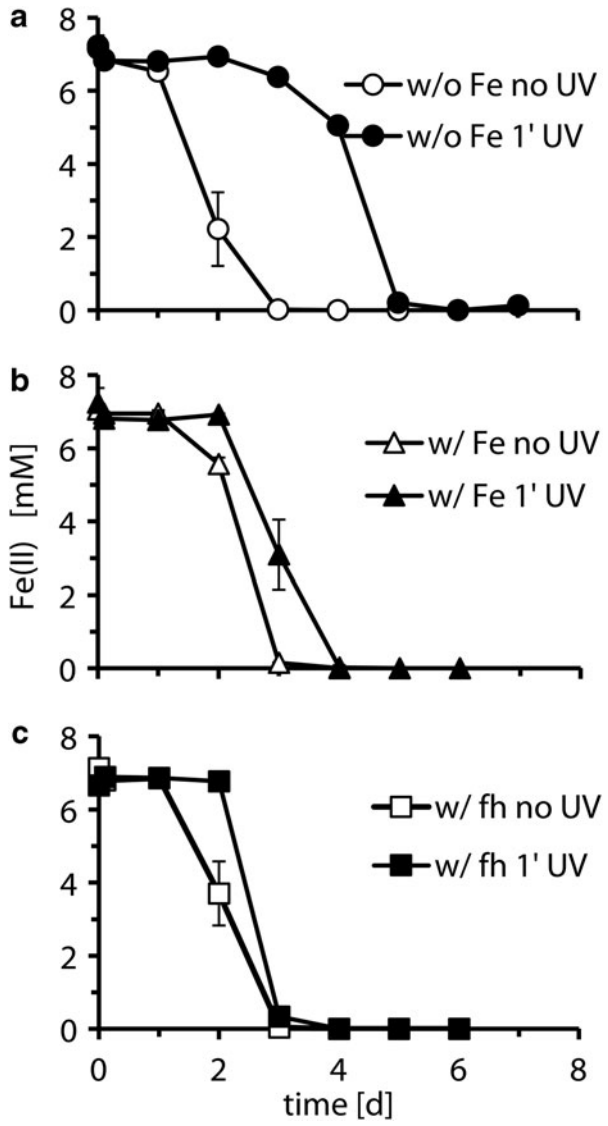


FIG. 3. Fe(II) oxidation over time by the nitrate-reducing Fe(II)-oxidizer *Acidovorax* sp. strain 2AN in cultures with acetate/nitrate/Fe(II) inoculated with inoculum that was either UV-treated (1′ UV; filled symbols) or non-UV-treated (no UV; open symbols). The inoculum stemmed from cultures that were grown with acetate/nitrate in the absence of Fe(II) (non-encrusted) (**a**); grown with acetate/nitrate/Fe(II) (encrusted) (**b**); or grown with acetate/nitrate without Fe(II) but with added abiogenic ferrihydrite (fh) (**c**). Error bars indicate standard deviation calculated from three parallels.

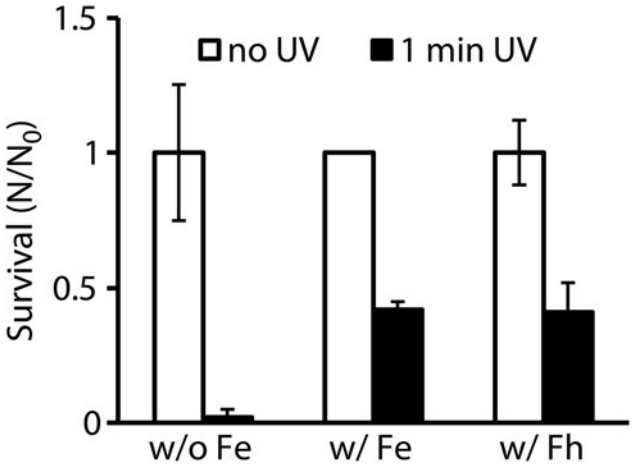


FIG. 4. Influence of UV treatment on cell growth. CFU analysis after UV treatment is shown of nitrate-reducing Fe(II)-oxidizing *Acidovorax* sp. strain BoFeN1 cultures grown without Fe(II) (left), with Fe(II) (middle), and with ferrihydrite (fh) added to a culture grown without Fe(II) (right). Survival was calculated by dividing CFUs after UV treatment (N) by CFUs before UV treatment (N_0). Error bars indicate standard deviation calculated from at least five parallels.

