Sample Collection and Return from Mars: Optimising Sample Collection Based on the Microbial Ecology of Terrestrial Volcanic Environments

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Abstract With no large-scale granitic continental crust, all environments on Mars are fundamentally derived from basaltic sources or, in the case of environments such as ices, evaporitic, and sedimentary deposits, influenced by the composition of the volcanic crust. Therefore, the selection of samples on Mars by robots and humans for investigating habitability or testing for the presence of life should be guided by our understanding of the microbial ecology of volcanic terrains on the Earth. In this paper, we discuss the microbial ecology of volcanic rocks and hydrothermal systems on the Earth. We draw on microbiological investigations of volcanic environments accomplished both by microbiology-focused studies and Mars analog studies such as the NASA BASALT project. A synthesis of these data emphasises a number of common patterns that include: (1) the heterogeneous distribution of

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biomass and diversity in all studied materials, (2) physical, chemical, and biological factors that can cause heterogeneous microbial biomass and diversity from sub-millimetre scales to kilometre scales, (3) the difficulty of *a priori* prediction of which organisms will colonise given materials, and (4) the potential for samples that are habitable, but contain no evidence of a biota. From these observations, we suggest an idealised strategy for sample collection. It includes: (1) collection of multiple samples in any given material type (∼9 or more samples), (2) collection of a coherent sample of sufficient size (∼10 cm³) that takes into account observed heterogeneities in microbial distribution in these materials on Earth, and (3) collection of multiple sample suites in the same material across large spatial scales. We suggest that a microbial ecology-driven strategy for investigating the habitability and presence of life on Mars is likely to yield the most promising sample set of the greatest use to the largest number of astrobiologists and planetary scientists.

**Keywords** Mars · Sample collection · Microbial ecology · Basaltic · Fumaroles

1 Introduction

The search for habitable conditions or life on any planetary body must necessarily be guided by what we know about life on Earth. A very large number of ‘analog’ environments on the Earth have been investigated to understand the factors that influence their microbial inhabitants and determine both the biomass and diversity of life they can support. These environments range from deserts to lakes with physico-chemical conditions, such as extreme aridity, acidity or salinity, that correspond to extant or ancient conditions on Mars (e.g., Dickinson and Rosen 2003; Navarro-González et al. 2003; Cabrol et al. 2007; Preston and Dartnell 2014; Hays et al. 2017; Lim et al. 2019).

Over the last two decades our view of Mars as a homogeneous ‘red planet’ has given way to a highly nuanced understanding of the great heterogeneity of past and present environments at kilometre scales (Cabrol and Grin 2001; Bibring et al. 2006; Ehlmann et al. 2009, 2011) down to millimetre scales, where individual mineral grains have been investigated (Weitz et al. 2006). Some geochemical records indicate paleoenvironmental conditions permissive for life (Grotzinger et al. 2013; Hurowitz et al. 2017). Despite this impressive expansion in our understanding of Mars, the fact remains that Mars is fundamentally a volcanic planet, with a meagre rock cycle (no plate tectonics) and no extensive surface biosphere, both of which vastly increase the range of viable habitats on the Earth. Even in the realm of volcanic lithologies, Mars is limited in its geological diversity compared to the Earth. For example the lack of a highly productive biosphere to generate large abundances of organic carbon (Field et al. 1998) limits the diversity and extent of volcanic soils, called ‘andosols’, that are a substantial habitat for microbial life on Earth. Although the Earth’s basaltic oceanic crust offers a number of lessons about microbial distribution (Edwards et al. 2003; Lysnes et al. 2004; Santelli et al. 2008), the specific chemical composition of Earth’s sea water and its continuous flow through the basaltic crust over million-year time scales makes even deep-ocean basaltic microbial habitats of potentially limited direct applicability to Mars. On Mars, the composition of hydrothermal fluids in the subsurface is poorly constrained, the former presence and extent of sustained ocean(s) to generate analogous deep ocean habitats is controversial (Head et al. 1999) and long-term hydrological activity appears to have been curtailed by the loss of most surface water in the late Noachian and Hesperian (Lasue et al. 2013).
There are, of course, environments on the Earth and Mars that are often considered to be independent of the geology of the crust and on Earth host life or preserve biosignatures. For example, ice-sheets and glaciers on Earth host life (Carpenter et al. 2000; Liu et al. 2015; Anesio et al. 2017), as do evaporitic salt deposits (Maturrano et al. 2006; Oren 2008; Robinson et al. 2015). These environments are known to exist on Mars (Head et al. 2003; Lasue et al. 2013; Osterloo et al. 2008, 2010). However, the aqueous and particulate geochemistry of these environments are influenced by the geological substratum and thus the nutrients and energy supplies in such settings on Mars would still be linked to a volcanic geological context. Nevertheless, this paper will not discuss the biological potential and sampling of ices and salts. We draw lessons on sample collection by focusing on primary volcanic rocks and derived weathering materials since they constitute the majority of materials and potentially habitable substrates that would likely be sampled by robotic and human explorers. Although we do not review the literature here, we note that the conclusions we will draw on the heterogeneity of microbial biomass and diversity in primary volcanic samples also apply to ices and salt deposits in which heterogeneous biomass and diversity distribution has been observed (e.g., Liu et al. 2015; Robinson et al. 2015).

