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Sulfur isotopes as biosignatures for Mars and Europa exploration

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Abstract

Sulfur (S) isotopes are used to trace metabolic pathways associated with biological S-cycling in past and present environments on Earth. These pathways (sulfate reduction, sulfur disproportionation, and sulfide oxidation) can produce unique S isotope signals that provide insight into biogeochemical S-cycling. The S cycle is also relevant for extraterrestrial environments and processes. On early Mars, sulfur existed in different redox states and was involved in a large range of surface processes (e.g., volcanic, atmospheric, hydrothermal, and aqueous brines). Sulfur compounds have also been detected on Europa's icy moon surface, with the S cycle implicated in Europa's surface and ocean geochemistry. Given the well-established utility of S isotopes in providing a record for past life on Earth, S isotopes are an valuable tool for identifying biosignatures on Mars and Europa. Here, we review S isotopes as a biosignature, in light of two recent advances in understanding the S cycle in both Mars and Europa: (i) the measurements of $\delta^{34}S$ *in situ* at Gale Crater and quadruple S isotopes (QSI) in Martian meteorites, and (ii) the identification of a likely exogenous origin of sulfur on Europa's surface. We discuss important considerations for unravelling QSI biosignatures in Martian environments, considering high and low sulfur environments, atmospheric S-MIF signals, and metabolic energy-limited niches. For Europa, we describe the potential for S isotopes to probe biogeochemistry, and identify key knowledge gaps to be addressed in order to unlock S isotopic tools for future life detection efforts. The resulting picture demonstrates how S isotopes will be a valuable tool for Mars Sample Return, and how future missions can focus on the search for environments where QSI signatures of microbial S-cycling processes have a greater chance of being preserved. For Europa, the first step will be to account for the S isotope composition of the various S pools, to recognise or rule out non-biologically mediated S isotope values, with a focus on experimental examination of potential S isotope signatures from exogenous sulfur sources.

1. Sulfur isotope biosignatures

Sulfur (S) is abundant in the Solar System, and speciates into geochemically extensive reservoirs on Earth, Mars, and the Jovian moons Europa, Io, Ganymede and Calisto (Carlson et al., 1999; Jessup et al., 2007; King et al., 2004; King & McLennan, 2010) Sulfur is a widely utilised source of energy for chemotrophic microbial metabolisms, due to its range of redox states. These redox states enable microorganisms to perform: (i) dissimilatory microbial sulfate reduction (MSR), (ii) microbial sulfur disproportionation of intermediate S compounds (MSD), and (iii) oxidation of sulfide and reduced sulfur species (SO) (Figure 1). Each of these metabolic processes produces a characteristic, but not unique, change in the S isotope values of the associated compounds within the reaction. When these values are preserved in the rock record they can provide evidence for past biological activity. Here, we review S isotope biosignatures within the context of S-rich environments on Mars and Europa, and consider how to distinguish S isotope biosignatures from abiotic signatures concurrently preserved in geologic deposits. We identify knowledge gaps to be addressed in order to interpret future isotopic measurements of sulfur phases from these and similar planetary environments, and evaluate the feasibility of interpreting *in situ* measurements versus samples from return missions.

1.1.   S isotope biosignatures on Earth

Sulfur (S) has four stable isotopes, with masses 32, 33, 34 and 36, and is one of the most abundant elements on Earth. The ^{3X}S isotope composition of sulfur species is presented using the standard delta (δ) notation, expressed in permil (‰):

$$\delta^{3X}S = [(^{3X}R_{SAMPLE} / {}^{3X}R_{VCDT}) -1]$$

where 3X is 32, 33, 34 or 36, and $^{3X}R_{SAMPLE} / {}^{3X}R_{VCDT}$ is the isotopic ratio of a sample ($^{3X}R= {}^{3X}S/ {}^{32}S$) relative to the standard, VCDT. Sulfur isotopes are influenced by biological and abiotic processes, and can be preserved in the rock record (e.g., Canfield, 2001a and references therein; Fike et al., 2015; Havig et al., 2011, 2017; Johnston, 2011). Effects on S isotopes produced by microbial processes, notably MSR and oxidative sulfur (re)cycling, have been used to reconstruct the evolution of S-based metabolisms and trace the oxygenation of Earth's surface through time (Canfield & Teske, 1996; Luo et al., 2016; Scott et al., 2008). The largest S isotope fractionation effects are generally produced by MSR, although oxidative recycling of sulfur species can increase these fractionations further (e.g., Canfield, 2001a,b).

Enzymatic reactions performed by S-utilizing microorganisms control the S isotope composition of S-bearing biomolecules produced during assimilatory processes (e.g., cysteine and methionine), and inorganic S compounds produced during dissimilatory processes (e.g., sulfate and sulfide). Enzymes perform reactions that happen at faster rates than if they were carried out abiotically by lowering the activation energy. The products (P) of these enzymatic reactions are typically depleted in the heavier S isotopes relative to the reactants (R), with the magnitude of the differences in isotopic compositions between the reactant and the product the result of the isotopic discrimination that happens during the multistep enzymatic reaction (Brunner & Bernasconi, 2005; Canfield, 2001a; Canfield & Thamdrup, 1994; Chambers et al., 1975; Fry et al., 1985). We express the magnitude of this discrimination, also called a fractionation or "isotope effect", as:

$$^{34}\varepsilon_{R-P} = (\alpha_{R-P} - 1) \times 1000$$

where $\alpha_{R-P} = (\delta^{34}S_P + 1000)/(\delta^{34}S_R + 1000)$ can help to identify the specific enzymatic reaction mechanisms responsible (Canfield & Teske, 1996; Johnston, 2005; Leavitt et al., 2013; Philippot et al., 2007; Shen & Buick, 2004). Where the S isotope values of both products and reactants can be measured, isotope effects between these species can provide evidence for biological activity (biosignatures) and clues to the biogeochemical S cycle during the time of their formation (Fike et al., 2015 and references therein).

MSR and MSD in particular produce H_2S depleted in ^{34}S, captured as pyrite and Fe-monosulfides with distinctively negative $\delta^{34}S$ values (e.g., (Fike & Grotzinger, 2008; Habicht & Canfield, 2001a; Johnston, Farquhar, Summons, et al., 2008). (Re)oxidation of H_2S generally produces much smaller fractionations in $\delta^{34}S$, but can further alter the minor S isotope composition (^{33}S and ^{36}S) of these S compounds (e.g.,Zerkle et al., 2009, 2016). Thus, the S isotope values of sedimentary pyrite and sulfate minerals can preserve evidence for these S-cycling metabolisms in past environments.

1.1.1. Microbial Sulfate Reduction (MSR)

Organisms that perform microbial sulfate reduction (MSR) are taxonomically and metabolically diverse, encompassing both archaea and bacteria capable of heterotrophy and/or autotrophy under anaerobic conditions (Anantharaman et al., 2018; Castro et al., 2000; Plugge et al., 2011). MSR in pure and enrichment cultures can produce S isotope fractionations larger than -65 ‰ between reactant SO_4^{2-} and product H_2S (Canfield et al., 2010; Sim et al., 2011a). Sulfur isotope fractionations produced during MSR are influenced by several environmental parameters, such as SO_4^{2-}, Fe, and NH_4^+ concentrations, type and

abundance of electron donors, and temperature (Figure 2) (e.g., Detmers et al., 2001; Hoek et al., 2006; Sim, 2012; Sim et al., 2011b, 2012).