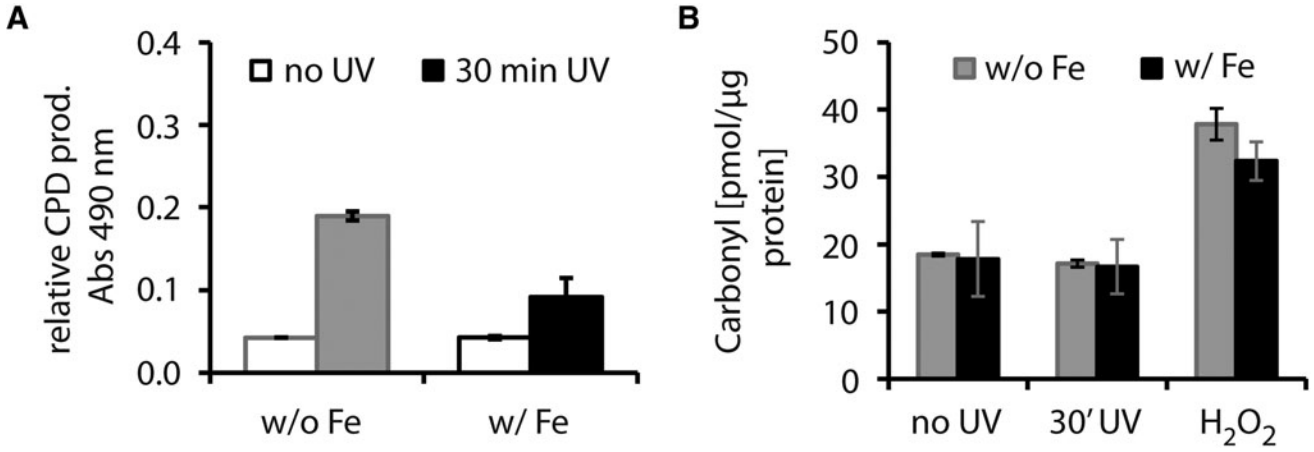


FIG. 5. Influence of UVC on DNA (**A**) and protein (**B**) damage in *Acidovorax* sp. strain BoFeN1. (A) shows relative production of CPD with (filled bars) or without (open bars) UV treatment in DNA extracts of the nitrate-reducing Fe(II)-oxidizing *Acidovorax* sp. strain BoFeN1 grown without Fe(II) (shown in gray) or with Fe(II) (shown in black). Error bars indicate standard deviation calculated from triplicate measurements. (B) shows carbonyl levels in proteins as indicator for protein oxidation by UVR. Carbonyl levels were determined under anoxic conditions in protein extracts of the nitrate-reducing Fe(II)-oxidizer *Acidovorax* sp. strain BoFeN1 grown without Fe(II) (non-encrusted culture, shown in gray) or with Fe(II) (encrusted culture, shown in black). Carbonyl content normalized to protein concentrations is shown for non-UV-treated controls (left), 30 min UV-treated cultures (middle), and H_2O_2-treated cultures as positive controls (right). Error bars indicate standard deviation of triplicate samples.

assessed by an increase in CO levels. Our results indicate that no ROS are produced under anoxic conditions (at least with UVC), and therefore, no protein oxidation occurred.

4.2. Effect of Fe(III) minerals on cell viability during UV exposure

We show that microbial activity is strongly affected by exposure to UVC radiation. Fe(II) oxidation and nitrate reduction rates were significantly delayed in cultures inoculated with cells irradiated without a UV shield. The lower metabolic rates point to a decrease in cell viability. In comparison, cultures inoculated with cells that were irradiated in the presence of biogenic and abiogenic minerals did not show lower metabolic rates.