The study of the microbial ecology of volcanic terrains relevant to Mars is surprisingly underdeveloped. For example, the microbial ecology of environments with sustained interactions between freshwater and volcanic rocks, including crystalline rocks and glasses, is poorly known. Indeed, limited knowledge exists regarding the overall thickness and extent of volcanic soils and other deposits derived by chemical weathering on Mars. However, a sufficient number of studies have been accomplished to extract knowledge that directly bears on what our most optimistic expectations for microbial ecology on Mars could be (assuming that there is or was life) and thus how one should go about collecting samples. In this paper, we do not attempt an exhaustive review of microbiology studies in volcanic environments. Instead, we provide examples used to draw out general principles. The selection of studies used in this paper are shown in Table 1.

In this paper, we have two objectives. First, we provide a review of microbial life in volcanic rocks to inform the reader about the typical distribution of life in a diversity of materials. This review provides insight into the types of samples of interest to a biologist. One might regard this as an introductory primer on microbial life in volcanic materials. Second, using these examples of the microbial ecology of volcanic materials, we draw out common patterns that allow us to suggest microbial ecology-driven sample collection approaches for investigating habitability and testing the hypothesis of life on Mars.

The conclusions we draw in this paper are applicable to the search for both extant and extinct life. The distribution of biomarkers such as biomolecules (e.g., hopanes) and isotopic signatures in volcanic paleohabitats will depend on the original distribution of biomass as well as its potential to be preserved in those environments (McMahon et al. 2018). Thus, the search for extinct life must also take into account taphonomic considerations relevant to the microbial habitats discussed here.

2 A Review of Igneous Petrology from an Astrobiological Perspective

Igneous petrology is a vast area of research, but from an astrobiological perspective, several points can be made about volcanism that are essential to the later discussion in this paper. Volcanic rocks (i.e. igneous rocks derived from the extrusion of lava at the Earth’s surface rather than cooling of magma in the subsurface) vary widely in composition. They
Table 1  Studies of the microbial ecology of volcanic environments relevant to Mars used in this paper to suggest a sampling strategy