Variations in SO_4^{2-} concentration play a major role in the S isotope fractionations produced during MSR, with larger S isotope effects generally associated with higher SO_4^{2-} concentrations (Habicht & Canfield, 1997). Moreover, S isotope fractionations during MSR have been shown to be dependent on strain-specific physiological parameters, such as affinity for SO_4^{2-} and electron donors (Bradley et al., 2016). MSR tends to produce larger S isotope fractionations when growing heterotrophically using organic electron donors than when growing autotrophically with H_2 (Figure 2; 6-44 ‰,Sim et al., 2011b). In addition, when MSR is limited by low concentrations of electron donors, the S isotope fractionations produced are generally larger (Chambers et al., 1975; Hoek et al., 2006; Sim et al., 2011b).

Concentrations of nutrients such as Fe and NH_4^+ can also indirectly control the extent of S isotope fractionation during MSR, through their influence on metabolic rates (Sim et al., 2012). Cell-specific sulfate reduction rates (csSRR, generally expressed as moles of SO_4^{2-} reduced/cell/time) respond to environmental parameters such as SO_4^{2-} concentration and source of electron donors, and the largest S isotope fractionations are generally produced at the lowest csSRR (Chambers et al., 1975; Habicht & Canfield, 1997; Kaplan & Rittenberg, 1964). Similarly, nutrient limitation can reduce overall growth rates, causing a decrease in csSRR and subsequently larger S isotope fractionations (Figure 2, Sim et al., 2011b). This response is similar to that seen for organic carbon limitation, as they are both key in controlling the proportional supply of electrons to the MSR pathway (Sim, 2012).

Temperature influences metabolic rates, which can affect S isotope fractionation effects, as described above (Canfield et al., 2006; Hoek et al., 2006). Temperature responses seem to be variable for different microbial strains, with larger S isotope fractionations generally shown for sub-optimal temperatures with correspondingly low csSRR, and smaller fractionations for optimal temperatures with correspondingly higher csSRR (Figure 2, Canfield et al., 2006). It is not known if pH directly affects S isotope fractionations during MSR. Indirectly, pH and O_2 concentrations determine aqueous speciation, and therefore Fe and SO_4^{2-} solubility, which can each impact S isotope fractionations as described above. This mechanism was recently suggested to be important in controlling S isotope fractionations observed in Icelandic hydrothermal systems spanning different pH and Fe concentrations (Moreras-Marti et al., 2021a).

More generally, the combined metabolic energy limitation within an environment can have an impact on S isotope fractionations through changing csSRR. A general decrease in

microbial metabolic rates has been observed in environments with limited electron donors, termed energy-limited or low-energy environments (Bowles et al., 2014; Hoehler & Jørgensen, 2013; Jørgensen & Marshall, 2016; Wenk et al., 2018). These metabolic energy-limited niches are widespread on Earth, and include the subsurface of intertidal mudflats, coastal environments, continental shelves, deep sea sediments, and euxinic water columns. Organisms that inhabit these environments have adapted to use electron carriers with modest negative reduction potentials, requiring less energy to grow (Wenk et al., 2018). The large S isotope fractionations associated with metabolic energy-limited niches have also been observed in cold hypersaline habitats (Moreras-Marti et al., 2021b).

1.1.2. Sulfur oxidation (SO)

Sulfur oxidation (SO) has a broad taxonomic distribution, being present in members of archaeal and bacterial phyla (Ghosh & Dam, 2009). Organisms that perform SO vary in terms of oxygen tolerance (facultative anaerobes or obligate aerobes) and strategies for carbon and energy acquisition (photoautotrophic, chemoautotrophic, or chemoheterotrophic) (Ghosh & Dam, 2009; Sorokin, 2003). Oxidative S-cycling proceeds via a variety of biological pathways in the environment, along with the abiotic pathways described below. The chemolithotrophic oxidation of reduced H_2S or S^0 can be coupled to the reduction of O_2 or NO_3^- for energy gain (Friedrich et al., 2001) (Figure 1). Sulfur isotope fractionations produced during chemolithotrophic SO are smaller than MSR and highly variable, ranging from -6 to +12‰ between H_2S and SO_4^{2-} or S^0 (Fry et al., 1988; Pellerin et al., 2019; Zerkle et al., 2016). The environmental parameters affecting S isotope fractionations during chemolithotrophic SO appear to be related to electron donor and acceptor availability, with larger fractionations linked to lower H_2S/O_2 ratios (Zerkle et al., 2016).

Anoxygenic phototrophy can oxidize reduced S compounds. In addition to sulfide, these organisms can oxidise bioavailable intermediate S compounds including thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3^{2-}), and elemental sulfur (S^0), although only S^0 is generally stable enough to build up in natural environments (Jørgensen, 1990; Troelsen & Jørgensen, 1982). Sulfur isotope effects during phototrophic SO are also small but highly variable, ranging from -5 to +5‰, depending on the S compounds reduced and the resulting products (e.g., Zerkle et al., 2009).

1.1.3. Microbial sulfur disproportionation (MSD)

In addition to MSR and SO, some S-cycling microorganisms are capable of sulfur disproportionation reactions(MSD) (Bak & Pfennig, 1987). MSD can utilize a number of intermediate sulfur species (S^0, SO_3^{2-}, or $S_2O_3^{2-}$) to produce both sulfate and sulfide (e.g.,

Frederiksen & Finster, 2003). The disproportionation of $S_2O_3^{2-}$ or S^0 is performed anaerobically, assimilating carbon from CO_2 or acetate (Widdel & Pfennig, 1977). For S^0 disproportionation to be thermodynamically feasible in an environment, sulfide concentrations must be less than ~1mM (Thamdrup et al., 1993). Moderate S isotope fractionations are associated with MSD, related to the recycling of reduced S components during metabolic processes (reviewed in Canfield et al., 2001a). Fractionations up to -34‰ have been measured on H_2S and SO_4^{2-} during S^0 disproportionation (disproportionation of S^0 to H_2S and SO_4^{2-}) by pure cultures at the cellular level (Habicht, 1997).

1.1.4. Minor S isotope biosignatures: $\Delta^{33}S$ and $\Delta^{36}S$ biosignatures

Minor S isotopes (^{33}S and ^{36}S), expressed as $\Delta^{33}S = \delta^{33}S - [(^{34}R_{sample}/^{34}R_{VCDT})^{0.515} - 1]$ and $\Delta^{36}S = \delta^{36}S - [(^{34}R_{sample}/^{34}R_{VCDT})^{1.90} - 1]$, have further contributed to our understanding of S-cycling processes on the early Earth (Farquhar et al., 2000a; Farquhar et al., 2000b; Farquhar & Wing, 2003). Following this notation, the exponents 0.515 ($^{33}\lambda$) and 1.90 ($^{36}\lambda$) represent reference values that approximate mass-dependent fractionations during thermodynamic equilibrium isotope exchange at low temperature (Farquhar & Wing, 2003; Hulston & Thode, 1965; Johnston et al., 2007a). Deviations from these reference values occur in biological systems due to a linear dependence of isotope ratios during redistribution of mass at the cellular or ecosystem level (e.g., via mixing or Rayleigh processes), which deviate from the predicted exponential relationship (Farquhar et al., 2003; Farquhar et al., 2007a; Johnston, 2005; Johnston et al., 2007). These deviations result in small magnitude anomalies in $\Delta^{33}S$ and $\Delta^{36}S$, termed mass conservation effects, that can be preserved in rocks and sediments alongside $\delta^{34}S$ (e.g., Johnston, et al., 2008).