The detrimental effect on microbial activity is also demonstrated by CFU analysis. Without a protecting UV shield only 2% of the population survived, but in the presence of an Fe(III)-mineral crust around the cells, or in the presence of abiogenic ferrihydrite in the medium, over one-third of the population survived. This indicates that in the presence of biogenic or abiogenic Fe(III) minerals either a higher amount of cells survived or the cells were less damaged by UVR.

The observed decrease in cell viability is due to the sterilizing properties of UVR. UVR is widely used as a disinfectant, as it inhibits proliferation of bacteria by damaging biomolecules in cells, particularly DNA and proteins (Bolton and Cotton, 2008; American Water Works Association and American Society of Civil Engineers, 2012). UV-treated cells obtain mutations that inhibit replication, or essential proteins are altered irreversibly (Pfeifer, 1997). In the presence of oxygen, ROS are produced (mainly by UVB and UVA) and cause damage to even more biomolecules, for example, lipids, and cause membrane porosity. If membranes are still intact after UVR exposure, the bacterial cells are inactive, as they are not able to multiply, but are technically still alive (Bolton and Cotton, 2008). We see that more cells survive or overcome UV treatment in cultures containing Fe(III) minerals and regrow their populations, possibly because of less damage and thereby shorter repair times or because fewer are dead, that is, completely inactive cells in these cultures.

In addition, we observed in our experiments that biogenic, as well as abiogenic, Fe(III) minerals are effective shields against UVC radiation. This indicates that it is not necessary for the microbial cells to be encrusted in Fe(III) minerals, but loose association with Fe(III) minerals produced in the course of microbial Fe(II) oxidation is sufficient to protect these microorganisms from UVR. Hence, for a protecting effect by Fe(III) minerals, it does not make a difference whether Fe(III) is formed directly by Fe(II)-oxidizing bacteria or indirectly (*i.e.*, abiotically) by the reaction of O_2 via oxygenic photosynthesis or other oxidants, such as nitrogen oxides from chemotrophic metabolisms (Klueglein *et al.*, 2014). Furthermore, this also implies that other Fe(II)-oxidizing microorganisms, for example, phototrophic Fe(II)-oxidizers, although not encrusted in Fe(III) minerals (Schädler *et al.*, 2009), have the possibility to produce their own UV shield.

In our study, we used UVC radiation, as it has the most detrimental effects on life and biomolecules. The next step for future studies would be to investigate the effects of other wavelength ranges, such as UVB and UVA, on nitrate-reducing Fe(II)-oxidizing bacteria and assess whether we observe the same protecting effect by the Fe(III) minerals they precipitate.

4.3. Implications for microbial ecology and evolution of a terrestrial biosphere on early Earth

The rise of atmospheric oxygen, frequently termed the Great Oxidation Event, is manifest in the rock record as the loss of sulfur mass-independent fractionations (S-MIFs) beginning at 2.45 Ga (Farquhar *et al.*, 2000) and culminating at 2.32 Ga (Bekker *et al.*, 2004). However, a number of recent papers have pushed back the timing for oxidative

continental weathering and, by extension, the onset of atmospheric oxygenation to as early as 3.0 Ga (Crowe *et al.*, 2013; Planavsky *et al.*, 2014). This apparent discrepancy can, in part, be resolved by the suggestion that recycling of crustal sulfur with a S-MIF might explain why S-MIF is preserved so late in the rock record (Reinhard, 2012). Another cause for the gap in time between the onset of oxidative weathering and the eventual loss of S-MIF is that the earliest oxidative weathering reactions occurred in benthic environments at profound redox disequilibrium with the atmosphere, such as BSCs. Lalonde and Konhauser (2015) calculated that oxygenic photosynthesis in these millimeter-thick ecosystems provided sufficient oxidizing equivalents to mobilize sulfate and redox-sensitive trace metals from land to the oceans already at 3.0 Ga (and perhaps earlier), while the atmosphere itself remained anoxic with its attendant S-MIF signature.