| Type of environment/material                                      | Location | Comments                                                                 | Reference                          |
|-------------------------------------------------------------------|----------|--------------------------------------------------------------------------|------------------------------------|
| Basaltic/andesitic crystalline rock                               | Italy    | Lichen colonisation of lava flows on Mount Vesuvius                     | Adamo and Violante (1991), Adamo et al. (1993) |
| Iceland                                                           |          | Studies on bacterial inhabitants of crystalline rocks (lava)             | Kelly et al. (2011), Cockell et al. (2013) |
| Iceland                                                           |          | Bacterial pioneer colonists of crystalline rocks (lava)                  | Kelly et al. (2014)                |
| Iceland                                                           |          | Inhabitants of lava flows of differing ages                              | Byloos et al. (2018)               |
| USA                                                               |          | Inhabitants of transects from fumarolic to non-fumarolic sites including unaltered and weathered basalts (Hawai‘i) | Cockell et al. (2019)             |
| Rhyolitic crystalline rock                                        | Iceland  | Bacterial inhabitants of crystalline rocks (lava)                        | Kelly et al. (2011)                |
| Basaltic glass                                                    | Iceland  | Bacterial inhabitants of weathered basaltic glass                        | Cockell et al. (2009)              |
| Iceland                                                           |          | Bacterial inhabitants of weathered basaltic glass                        | Kelly et al. (2010)                |
| Obsidian (silica-rich glass)                                      | Iceland  | Bacterial inhabitants of obsidian                                        | Herrera et al. (2008, 2009)        |
| Iceland                                                           | Iceland  | Bacterial inhabitants of weathered glasses                               | Kelly et al. (2010)                |
| Volcanic fumaroles/active geothermal substrates/geothermal lakes  | Italy    | Endolithic inhabitants of rocks and soils in active geothermal area      | Gross et al. (1998)                |
| USA                                                               | USA      | Endolithic inhabitants of siliceous geothermal deposits                 | Walker et al. (2005)               |
| New Zealand                                                      |          | Endolithic inhabitants of siliceous deposits in active geothermal springs | Gaylarde et al. (2006)             |
| Chile                                                            |          | Inhabitants of fumaroles and surrounding soils                           | Costello et al. (2009)             |
| Antarctica                                                       |          | Inhabitants of fumarolic soils                                           | Soo et al. (2009)                  |
| USA                                                               | USA      | Inhabitants of nonsulphur, sulphur and iron geothermal steam vents       | Benson et al. (2011)               |
| USA                                                               | USA      | Inhabitants of fumaroles                                                 | Wall et al. (2015)                 |
| Iceland                                                           |          | Geothermal spring microbial and isotopic gradients                        | Cousins et al. (2018)              |
| Costa Rica                                                       |          | Microbial diversity in Laguna Caliente lake                             | Hynek et al. (2018)                |
| Chile                                                            |          | Inhabitants of fumarolic soils                                           | Solon et al. (2018)                |
| USA                                                               | USA      | Inhabitants of transects from fumarolic to non-fumarolic sites (Hawai‘i) | Cockell et al. (2019)             |
| Type of environment/material | Location | Comments | Reference |
|------------------------------|----------|----------|-----------|
| Mud volcanoes                | Romania  | Microbial diversity of mud volcanoes | Dahigaonkar and Chavan (2018) |
|                              | China    | Microbial diversity of mud volcanoes | Huang et al. (2016) |
| Ash/ash soils/tephra         | Iceland  | Colonisation of Surtsey Island | Schwabe (1970), Schwabe and Behre (1972); Kristinsson (1970, 1974); Brock (1973); Henriksson and Henriksson (1982); Stefansdottir et al. (2014) |
|                              | Iceland  | Colonisation of Heimaey Island | Englund (1976) |
|                              | USA      | Cinder cone colonisation (Hawai`i) | Carson and Brown (1978) |
|                              | USA      | Soil microbial communities (Hawai`i) | Nüsslein and Tiedje (1998) |
|                              | USA      | Recent colonisation of ash and their lithotrophic metabolisms (Hawai`i) | King (2003) |
|                              | USA      | Recent colonisation of ash and their lithotrophic metabolisms (Hawai`i) | Nanba et al. (2004) |
|                              | USA      | Inhabitants of 42–300 year old Hawaiian volcanic deposits (Hawai`i) | Dunfield and King (2005) |
|                              | USA      | Inhabitants of numerous soil and ash deposits of different ages (Hawai`i) | Gomez-Alvarez et al. (2006) |
|                              | Antarctica | Inhabitants of deposits on geothermally active volcano | Ferrani et al. (2007) |
|                              | Italy    | Inhabitants of different aged soils | Hopkins et al. (2007) |
|                              | USA      | Inhabitants of different aged soils | Ibekwe et al. (2007) |
|                              | Iceland  | Biomass and microbial community in fresh volcanic tephra | Amador et al. (2015), Gentry et al. (2017) |
are classified into fifteen different compositional groups depending upon their silica and alkali content (in this context alkali means the concentration of Na and K) following convention established by the International Union of Geological Sciences (IUGS) (Le Bas et al. 1992). Ultimately, these categories largely reflect the source composition and thermal evolution of the magma, with more mafic (iron- and magnesium-rich) minerals crystallising from the melt at higher temperatures. Mafic and ultramafic rocks (also referred to as ‘basic’) are formed between \( \sim 1000–1500 \) \( ^\circ \)C and contain elevated concentrations of pyroxenes, olivines and plagioclase (predominantly Mg, Fe and Ca-containing minerals). The precipitation of these dense minerals within a cooling magma chamber drives the composition of the residual magma towards higher silica content; thus high-silica volcanic rocks (also referred to as ‘acidic’ or ‘felsic’ rocks) generally represent lower temperatures (typically about 700 \( ^\circ \)C). They also have a higher content of orthoclase feldspar (a K-containing silicate mineral) and quartz. Although rare felsic lavas have been detected on Mars, the martian crust is dominated by the mafic rock, basalt. In this paper, we will not consider samples spanning the entire ultramafic-felsic continuum, but we will provide examples of the microbial ecology of materials, focusing on basaltic rocks. We also provide comparisons to high-silica obsidian where this is useful to illustrate factors that influence colonisation.