Experimental studies have shown that each of the biological S metabolisms discussed above produce characteristic minor isotope patterns, resulting from the individual steps controlling the sulfur flowing through the metabolic pathways (Figure 3; (Zerkle et al., 2016). MSR in pure cultures has been observed to produce $^{33}\lambda$ values between 0.5077 and 0.5125; MSD produce higher $^{33}\lambda$ values ranging from 0.5145 to 0.5187 (Johnston, 2005; Johnston et al., 2007) ; and chemolithotrophic SO produce $^{33}\lambda$ values ranging from 0.513 to 0.515 (Pellerin et al., 2015, 2019; Zerkle et al., 2016). These differences in the minor isotopes can help to decipher complex environmental isotope records, where MSR, MSD and SO can co-occur, producing $\delta^{34}S$ values that overlap. For example, biological variations have been shown to produce distinctive $\Delta^{33}S$ values in both hydrothermal and hypersaline Mars analogue environments, even when $\delta^{34}S$ values are small or indistinguishable (Moreras-Marti et al., 2021b).

1.1.5. Abiotic fractionation of S isotopes

Abiotic processes act alongside biology to cycle sulfur in natural systems. Therefore, to use S isotopes as a biosignature for S-cycling metabolisms in extra-terrestrial environments, it is important to examine any abiotic processes that could modify or mask the biogenic S isotope values. Sulfate can be reduced abiotically by thermochemical sulfate reduction (TSR), requiring high temperatures (80 to 200°C) and organic matter. TSR produces δ^{34}S fractionations of up to -20‰ between reactant sulfate and product sulfide (Machel et al., 1995). Sulfide can also be oxidized abiotically by reacting with Fe (III), Mn (IV) or by rapid reaction with molecular oxygen, producing mean fractionations in δ^{34}S of -5.5‰ between H_2S and intermediate S products (Eldridge et al., 2021; Eldridge & Farquhar, 2018; Fry et al., 1988). Small pH-dependent equilibrium isotope fractionations have also been measured between aqueous and gaseous H_2S, with a fractionation of -1‰ at pH < 6 and +2‰ at pH of 8 (Sim et al., 2019). Theoretical calculations predict that abiotic equilibrium isotope effects in ^{34}S-^{32}S between sulfur bearing species can be as large as 58‰. These types of effects are rarely seen at Earth's surface but have been hypothesized to contribute to the δ^{34}S values measured at Gale Crater (Franz et al., 2017).

Abiotic S-cycling processes generally produce negligible changes in minor S isotopes (e.g., (Eldridge et al., 2021; Eldridge & Farquhar, 2018; Johnston, 2011). Two exceptions are TSR, which can induce large Δ^{33}S values up to +13‰ via magnetic isotope effects (Oduro et al., 2011), and mass-independent fractionation of sulfur isotopes (S-MIF) that occurs via gas-phase reactions during atmospheric sulfur photochemistry. S-MIF signatures are commonly observed in sulfide and sulfate minerals in sedimentary rocks older than 2.4 Ga, and attributed to the interaction of ultraviolet (UV) photons with SO_2 and other sulfur gases in the absence of an ozone layer (e.g., Farquhar et al., 2000a). Biomass burning has also been shown to impart small-magnitude S-MIF, with Δ^{33}S values as low as -0.19‰ observed in combustion experiments and associated aerosols (Lee et al., 2002; Lin et al., 2018; Shaheen et al., 2014); however, organic sulfur was very unlikely to have been extensive on early Mars. S-MIF signatures are also associated with large stratospheric eruptions (e.g., Crick et al., 2021) and volatilization of massive amounts of sulfur during the end Cretaceous meteorite impact (Junium et al., 2022), and have previously been measured in Martian meteorites (e.g., (Franz et al., 2014). We explore the implications for these abiotic fractionation mechanisms in altering S isotope biosignatures below.

2. Sulfur Isotope Biosignatures on Mars

2.1. Sulfur cycling on Mars

Sulfur is one of the most abundant elements on the Martian surface (Baird et al., 1976; Franz et al., 2019a; King & McLennan, 2010). The main sulfur oxidation states found on Mars are: S^{2-} (pyrrhotite, FeS_{1-x}), S^{1-} (e.g., pyrite, FeS_2), S^{4+} ($SO_2(g)$, SO_2^{2-}, SO_3^{2-} bisulfite), S^{6+} (SO_3, SO_4^{2-} sulfate, SO_2^{2+} sulfone) and S^+, S^{2+}, (S_3) (Franz et al., 2019a and references therein), with S^{6+} likely to be dominant (King & McLennan, 2010). Reduced sulfur is found in primary igneous phases from Martian meteorites, but also in the Martian sediments at Gale Crater (Franz et al., 2017; McAdam et al., 2014, 2020; Wong et al., 2020). In meteorites, the reduced S is mainly in the form of sulfides, including some reduced Fe-S minerals (e.g., pyrite and pyrrhotite) (Meyer, 2012 and references therein). High concentrations of sulfur on the Martian surface are evident through observations and measurements of high SO_3 concentrations in the Martian soil, including 5.12 wt. % at Gale Crater (Berger et al., 2016) and 6.16 wt. % in average Martian soil (Berger et al., 2016; Taylor & McLennan, 2009). This abundance of surface sulfur species on Mars ultimately derives from an S-rich mantle. Measurements of Martian meteorites suggest the presence of high sulfur concentrations in the Martian interior, with average concentrations of 6 wt. % SO_3 found in shergottites, which are Martian basaltic meteorites (Meyer, 2012). The prolific volcanism that extended from the Noachian to Hesperian led to significant amounts of outgassing to the surface (Figure 4) (Halevy et al., 2007; King & McLennan, 2010; Righter et al., 2009). The outgassing involved a large injection of sulfur gases (H_2S and SO_2) into the atmosphere (Gaillard et al., 2013; Settle, 1979; Tian et al., 2010). Mass-independent fractionation of S isotopes (S-MIF) from SO_2 and H_2S measured in Martian meteorites suggests that atmospheric photochemistry was a key influence on the early Martian S cycle (Farquhar et al., 2000b; Franz et al., 2014). Volcanic H_2S released into the atmosphere would have photo-oxidised to SO_2, where SO_2 was then either precipitated or further photo-dissociated, further deposited on the Martian surface with a distinctive S-MIF signal (Franz et al., 2014). It is still unknown how S-MIF signals on the Martian surface were affected by environmental factors, or if they have regional or local variations, providing a target for future analysis. Both hydrothermal circulation and meteorite impacts into S-rich sediments could have provided an active geochemical cycle that could have subsequently homogenised the S-MIF signal on the Martian surface (Figure 4). The delivery of dust into the atmosphere could have also played a role in homogenizing S-MIF between the atmospheric sulfur species and surface sulfates (Farquhar et al., 2000b).