If terrestrial life began in a BSC, then it would have been directly exposed to incoming UVR. Modeling approaches by Cockell (2000) suggested that UVR fluxes that reached Earth's surface during the Archean were 3 orders of magnitude higher than today. In environments where Fe(II) was present, such as in association with mafic and ultramafic soils/outcrops, the production of Fe(III) minerals, as a by-product of their metabolism, constituted an obvious advantage. At what point in time such Fe(II)-oxidizing bacteria were linked to nitrate reduction is unclear, but it has been suggested that they are potentially the most ancient Fe(II)-oxidizers (Ilbert and Bonnefoy, 2013). This is based on the hypothesis that the use of nitrate as an electron acceptor for anaerobic respiration (denitrification) occurred before aerobic respiration, as it is a phylogenetically widespread metabolism in Bacteria and Archaea (Mancinelli and McKay, 1988), and its major enzyme, for example, the nitrate reductase Nar, was suggested to even have been present in the last universal common ancestor, LUCA (van Lis *et al.*, 2011). Moreover, it is certainly feasible that the presence of localized O_2 in BSCs meant that an oxidative N cycle could already have been in place, such that nitrifying and denitrifying bacteria were already in existence on land; by 2.72 Ga, the existence of an aerobic N cycle is recorded in the lacustrine sediments from the Tumbiana Formation in Western Australia (Thomazo *et al.*, 2011).

Despite its damaging effects, UVR might also have had positive effects on microbial life, in particular for microorganisms involved in Fe metabolism. It has been shown, for instance, that under anoxic conditions siderite ($FeCO_3$) can be photooxidized by UVR, generating molecular hydrogen (Kim *et al.*, 2013). This H_2 could have been used as electron donor by a variety of microorganisms, including nitrate-reducers or Fe(III)-reducing microbes using the Fe(III) that is produced by the nitrate-reducing Fe(II)-oxidizers as electron acceptor for anaerobic respiration. It would even allow the same microorganisms to oxidize Fe(II) and reduce Fe(III) under alternating, dynamic geochemical conditions, similar to what has been shown for *Geobacter sulfurreducens*, which is able to switch between nitrate-reducing Fe(II) oxidation and respiration using Fe(III) as electron acceptor (Finneran *et al.*, 2002). A second positive effect of UVR for Fe(II)-oxidizing bacteria could come from its ability to photoreduce Fe(III) to Fe(II), in particular in the presence of natural organic matter and organic matter–complexed Fe(III) (Stumm and Sulzberger, 1992; Johnson *et al.*, 1994; Borman *et al.*, 2010; Melton *et al.*, 2014) but also involving superoxide radicals (Emmenegger *et al.*, 2001). Therefore, the UVR could regenerate the electron donor for nitrate-reducing (and other) Fe(II)-oxidizers and could lead to efficient local iron cycling.

Acknowledgments

This work was supported by the German Research Foundation (DFG)–funded research training group RTG 1708 ''Molecular principles of bacterial survival strategies.'' K.O.K. was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). We would like to thank E. Struve for nitrate/nitrite measurements, A. Piepenbrock and M. Wüstner for supplying ferrihydrite, N. Hagemann and F. Zeitvogel for SEM work, and the work group of S. Grond for providing one of their UV lamps. We also thank A. Chakraborty and F. Picardal for providing *Acidovorax* sp. strain 2AN. M. Schad is acknowledged for experimental support and Y. Wang for help with the ELISA protocol.

Author Disclosure Statement

No competing financial interests exist.

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Submitted 17 June 2015
Accepted 18 November 2015

Abbreviations List

BSA = bovine serum albumin
BSCs = biological soil crusts
CFUs = colony-forming units
CO = carbonyl
CPD = cyclobutane pyrimidine dimers
DNPH = 2,4-dinitrophenylhydrazine
DSB = DNA strand breakages
ELISA = enzyme-linked immunosorbent assay
HRP = horse radish peroxidase
PBS-T = phosphate-buffered saline with Tween
ROS = reactive oxygen species
SEM = scanning electron microscopy
S-MIFs = sulfur mass-independent fractionations
UVR = ultraviolet radiation