A second factor of great importance for a biota is the glassiness or crystallinity of the material. Volcanic melt that comes into contact with ice or water is rapidly quenched before crystals can form, resulting in a homogeneous glass, in contrast to other rocks that are formed subaerially and cool slowly, forming crystalline materials. This is significant in the Martian context because widespread glaciers and ground ice in contact with volcanically active regions (Cousins and Crawford 2011) may imply that past episodes of volcanism have generated substantial quantities of glass and their weathering products (Horgan and Bell 2012).

Why is distinguishing crystalline rock and glass important? First, glasses present a material with a more homogenised composition to a potential biota, whereas in crystalline materials, bioessential elements are generally localised to particular crystals, meaning that fewer bioessential elements can be found in one location at the micron-scale. Second, basaltic glass, in contrast to crystalline rocks, has a distinctive capacity for weathering to palagonite, a soft alteration product (Thorseth et al. 1991; Stroncik and Schminke 2002) that tends to form within basaltic vesicles and fractures and may be a more bio-accessible source of nutrients than the crystalline matrix. Third, in crystalline high-silica rocks, the silica is primarily bound up in quartz. However, in glass, this silica (which has no biological use) is distributed throughout and may retard the release of more biologically useful elements such as Fe (Wolff-Boenisch et al. 2004). A priori therefore, in parent rocks on Mars we would expect the concentration of silica and the glassiness of the material to be important considerations in their potential as habitable substrates and how microorganisms would be theoretically distributed within them. These factors are explored in later sections.

The weathering of these primary rock types (and of volcanic ash deposits) in aqueous environments leads to the formation of a variety of cation-rich aluminosilicate clays (or palagonite in the case of basaltic glass). The types of clays formed depend on the thermal regime and aqueous chemistry. On Mars, Fe- and Mg-rich smectites, serpentine, and chlorite, resulting from the alteration of basaltic precursor minerals, are particularly widespread (e.g., Poulet et al. 2005; Milliken and Bish 2010; Ehmann et al. 2011; Bristow et al. 2018). Interactions with clay particles, including smectite, can profoundly influence the fate and residence time of organic matter in the environment and inhibit or promote the growth of different microbial groups. However, little is currently known about the correlation of microbial diversity and biomass to different clay types formed in volcanic
environments, particularly in environments where organic input is low. Most analyses of volcanic clays have occurred in the critical zone (soil-rock interface) in regions of the world where the formation of economically important volcanic soils (andosols) is associated with generally high organic/humic substance concentrations (Kimble et al. 2000). Nevertheless, the studies that have been undertaken in volcanic terrains are sufficient to draw the conclusions we make here.

We recognise that present-day surface conditions on Mars are very different from the Earth. The desiccated surface, high-ultraviolet and ionising radiation and the presence of perchlorates (Cockell et al. 2000; Dartnell et al. 2007; Hecht et al. 2009; Wadsworth and Cockell 2017), among other factors, make the surface inimical to life and may also impact the preservation of ancient biosignatures (particularly organic biomarkers). In this synthesis, we are assuming that martian samples will be collected from environments that experienced sustained contact with water in the past and have either been drilled from the subsurface or were only exposed to martian atmosphere relatively recently, for example by wind erosion (e.g., Farley et al. 2014; Hurowitz et al. 2017). We also note that more extreme conditions found on Mars, particularly in later stages of its hydrological evolution, if they were to theoretically reduce the potential biomass and diversity of life, would merely strengthen the conclusions we draw about low biomass environments and the need for large sample numbers required to test for life or affirm its absence.