The Sample Analysis at Mars (SAM) instrument on board the Curiosity rover detected SO_2, H_2S, OCS, and CS_2 at Gale crater. The SO_2 is likely thermally derived from Fe sulfates, Ca

sulfites, oxidation of sulfide (e.g. pyrite or pyrrhotite) (McAdam et al., 2014), or from Mg sulfates (McAdam et al., 2020; Sutter et al., 2017). The OCS and CS_2 are likely a product of a reaction involving reduced sulfur and some C source (McAdam et al., 2014; Wong et al., 2020). Sulfide minerals at Gale crater have been hypothesised to be the product of hydrothermalism and groundwater transport (Franz et al., 2017; Wong et al., 2020).

2.2.   Sulfur-rich habitats on early Mars

The presence of sulfur species and suitable electron donors and acceptors, including organic C (Eigenbrode et al., 2018), NO_3^- (Stern et al., 2015), H_2 (Feldman et al., 2004a), Fe^{2+} and Fe^{3+} (Banin et al., 1993; Morris et al., 2008), on Mars raises the question of whether a Martian sulfur biogeochemical cycle has ever been viable. Both SO and MSR have been proposed as feasible metabolisms under Martian chemical conditions, based on the chemistry of the Martian regolith (Nixon et al., 2013), Mars simulation experiments (Denson et al., 2009; Oliver et al., 2022), Gibbs free energy calculations (Macey et al., 2020; Ramkissoon et al., 2021), and their detection in a range of Mars analogue environments (Cousins et al., 2018; Lay et al., 2013; Macey et al., 2020; Moreras-Marti et al., 2021a,b; Perreault et al., 2007; Pontefract et al., 2017; Singh et al., 2019). The widespread presence of sulfur species would have created several potentially habitable S-rich environments on the Martian surface with different sulfur species available for S metabolic reactions. Two systems of particular relevance are: (i) hydrothermal systems accumulating reduced and oxidised species delivered directly from volcanism, and (ii) evaporative systems accumulating oxidised species from both volcanism and atmospheric deposition. These two types of environments were widespread on early Mars and could have supported a Martian biogeochemical S cycle in a habitat space conducive to biology with regards to other factors (e.g., water activity and availability of other bio-essential elements). These are discussed below.

During the Noachian-Hesperian transition, large amounts of heat were released through endogenic volcanic activity, directly linked to the formation of localised hydrothermal systems (Abramov & Kring, 2005; Gulick, 1998; Osinski et al., 2013). Exogenic impacts also formed hydrothermal systems within the impact craters themselves (Abramov & Kring, 2005; Rathbun & Squyres, 2002; Schwenzer et al., 2012). Evidence for hydrothermal systems can be found both on the surface (e.g., Ojha et al., 2021; Rathbun & Squyres, 2002) and within the subsurface (Ehlmann et al., 2011). One example is the relict surficial hydrothermal system at Home Plate (Columbia Hills, Gusev Crater), explored by the MER-A Spirit rover (Squyres et al., 2008). Here, opaline silica associated with volcanic material indicates past

hydrothermal activity (Ruff et al., 2007, 2020; Ruff & Farmer, 2016a, 2016b). The nearby Columbia Hills have sulfur-rich soils attributed to fumarolic activity of Home Plate (Squyres et al., 2008). A contrasting example of Martian hydrothermal activity can be found in the Eridania Basin, where the presence of both chloride evaporites and hydrothermal alteration mineral assemblages point towards the existence of a seafloor hydrothermal system (Michalski et al., 2017). Endogenic Martian hydrothermalism has also been linked to glacial surface deposits, such as Arsia Mons (Scanlon & Head, 2014) and Sisyphi Montes (Ackiss et al., 2018).

Mineralogical evidence for evaporitic brines on Mars has been found in many regions, including evaporite outcrops in Valles Marineris, Terra Meridiani, Margaritifer Sinius, Gusev Crater, Meridiani Planum, and North Polar regions (Gendrin et al., 2005; Langevin et al., 2005; Squyres et al., 2004), and observations of salt minerals in the equatorial and mid-latitudes (Feldmanet al., 2004b; Karunatillake et al., 2014). Evaporites on Mars are commonly associated with Hesperian aged terrain, a period between approximately 3.5 and 3.0 Ga (Hurowitz & McLennan, 2007). Sulfates (Mg, Fe and Ca) represent a major component of Martian evaporites, both in outcrops (Bibring et al., 2006), and in the globally distributed dust (Yen et al., 2005). Furthermore, Ca-sulfate deposits are a diagenetic alteration feature identified at both Gale Crater (Schwenzer et al., 2016) and Endeavour crater (Arvidson et al., 2016). These data imply a global aqueous chemistry at the time dominated by varying ratios of $Mg^{2+}/Fe^{2/3+}/SO_4^{2-}/Cl^-$ present in evaporite-forming brines, where local variations allowed $(H)CO_3$ to accumulate. At a neutral to alkaline pH, the presence of aqueous $(H)CO_3$ would likely have removed Fe^{2+}, Mg^{2+} and Ca^{2+} during evaporation. Therefore, the widespread presence of Mg, Fe and Ca-sulfate minerals indicates acidic sulfate-enriched brines.

2.3.   Current and future prospects for the detection of S isotope biosignatures on Mars

To test the utility of S isotope biosignatures, we consider S isotope values measured on Mars's surface and in Martian meteorites (Franz et al., 2014; 2017; Chela-Flores, 2019), within the context of S isotope measurements from terrestrial Martian analogue environments. We show that biogenicity is difficult to assess from major S isotopes alone and propose an important role for minor S isotopes in future life-seeking missions.

The S isotope values of sulfide and sulfate minerals have been measured from shergottite and nakhlite Martian meteorites (Figure 5) (Farquhar et al., 2007b; Farquhar et al., 2000b; Franz et al., 2014; Franz et al., 2019b), with shergottite meteorites providing the closest

available representation of Mars's mantle composition (Franz et al., 2019b). The δ^{34}S values of both types of meteorites vary from –3.34 to +0.7 ‰ for sulfides, and from -4.98‰ to +12.8‰ for sulfates (±0.15‰ for all δ^{34}S values). In contrast, a significant *in situ* discovery from the SAM instrument on board the Curiosity rover has been the identification of a large variability in the S isotope compositions measured in the sediments from Gale Crater (Franz et al., 2017). The first δ^{34}S values measured at Gale Crater from mudstones show a large δ^{34}S range of sulfides and sulfates, from -47 ± 14‰ to +28± 7‰. These large S isotope variations are proposed to have resulted from equilibrium fractionation between sulfate and sulfide within an impact-driven hydrothermal system, with related atmospheric processing of sulfur gasses during warm periods (Franz et al., 2017). These variations are larger than the δ^{34}S values seen in S-phases from Martian meteorites and overlap with variations produced on Earth by microbial sulfur metabolisms. Sulfur isotope fractionations ($^{34}\varepsilon_{CRS-SO4}$) in Mars analogue hydrothermal environments have been observed to be small, from -9.1 to -3.7 ‰ between water sulfate and CRS (Chromium Reducible Sulfur: pyrite and elemental S) from sediments (Cousins et al., 2018; Moreras-Marti et al., 2021b; Szynkiewicz et al., 2012). Conversely, hypersaline Mars analogue environments that support both MSR and MSD have larger $^{34}\varepsilon_{CRS-SO4}$ between -49.5 to -43.5 ‰ (Moreras-Marti et al., 2021b) (Figure 5). As described in Section 1, the occurrence of MSR with the addition of an oxidative S cycle, supporting SO and/or MSD, generally increases the S isotope fractionations expressed in natural environments, and could explain the larger fractionations in the hypersaline spring. Another important factor contributing to this disparity is the relative availability of metabolic energy in these environments. Hydrothermal environments provide functionally unlimited energy for MSR, MSD and SO in the form of abundant electron donors/acceptors. Under such energy-unlimited conditions on Earth, MSR use electron carriers with strongly negative reduction potentials which have been observed to produce fractionations smaller than -22 ‰ (Wenk et al., 2018). The opposite has been observed for energy-limited or low-energy niches, where MSR is associated with large S isotope fractionations (Sim et al., 2011a and references therein).

Minor S isotope data from both types of Mars-analogue sites show small-scale Δ^{33}S and Δ^{36}S variations consistent with mass conservation effects formed during biological sulfur transformations. Inclusion of the minor isotope analyses reveals that complex biological S-cycling, consisting of MSR and further oxidative recycling of sulfur species, are occurring in both environments. Particularly in hydrothermal environments with small variations in δ^{34}S, it is only when the minor S isotope values are included that biological and abiotic hydrothermal processes can be decoupled (Moreras-Marti et al., 2021b). This decoupling is of importance

when searching for biosignatures within a highly active abiotic S cycle. Likewise, these values form a small range in comparison to Martian meteorites, which instead show large $\Delta^{33}S$ values consistent with an atmospheric S-MIF signal (-1.25‰ ± 0.01‰ to 0.260‰ ± 0.008‰ for sulfates, and -0.538‰ to 0.093‰ for sulfides), and a smaller range of $\Delta^{36}S$ values (-0.67‰ to 2.4‰ ± 1.4‰ for sulfates, and -0.24‰ ± 0.2‰ to 2.6 ± 1.6‰ for sulfides) (Farquhar et al., 2007b; Farquhar et al., 2000b; Franz et al., 2014; 2019b). One observation from these meteorites is the co-existence of large $\Delta^{33}S$ anomalies with near-zero $\Delta^{36}S$ values, which implies Martian S-MIF formed by different pathways than those that operated on the early Earth, since Archean S-MIF values show a distinctive covariation between $\Delta^{33}S$ and $\Delta^{36}S$ (Farquhar et al., 2000a; Franz et al., 2014).

Future prospects for the detection of Martian S isotope biosignatures: unravelling abiotic from biotic QSI signatures in Martian materials

As described above, the addition of minor S isotopes ($\Delta^{33}S$ and $\Delta^{36}S$, QSI) can provide a third and fourth dimension for interpreting $\delta^{34}S$ values in natural systems. QSI can help to remove ambiguity between abiotic and biotic S-cycling processes, given that mass conservation effects are distinguishable from abiotically produced QSI values (e.g., Ono, 2008). In this section we consider the abiotic processes that could contribute to QSI values in Martian systems, and prospects for unravelling these from microbial S isotope signals.

Theoretical calculations predict that equilibrium isotope effects in ^{34}S-^{32}S between sulfur bearing species can be as large as 58‰ (Farquhar & Wing, 2003; Johnston et al., 2007; Ono et al., 2007). The large range in $\delta^{34}S$ values at Gale Crater (from -47‰ to +28‰) have been interpreted to reflect a combination of equilibrium isotope effects and atmospheric processing of S-bearing gases that were incorporated into minerals (Franz et al., 2017). Future QSI analyses could help to distinguish between these scenarios, since changes in $\Delta^{33}S$ values associated with equilibrium fractionations (between -0.02 and 0.03 ‰) are small in comparison with $\Delta^{33}S$ effects produced during biological transformations (> 0.05 ‰), providing a diagnostic biosignature (Johnston et al., 2011). TSR can also contribute to QSI values, particularly in hydrothermal systems, however, TSR requires the interaction of sulfate with organic matter, which does not seem to be prevalent for the majority of Martian history.

Mass independent fractionation of sulfur isotopes (S-MIF) during atmospheric photochemistry provides the most challenging abiotic signal to untangle from biological QSI values. Martian meteorites (sulfide and sulfate) show a larger range of $\Delta^{33}S$ (from -1.25 to 0.260‰) and $\Delta^{36}S$ values (from -0.67 to 2.6 ‰), interpreted to reflect S-MIF (Figure 5 A,B).

$\Delta^{33}S$ values from Martian meteorites are outside the range of values produced during mass conservation effects and mass dependent processes. Variations in $\Delta^{36}S$ are considered insignificant for Mars due to the relatively large uncertainties in the measurements, thus these values appear to show a lack of covariation between $\Delta^{33}S$ and $\Delta^{36}S$ (Farquhar, 2000b; Franz et al., 2014). Sulfur species processed by biology form coherent mass fractionation arrays in $\Delta^{36}S/\Delta^{33}S$ (Farquhar et al., 2000b). For example, biologically-influenced samples from Lost Hammer and the Icelandic hydrothermal pools show co-variation in $\Delta^{33}S$ and $\Delta^{36}S$, with $\Delta^{36}S/\Delta^{33}S$ slopes between -5.6 to -7.5, consistent with mass dependent processes (Figure 5). The $\Delta^{36}S/\Delta^{33}S$ relationship could therefore provide a powerful tool to help decipher mass dependent fractionations from S-MIF. Notably, if large atmospheric S-MIF signals are globally distributed at Mars' surface, these could effectively mask small mass-dependent microbial S isotope signals, unless $\Delta^{36}S/\Delta^{33}S$ slopes are discernible. In addition, even if S-MIF is globally distributed at the Martian surface, microbial S-cycling can dilute the S-MIF signal by mixing sulfur reservoirs in the environment, leading to characteristic mixing trends (Ono, 2008). This process has already been observed in late Archaean rocks, revealing trends that reflect S-MIF signals were overprinted by S-cycling microorganisms (Ono et al., 2003; Zerkle et al., 2021). In order to understand mixing processes and resulting dilution effects, the QSI composition of the different sulfur reservoirs would need to be characterised (e.g., volcanic SO_2, S_8 and H_2SO_4 aerosols, SO_4^{2-} minerals, sedimentary Fe_2S, etc), and considered within the depositional setting of the system (e.g., closed versus open system processes).