A further complication is the aeolian transport of material that becomes entrained in rocks. Microorganisms are known to be transported in atmospheric dust on Earth (Gat et al. 2017). As well as providing a microbial inoculum to new habitats, the aerially transported dust can potentially introduce extraneous geological material into samples, confounding geological analysis. On Mars, aeolian dust transport and mixing over the planetary scale is ubiquitous (Lasue et al. 2018). Aerial transport of particulate matter is also widespread in many volcanic environments on the Earth, including those in which microbial ecological analysis described in this paper have been undertaken, such as environments in Iceland (Arnalds et al. 2016). This transport does not compromise geological analysis of the origin of samples or the conclusions about the heterogeneous distribution of biomass and diversity within the sample that is linked to the primary sample type. This is likely to be the case for sample selection on Mars, particularly if samples are cleaned of dust or collected from the near-surface or interior of outcrops.

3 Limits of ‘Analogue’ Materials

Not all volcanic environments and materials on Earth are useful analogues to Mars. Two examples are andosols and marine-influenced volcanic rocks. We discuss these here to emphasise caution in drawing lessons about Mars from some volcanic materials on Earth.

Volcanic rocks ultimately weather to soils called andosols, which form an important component of terrestrial volcanic environments (Kimble et al. 2000). Andosols exist in a variety of states such as extreme pH in soils surrounding geothermal pools, hot and cold desert soils, as well as humid andosols (Oskarsson et al. 2004; Parfitt and Kimble 1989; Arnalds 2004; Belobrov and Ovechkin 2005; Hopkins et al. 2007). However, apart from regions free of vegetation and with low precipitation (deserts), most andosols are fertile because of many years of microbial and floral amelioration. They are generally high in Fe and Al. These metals, in addition to high concentrations of humic acids, make andosols highly effective at immobilising phosphate (Lukito et al. 1998). This retention of biologically available phosphorus combined with the precipitation of phosphorus, impedes microbial and floral growth
on younger andosols (Kimble et al. 2000). It is not currently known what the initial microbial colonists are for many andosols as it depends on the chemical composition of each individual soil. Richardson (2001) showed that microorganisms were key to the release of organic and inorganic phosphorus. Nevertheless, even young andosols, although relatively hostile, are capable of sustaining highly diverse microbial communities. Nüsslein and Tiedje (1998) investigated a 200 year-old andosol in Hawai‘i with high diversity. They showed that the soil biomass was dominated by a distinct microbial diversity of *Pseudomonas*, *Rhizobium-Agrobacterium* and *Rhodospirillum* species. On many andosols, plants strongly influence microbial colonisation. Some of the first floral species to inhabit Mount St. Helens soils following the 1980 eruption were *Lupinus* spp., which provide support for nitrogen-fixing cyanobacteria (Halvorson et al. 1992). Although these data are interesting, they nevertheless illustrate that many terrestrial andosols are likely poor analogues to past or present Martian volcanic environments because of the extent of microbial and plant colonisation and the relatively rich organic carbon cycle established within them.

An extensive body of literature exists on the microbial ecology of the basaltic oceanic crust. Although superficially this carbon- and energy-poor environment might appear to be a good analogue for the Martian deep subsurface, there is uncertainty as to how much the communities associated with ocean crust, their biomass and diversity, are influenced by ocean chemistry and factors linked with the Earth’s unusual plate tectonic regime. During the rifting that occurs at plate boundaries in the oceans, basaltic glass is produced. The rate of crustal production may be well over a billion tonnes a year (Bach and Edwards 2003). Insofar as basaltic glass contains many major cations that are biologically useful, including Ca, Mg, K, Na and Fe, then it would be expected to be a source of material for microbial growth (Staudigel et al. 1995, 1998; Thorseth et al. 2001; Santelli et al. 2008).

The extent to which oceanic basaltic glass is used by a biota *in-situ* is unclear (Staudigel et al. 1998, 2006, Torsvik et al. 1998). There is evidence for the deep-ocean biological alteration of glass. In investigations of 0–30 Myr old basalts from the region of mid-ocean ridge known as the Australian–Antarctic Discordance, Thorseth et al. (2003) recorded the presence of Mn-rich encrustations which they suggested could be linked to biological Mn-yielding reactions. Partially fossilised endolithic microorganisms were associated with rims of altered glasses. Basaltic glass from the Knipovich Ridge, Arctic shows pervasive colonisation of fractures, including stalked, coccoid, rod and filamentous forms. Organisms were found to be associated with the alteration products in the fractures and they were often covered in Fe- and Al-rich precipitates (Thorseth et al. 2001).