We suggest four considerations for unravelling biogenic vs abiogenic QSI values from sedimentary sulfur species preserved on Mars. First, S isotope signals indicative of biological S-cycling would be more clearly detected in systems largely free of contamination by atmospheric S-MIF signatures. Second, if a S-MIF signal was globally widespread on Mars, microbial mixing of sulfur could dilute the S-MIF signal and produce characteristic mixing arrays (Ono, 2008). Third, $\Delta^{36}S/\Delta^{33}S$ slopes could be useful in distinguishing between S-MIF and mass dependent S isotope fractionations. Lastly, S-cycling microorganisms inhabiting extreme environments that exert physiochemical stressors to life are more likely to produce larger S isotope fractionations (see Figure 2) that can be distinguished from non-MIF-forming abiotic processes. Like any biosignature, QSI can only be used together with other life-detection approaches. Carbon isotope analysis of organic matter (OM) for example pairs well with S isotopes, in addition to characterisation of the organic molecules present (Hays et al., 2017). This combination can further constrain the type of environments that are most likely to preserve microbial biosignatures.

Environmental targets for the preservation of QSI biosignatures

Given the four considerations outlined above, we propose that high sulfur environments, such as relic hydrothermal systems, impact craters and subsurface brines, would have provided the ideal setting for S-cycling organisms to interact directly with mantle-derived sulfur reservoirs, such as volcanic or hydrothermally-sourced sulfur (Figure 6). For example, sulfur delivered directly from inputs of volcanic sources could have had ~0‰ S isotope values, reflecting a high contribution of mantle-derived sulfur and a small S-MIF contribution (Labidi & Cartigny, 2016). Further, where high-S environments with large mantle input had a physical barrier to the surface, such as in subglacial hydrothermal environments and deep crustal hydrothermal systems, they would also have had a smaller S-MIF contribution (Figure 6). The Martian meteorites bearing a S-MIF signal only represent a subset of all surface and subsurface processes from the S cycle on Mars, thus it is reasonable to assume that relatively small fluxes of atmospheric S-MIF could have been mixed with sulfur reservoirs dominated by mantle sulfur sources. Conversely, environments with a relatively low flux of mantle-derived S, such as non-hydrothermal, non-sulfatic lacustrine, flood and groundwater environments would have been more susceptible to overprinting by deposition of S-MIF-bearing atmospheric sulfur. In addition, hydrothermal and volcanic systems would provide excess electron donors and acceptors, whereas settings such as brines would be energy-limited niches, which could promote larger S isotope fractionations. For settings such as lacustrine habitats and flood plains, it is likely the subsurface sediments presented energy limitation compared with hydrothermal systems, like the Jezero paleo-lake, however the paleochemistry of this lacustrine habitat is yet to be determined (Figure 6). We therefore suggest that the subsurface of lakes and floodplains could be systems to prioritise when searching for QSI biosignatures, with the likely challenge of having to unravel from S-MIF signals.

QSI analysis on returned samples

Currently, there are limitations with the analytical technique for measuring the four masses of S isotopes with a lander spacecraft. The process to analyse bulk QSI is intricate and requires a fluorination step, vacuum line to purify samples, gas chromatograph and mass spectrometer to enable simultaneous measurement of the four sulfur isotopic abundances. Alternatively, with Secondary Ion Mass Spectrometry (SIMS), a complex sample preparation process is required involving sample polishing, grinding, and gold coating, followed by analysis in an ion probe. Spacecraft measurements also involve larger errors due to the inability to control for measurement conditions, for example, $\delta^{34}S$ measurements at Gale Crater involve errors larger than ± 4‰ and as high as ± 14‰ (Franz et la., 2017). Considerable engineering efforts are needed to develop faster and smaller versions of these processes for inclusion in a future spacecraft payload. Mars sample return efforts circumvent this issue by allowing laboratory QSI measurements. Laboratory analysis of QSI through fluorination, vacuum and mass spectrometer only requires between 0.3 to 0.5 mg of Ag_2S. The SAM instrument analysed for S isotopes around ~45 to ~135 mg for S isotopes, of which 1 % in wt (0.45 to 1.35 mg) of these samples comprised pyrrhotite and possible pyrite in the JK sample (Franz et al., 2017). QSI measurements are also possible via grain-scale SIMS analysis, which can be conducted on 6μm to 10μm spots of sulfate or barite grains (e.g. Grema et al., 2022).

The Perseverance rover landed in Jezero crater in February 2021 to study Jezero's ancient lake system and to drill and store geological sample cores for future return to Earth. Perseverance has been studying the crater floor units, Maaz and Séítah formations, basal units of the lake identified as igneous in origin and affected by aqueous alteration (Gupta et al., 2022; Mangold et al., 2022; Sun et al., 2022). Hydrated Ca and Mg-sulfates suggest contact with briny waters (Meslin et al., 2022). Perseverance has already taken core samples from these crater floor units, and will sample the delta units (Mangold et al., 2022). These samples will eventually be recovered and returned to Earth (Muirhead et al., 2020). We argue the importance of performing QSI analysis on these returned samples as a tool to characterise the local S-cycling in Jezero's paleolake, in addition to contributing to deciphering the wider S cycle on Mars. For example, analysing QSI on S minerals in the crater floor units can identify S-MIF process captured here, together with the extent of its incorporation into magmatic materials. Where the deltaic environment has captured sedimentary sulfide and sulfate, either transported or formed *in situ*, QSI measurements can inform about their origin, or any putative biological S-cycling involved. There is a need for QSI studies on Jezero paleolake analogues on Earth to better understand the microbial signals in such settings. This analysis is important not only for QSI but also to improve

understanding of the general processes constraining the preservation of biosignatures in Jezero-like environments with deltaic mudstones, authigenic or detrital clay minerals, hydrated silica and magnesium carbonates (Bosak et al., 2021).

3. Sulfur Isotope Biosignatures on Europa

It is likely that oceans of liquid water exist beneath the icy surfaces of several moons of the gas giant planets, including Europa, Ganymede and Callisto (Jupiter), Enceladus and Titan (Saturn), and potentially elsewhere, including dwarf planets Ceres and Pluto, and Neptune's moon Triton (Nimmo & Pappalardo, 2016). While both Enceladus and Europa have received significant attention as potential habitats for life, due in large part to the likelihood of hydrothermal water-rock interaction maintaining redox disequilibria in their oceans, only at Europa have S compounds been detected.

3.1.   Potential sulfur cycling on Europa

Europa's ocean chemistry is currently not well constrained. Models for Europa's formation and differentiation into silicate core, ocean and ice shell have predicted that sulfate should be one of the dominant ions in the ocean (Fanale et al., 2001; J. Kargel, 2000; Zolotov & Shock, 2001a). Sulfate is a major detected component of Europa's surface, which displays extensive evidence for resurfacing (Figueredo & Greeley, 2004; Leonard et al., 2018). However, other theoretical approaches have argued that the oxidation of accreted sulfides may not have occurred if rates of H_2 escape were low enough to maintain reducing conditions (Mckinnon & Zolensky, 2003). Furthermore, it is possible that most (if not all) S on Europa's surface is exogenous - mapping of sulfate spectral signatures shows that their distribution is centred at the apex of Europa's trailing hemisphere (Brown & Hand, 2013; Carlson et al., 2005; Ligier et al., 2016), closely matching the intense flux of exogenic sulfur ions deposited on Europa from the high-energy plasma environment within Jupiter's magnetic field (Hendrix et al., 2011). Experimental work has shown that sulfate anions are generated via S ion implantation into ice (Strazzulla, 2011), and by radiolysis and thermal processing of water ice in the presence of S (Carlson et al., 2002; Loeffler & Hudson, 2010), all conditions that are met on Europa's trailing hemisphere.

By contrast, spectra from the leading hemisphere, which experiences a far lower flux of S ions (Hendrix et al., 2011), can be satisfactorily explained without contribution from sulfates (Brown & Hand, 2013; Ligier et al., 2016; Trumbo et al., 2019). Instead, leading hemisphere non-icy materials appear to be dominated by Na and Mg (and possibly K) chlorides, which must originate from water-rock interaction in the interior. Lack of endogenous sulfates on Europa's surface need not rule out a sulfate- rich ocean, as sulfate-rich fluids can evolve towards a chloride-dominated endmember during freezing (Vance et al., 2019; Zolotov & Shock, 2001a). However, this explanation assumes an as-yet unknown mechanism within

Europa's ice shell that efficiently returns precipitated sulfates to the ocean, preventing them from being expressed at the surface.

Regardless, it is reasonable to expect a moderate level of bioavailable S in the ocean (Figure 7). Europa's ice surface is thought to be less than 100 million years old, and in many regions significantly younger than this (Figueredo & Greeley, 2004). Resurfacing via exhumation and burial of subsurface materials has likely resulted in the delivery of radiolysis products, including sulfates, to the ocean over these timescales (Greenberg, 2010; Hand et al., 2007). Resurfacing also serves to expose fresh, unirradiated ice, ensuring the continued radiolytic production of oxidants. Estimates of the flux of oxidised S into the ocean through this route suggest that a minimum of 2×10^9 moles of SO_4^{2-} per year could be transferred into the ocean (Hand et al., 2007), with other estimates suggesting that rates of total oxidant flux (including SO_4^{2-} as well as H_2O_2, O_2 and other minor compounds) could be as high as 3×10^{11} moles yr^{-1} (Greenberg, 2010). Hydrothermal water-rock interaction predicted to occur at Europa's core-ocean boundary can supply the ocean with reductants such as H_2 at rates of between $\sim 10^8$ and $\sim 10^{10}$ mol yr^{-1} (Vance et al., 2016), which, when coupled with exogenous SO_4^{2-}, can serve as electron donors for MSR. The delivery through the ice of other oxidants such as H_2O_2 and molecular oxygen, (Hand et al., 2007), can provide further electron acceptors for microbial sulfide oxidation (Figure 7).

Because the ultimate source of S ions in Jupiter's magnetic field is Europa's neighbouring moon Io, a plausible biogeochemical S cycle on Europa therefore encompasses reductants and oxidants sourced from two separate planetary bodies (Figure 7), a significant contrast to S cycles on Earth and Mars. The existence of endogenous salts such as Na and Mg-chlorides on Europa's leading hemisphere, which must originate from water-rock interactions in the subsurface, demonstrates that ice shell overturn transports oceanic material upwards as well as downwards. Products of biological metabolisms, such as reduced or oxidised S pools, may therefore be incorporated into surface-accessible materials.

3.2 Future prospects for the detection of S isotope biosignatures at Europa

Upcoming missions such as the Europa Clipper and the proposed Europa Lander will have the capability to make isotopic measurements (at least δ^{34}S) to interrogate surficial S compounds for evidence of biological processes. In the latter case, this will occur by directly sampling the surface (Hand & Europa Lander SDT, 2017), and in the former case by

encountering eruptive plumes or micrometeoroid impact ejecta during fly-bys (Postberg et al., 2011). Recognising or ruling out biologically mediated isotopic signals in these materials requires accounting for the S isotope composition of the various pools of S both on and within Europa and their potential for diluting or overprinting biological processes, as discussed for Mars.

Sulfur on Europa's surface could originate from three reservoirs: (i) S compounds that have been recently delivered exogenously, (ii) exogenous S compounds that were delivered to the ocean via ice shell overturn, processed by (bio)geochemical cycling, and subsequently returned to the surface, or (iii) S compounds which are endogenous to Europa (*i.e.*, sourced from the silicate core) (Figure 7). Scenarios (i) and (ii) represent an exotic case for which new knowledge will be required. Specifically, two distinct processes potentially capable of imparting isotopic fractionations should be accounted for: ionisation of neutral molecules at Io, and radiolytic/thermal production of oxidised S compounds on Europa's surface. Sulfur ions impacting Europa's surface originate as neutral S molecules (*e.g.*, SO_2, SO) ejected from Io by volcanic activity, which are then ionised by high-energy electrons in Jupiter's magnetosphere within tens of hours after ejection (Yoshioka et al., 2017). The production efficiency of S ions (including S^+, S^{2+}, S^{3+} as well as O^+) differs markedly, with S^{2+} at least two orders of magnitude more abundant in Io's plasma 'torus' (Smyth & Marconi, 2003; Yoshioka et al., 2017). Ionisation of SO_2 by electrons has been demonstrated experimentally (Fletcher et al., 2013), but new experiments are required to measure isotopic distribution across ionic products. In addition, theorical predictions and new experiments are required to understand S isotope fractionations during the production of oxidised S compounds on Europa's surface. A review of ice-hosted thermal and radiolytic conversion of S compounds can be found in Mifsud et al.,(Mifsud et al., 2021). The radiolytic and thermally induced chemistry on Europa's surface can cycle S through a range of species, including SO_4^{2-}, SO_2, HSO_3^-, $S_2O_5^{2-}$, elemental S, S polymers, and even H_2S (Carlson et al., 2002; Kaňuchová et al., 2017; Loeffler et al., 2011; Loeffler & Hudson, 2010). If these compounds are delivered to the ocean *via* ice shell overturn, they could each participate in different biogeochemical processes including MSR, MSD and sulfide oxidation, with different implications for the extent of biological S isotope fractionations (as described in Section 1). It is important therefore to understand the $δ^{34}S$ S isotope composition of each product of surface chemistry, and how this relates to the S isotope composition of incoming S ions.

Discriminating between biological and abiotic S isotope fractionations in Europan surface materials also requires understanding the endogenous S content of the ocean. If Europa's ocean has low endogenous S, then the oceanic S inventory will be limited by the flux of S compounds from the ice shell, and exogenous S will dominate the S cycle, biological or

otherwise. If Europa's ocean contains abundant endogenous sulfate, as predicted by several models (Kargel et al., 2000; Melwani Daswani et al., 2021; Zolotov & Shock, 2001b), then exogenous S may represent a very small relative contribution. The contemporary delivery of exogenous S risks overprinting any biological S isotope signal that has been exhumed at the surface. This is particularly relevant for the trailing hemisphere, where the highest abundances of exogenous S compounds are found (Brown & Hand, 2013; Hendrix et al., 2011). Landed missions can strategically avoid these regions, and instead target locations such as the leading hemisphere, that experience low exogenous S flux. However, fly-by spacecraft such as NASA's Europa Clipper, which aim to analyse dust ejected from the surface by micrometeoroid impact, have less ability to target specific regions. Finally, if eruptive plumes are active on Europa, as have been tentatively identified (Jia et al., 2018; Sparks et al., 2017), ejected materials would presumably have no contribution from contemporary exogenous S, and therefore make a high priority target for probing subsurface (bio)geochemistry.