The organisms that might be involved in weathering glass have been the subject of several investigations. Templeton et al. (2005) showed the presence of Mn-oxidising bacteria in basalts from Loihi Seamount, although autotrophic Mn-oxidisers were not recovered. This suggests Mn oxidation is a secondary process occurring in heterotrophic organisms (Tebo et al. 2005) that might be using organic carbon compounds available in seawater. Diverse neutrophilic Fe-oxidising bacteria have been isolated from oceanic environments (Edwards et al. 2003), although it is not clear to what extent they might be accessing iron from basaltic glass or from the surrounding sea water. The Fe oxide minerals found associated with deep-ocean basalts have been shown to be similar to minerals produced by iron-oxidising bacteria in culture (Daughney et al. 2004).

In a study of the microbial community of the basaltic glasses of the Knipovich Ridge, Arctic, Thorseth et al. (2001) identified heterotrophs and some chemolithotrophs including phylotypes closely matching with S-oxidisers. Fe-reducing organisms were cultured from Arctic Ridge seafloor basaltic glasses by Lysnes et al. (2004) and these authors suggested that this is evidence for an Fe-cycle within seafloor basalts. Phylogenetic analysis of seafloor
basaltic glasses around Hawai’i show high diversity (Santelli et al. 2008; Orcutt et al. 2009) which is hypothesised to be linked to the chemolithotrophic use of basaltic glass alteration products (Santelli et al. 2008).

In summary, there appears to be good evidence for microbial use of elements from basaltic glass directly, particularly Fe, that may suggest that these rocks provide an analogue for habitable substrates on Mars. However, the extent to which resources in seawater are used, such as organics and other ions, and the extent to which the biomass and diversity of these habitats is established by continuous water flow in the oceanic crust together with the high production rate of fresh substrate through plate tectonics is not known. Plate tectonics, if it existed on Mars at all, ceased early (Yin 2012). Although there is controversial evidence for a northern ocean (Head et al. 1999), even if it did exist, its chemical composition and the degree to which it might have sustained crustal hydrothermal cycling to generate habitats analogous to the terrestrial oceanic crust is not known.

For these reasons, in this paper we focus on volcanic materials in contact with freshwater, primarily land-based crystalline and glassy volcanic rocks, including materials in land-based volcanic hydrothermal systems. However, heterogeneous distribution of biomass and diversity is observed in ocean crust materials (Santelli et al. 2008), so although we do not consider these to be necessarily the best analogue materials to examine here, the consideration of their microbiology would not change our conclusions.

4 Microorganisms Are Heterogeneously Distributed—the Need for Appropriate Sample Size

Observations of microorganisms in contiguous cryptoendoliths biofilms (e.g., Bell 1993; de la Torre et al. 2003) might give the impression that organisms are generally uniformly distributed in rocks. Although these distinctive endolithic communities can be observed in volcanic geothermal materials (Gaylarde et al. 2006; Gross et al. 1998; Walker et al. 2005) and cooled volcanic rocks (Herrera et al. 2009), organisms within volcanic materials are frequently highly heterogeneously distributed at millimetre and sub-millimetre scales, with implications for sample collection (Fig. 1). As a case study, it is worth considering the colonisation and distribution of microbes in basaltic glass and its felsic counterpart, obsidian. In both materials, microbes are observed to be distributed heterogeneously, but apparently caused by different factors. The distribution of microbes in obsidian, although not directly relevant to Mars as this material has not be detected, provides insights into the factors that influence microbial distribution in surface and near-surface volcanic materials more generally.

Basaltic glass weathers to palagonite, a relatively soft weathering product, which forms rinds on the glass (Stroncik and Schminke 2001, 2002). Although weathering of obsidian occurs, it is much slower than the weathering of basaltic glass (Wolff-Boenisch et al. 2004, 2006). The higher silica concentration in obsidian impedes the degradation of the material since the Si-O bonds result in a more extensive silica network that is thought to retard leaching of cations, including bioessential cations, from the rock.

These different weathering processes are consistent with observations of varying microbial abundance. Cockell et al. (2009) observed high cell numbers in basalt glass samples with bulk cell numbers of $\sim 10^7$ cells/gram. In this material, most of the observed cells were associated with the weathered palagonite surfaces and potentially a population beneath the palagonite. Very few cells were associated with the unweathered glass surface. In obsidian, which does not so readily form weathered rinds, Herrera et al. (2008), using FISH
Fig. 1 Cells are distributed heterogeneously in materials for different reasons. Examples of heterogeneity in microbial distribution in volcanic glasses. Image 1 shows cells in obsidian. In (A), unaltered glass (G) and altered glass (AG) at the edge of a vesicle (V) are shown. Subimage B shows the region located in the square in image A. Cells were detected after DAPI staining under UV light (subimage C) and after fluorescence in situ hybridisation (FISH) with bacterial Cy3-EUB338I-III primers targeting most of bacteria (subimage D). Observations B, C and D correspond to an identical area on the thin section (data from Herrera et al. 2008, 2009). Image 2 shown cells in basaltic glass. Subimage A shows SYBR Green I (DNA) staining of microorganisms on a fragment of palagonite also shows in bright field (scale bar 20 µm) (subimage B). Subimage C shows a SYBR Green I stained section of a vesicle in weathered basaltic glass, arrow indicates DNA staining at the rock–palagonite interface with staining also apparent on the surface of the palagonite (scale bar 40 µm) (data from Cockell et al. 2009). (Fluorescence In-Situ Hybridisation), found that cells were localised to altered regions in the obsidian glass (Fig. 1). The cells were associated with iron oxide crystals. Subsequent cell enumeration found total bulk cell numbers less than $10^4$ cells/gram. Because the cells were so localised within the obsidian, whole rock cell numbers were unrepresentative. Significantly, entire chunks to cm$^3$ scales, contained no detectable cells. The localised biofilms associated with alteration zones were often only found in exposed rocks or weathered regions where water had penetrated into the material.

Similarly heterogeneous distribution of biomass has been observed in other volcanic environments. The NASA project BASALT (Biologic Analog Science Associated with Lava Terrains; Lim et al. 2019) carried out a study of microbial diversity and biomass in basaltic fumaroles, weathered rocks and unweathered rocks in Hawai‘i. Consistent with other studies cited here, all materials were found to host a low diversity, and biomass was highly heterogeneous, ranging from $6.25 \times 10^5$ to $4.06 \times 10^7$ cells/gram dry weight based on DNA concentration (means shown in Fig. 3). Lipid biomarker-based estimates of biomass also demonstrated heterogeneous distribution within BASALT samples (Brady et al. 2017). The heterogeneous distribution of biomass in basaltic terrains has been noted in both fresh lava (Kelly et al. 2014) and in dispersed volcanic tephra of varying ages (Gentry et al. 2017).

Factors that are likely to account for the observed heterogeneity in microbial biomass in volcanic materials include physical influences such as variations in the availability of fracture surfaces and pore/vesicle space for growth, differences in permeability and thus
fluid flow through fractures, albedo and therefore tendency to absorb sunlight and heat; chemical factors such as the proportion of glass, heterogeneity in leaching of elements from the composite minerals or glasses available for redox couples or as nutrients, variations in the chemical weathering reactions mediated by constituent minerals and elements, differences in the kinetic release rates of elements from rocks (Cockell et al. 2011); and biological factors, e.g. priority effects, whereby first colonisers influence subsequent colonists (Lee et al. 2013). Some of these factors are known to be important determinants in the colonisation of soils (Garcia-Pichel et al. 2003; Vos et al. 2013; O’Brien et al. 2016).

These observations across a variety of volcanic materials emphasise the need for appropriate sample size. It is not possible to state an exact size, since the optimal sample size depends on the material. However, the presence of organisms inhabiting millimetre-scale vesicles, crystals and fractures in many volcanic materials shows that samples with integrity at centimetre scales are required. These observations of cm³ scale samples or segments of samples devoid of life even in materials on Earth, such as obsidian, shows that typical minimum sample sizes of volcanic rock collected in the field for microbiological analysis should be of size ~10 cm³ and this sample size applies to Mars exploration.

5 Every Sample Is a ‘Microbial Island’—the Need for Multiple Samples from the Same Material Type

Like biomass, the microbial diversity of volcanic materials varies enormously as a function of physical, chemical and biological factors that relate to the functional requirements of life in that environment and/or the specific organisms that first successfully colonise the material.

Microbial diversity is significant to the search for life on Mars independent of biomass. For example higher-diversity environments could include more organisms with particularly good preservation potential compared with lower-diversity environments simply because such environments contain a larger cross-section of microbial taxa. For an extant biota, microbial diversity provides critical information about the range of functional capabilities that an environment can support and so diversity can be used as a measure of the habitability of any given environment.