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4. Conclusions

- Sulfur isotope measurements represent a powerful tool with which to probe both abiotic and putative biological processes on Mars and Europa. QSI has the potential to delineate these processes on Mars by taking into account the four considerations suggested in Section 2.3.

- Analysis of these measurements needs to be conducted within the context of Sulfur reservoir inputs and outputs, many of which are still unconstrained, especially for Europa.

- On Mars, there is an apparent trade-off between environments most energetically favourable for S-based microbial metabolisms and those that are more likely to capture detectable S isotope biosignatures through exerting environmental stressors. This has implications for future landing site selection and mission targeting.

- QSI analysis on future returned samples from Jezero Cater can play a significant role in deciphering the local, and wider, Martian S cycle.

- For Europa, significant knowledge-gaps currently exist which hamper the utility of S isotopes as an effective biosignature for S-cycling microorganisms, despite the likely bioavailability of S in the ocean. This includes (i) understanding the S isotope signatures of space-based processing and (ii) establishing the relative contributions of exogenous and endogenous S to the Europan ocean environment.

- New experiments are required to measure S isotope distribution across products of ionisation of S neutrals in Io's plasma torus and radiolysis of implanted S compounds on Europa's surface

- Measuring $\delta^{34}S$ on Europa could be feasible on a lander or flyby mission. Furthermore, measuring $\delta^{34}S$ will help untangle the S cycle on Europa, and ultimately differentiate the different S pools.

5. Acknowledgements

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Figure 1. Schematic of S redox transformations and resulting fractionations in $\delta^{34}S$ by biotic and abiotic process. Fractionations are expressed as $^{34}\varepsilon_{R-P}$, where R = reactant and P = product. Green lines reflect microbial S-cycling processes, including: microbial sulfate reduction (MSR; Canfield et al., 2010; Sim et al., 2011a), microbial sulfur disproportionation (MSD; Canfield & Thamdrup, 1996), and sulfur oxidation (SO; (Pellerin et al., 2019; Zerkle et al., 2016). For SO, fractionations are summarized for the range of S oxidation pathways between reduced sulfur species (redS) and oxidized sulfur species (oxS). MSD can utilize a

number of intermediate sulfur species (S^0, SO_3^{2-}, or $S_2O_3^{2-}$) to produce both sulfate and sulfide (e.g., Frederiksen & Finster, 2003). Dashed brown lines reflect abiotic S-cycling processes, including: abiotic sulfur oxidation (Eldridge et al., 2021; Eldridge & Farquhar, 2018; Fry et al., 1988) and thermochemical sulfate reduction (TSR), which happens at temperatures between 80 to 200°C (Machel et al., 1995). The dashed purple line represents the range of $^{34}\varepsilon_{R-P}$ measured at Gale crater (Franz et al., 2017).

Figure 2. Main environmental controls on S isotope fractionation during MSR, modified from Fike et al. 2009 with updated parameters. Arrows indicate the magnitude of the fractionation effect (larger or smaller) for the indicated parameter. Parameters denoted with an asterisk(*) demonstrate strain-specific behaviour, including SO_4^{2-} concentrations, temperature, and cell specific sulfate reduction rates (csSRR). Based on measured changes in S isotope fractionation effects at differing SO_4^{2-} concentrations (Bradley et al., 2016; Canfield, 2001a, 2001b; Habicht et al., 2002) substrate effects (Hoek et al., 2006; Sim et al., 2011b, 2012) temperature (Canfield et al., 2006; Hoek et al., 2006), increasing csSRR (Chambers et al., 1975; Habicht & Canfield, 1997; Sim, 2012; Sim et al., 2011b), Fe and NH_4^+ limitation (Sim et al., 2012), and metabolic energy limitation (Wenk et al., 2018).

Figure 3. $\delta^{34}S$ vs $\Delta^{33}S$ systematics for H_2S and S^0 produced by different sulfur cycling metabolisms. MSD pure cultures from Johnston et al. (2005a); MSR Sim et al. (2011b), phototrophic SO from Zerkle et al. (2009). Natural systems: MSR from euxinic lake from Canfield et al. (2010), MSR+MSD also from euxinic lake from Zerkle et al., 2010.

Figure 4. Schematic describing the S cycle on the Martian surface in the Noachian-Hesperian time period, modified from Farquhar et al. (2000b) and Franz et al. (2017). Volcanic H_2S and SO_2 underwent UV photolysis in the atmosphere. These sulfur species were then deposited on the Martian surface and incorporated into the basalt or sulfate deposits through a variety of processes. H_2S and SO_2 could also have degassed from subsurface hydrothermal systems. Reprinted by permission from Springer Nature, Nature (Evidence of atmospheric sulphur in the Martian regolith from sulphur isotopes in meteorites, Farquhar *et al.*) © 2000, and Springer Nature, Nature Geoscience (Large sulfur isotope fractionations in Martian sediments at Gale crater, Franz *et al.*) © 2017.

Figure 5. A) $\Delta^{36}S$ vs $\Delta^{33}S$ for S-rich hypersaline ('LH'), and Icelandic hydrothermal (Kerlingarfjöll, and Kverkfjöll) environments (data from Moreras-Marti et al., (2021b), and Martian meteorites (from Franz et al. 2014, 2019b; Farquhar et al., 2000b, 2007b). B) Expansion of plot A. C) $\delta^{34}S$ vs $\Delta^{33}S$ for hypersaline (LH), Icelandic hydrothermal (Kerlingarfjöll, and Kverkfjöll), compared with microbial S-cycling from Figure 3, and Martian meteorites. $\delta^{34}S$ values for Gale Crater range from -47 ± 14‰ to -28± 7‰ (Franz et al. 2017). AVS for Acid Volatile Sulfur: H_2S, HS^- and other polysulfides, CRS for Chromium Reducible Sulfur: pyrite and elemental S.

Figure 6. Summary figure with the different environmental factors affecting QSI biosignature preservation in high sulfur environments and low sulfur environments on Early Mars. Scenarios consider metabolic energy unlimited vs metabolic energy limited subsurface niches. High sulfur environments feature a significant mantle sulfur signal in volcanically-driven systems, the opposite for low sulfur environments. This scenario is hypothesised on a past Martian atmosphere with ongoing S-MIF processes, resulting in high S-MIF signals present in environments with direct atmospheric access. Subsurface or subglacial environments present low S-MIF signal due to their partial disconnection with the surface.

Figure 7. Schematic describing the potential S cycle on Europa profile, from the rock interior, to the surface, with delivery of exogenous SO_2 particles from Io. Figure modified from Europa Lander Study report 2016.