Studies have been undertaken on the diversity of volcanic rocks to investigate the role of elemental composition in their colonisation. For example, statistically significant differences were observed in the microbial diversity between basaltic and more silica-rich obsidian glass in Iceland and between basaltic and more silica-rich rhyolitic rocks in juxtaposed locations where environmental factors were unlikely to influence the results (Kelly et al. 2010, 2011).

It is now well established that gradients in both physical parameters such as temperature (Soo et al. 2009) and chemical parameters such as pH and sulphur content (Benson et al. 2011) cause small-scale variations in microbial diversity in volcanic environments. These spatial variations in microbial diversity are observed in all volcanic environments investigated (Table 1) ranging from geothermal fumaroles (Soo et al. 2009; Benson et al. 2011; Wall et al. 2015) to weathered exposed crystalline and glassy volcanic rocks (Herrera et al. 2009; Kelly et al. 2010, 2011), ash and soils (Gomez-Alvarez et al. 2006; Ibekwe et al. 2007), and mud volcanoes (Huang et al. 2016; Dahigaonkar and Chavan 2018). Variations in the microbial ecology caused by temperature and geochemical spatial gradients were examined in detail in a geothermal setting in Iceland, where differences in microbial phylogeny, function and their associated isotope fractionation values were found in single geothermal outflows (Cousins et al. 2018).
Fig. 2  Diversity varies across materials, but also between samples of the same material. (A) Microbial diversity of basaltic glass and obsidian. (B) Each sample is a ‘microbial island.’ DGGE (Denaturing Gradient Gel Electrophoresis) shows that each rock hosts a distinct community. Although particular volcanic materials host distinct types of community, variations occur within those communities at small scales. Thus, multiple samples are required to investigate microbial diversity. In image B, cluster analysis of glass microbial communities was based on DGGE (30–70% denaturant) community fingerprints. The Jaccard UPGMA dendrogram was generated from the presence-absence matrix in GelComparII. Values at nodes represent cophenetic correlations. Pal represents basalt glass samples from Valafell, Obs represents rhyolitic glass/obsidian samples from Dómadalshraun. Samples ending in C are composites for the given site (data from Kelly et al. 2010, 2011). Note that obsidian and basalt glass samples tend to cluster into two separate groups, but within each group, the microbial community of each individual sample is distinctive.

These published findings suggest that certain factors reliably influence microbial diversity, but that combinations of local environmental factors, such as specific mineralogy and age, uniquely determine the microbial community in any given environment making the a priori prediction of which organisms will colonise a given type of environment difficult to achieve. These factors are compounded by biogeographical stochasticity caused by the variations in local microbial populations and their dispersal (Whitaker et al. 2003; Fierer and Jackson 2006).

With respect to sample collection, a notable observation is that in all these studies individual samples of the same material exhibit variations in microbial diversity (Fig. 2). In that sense, we can regard each rock or sample as a microbial island. As sample size becomes smaller, especially in outcrops where rock heterogeneities are on a smaller scale compared to sample size, one might expect that diversity differences could increase as smaller patches of colonies are targeted. At large scales, biogeographical factors (such as environmental differences) will come into play. Thus, within any given sample size, physical, chemical and biological factors are likely to make it extremely unlikely that two samples will exhibit exactly the same microbial diversity.
As with biomass, much of the diversity of surface volcanic environments on Earth is either directly influenced by the oxygenated environment on Earth (e.g., containing aerobic microorganisms) or indirectly (e.g., the presence of electron donors such as organic matter or electron acceptors such as sulfate or oxidised iron). Some of these factors are irrelevant for Mars (such as widespread oxic conditions), while others are relevant; for example, although Mars is generally anoxic, it does host very abundant sulfate and iron oxides (Gendrin et al. 2005; McSween et al. 2009; Grindrod et al. 2012). Extrapolating a theoretical pattern of colonisation of Martian volcanic materials is not possible, since Martian volcanic materials are in some sense uniquely Martian. However, the general lessons we suggest about these environments on the Earth (such as heterogeneous microbial biomass and diversity) would be applicable since physical, chemical and biological factors will influence microbial colonisation of rocks at sub-millimetre to kilometre scales, even if the specific concatenation of those factors was uniquely Martian.

From the point of view of sample collection, these observations emphasise the need for multiple samples to gain statistically meaningful insights into microbial diversity and its variation. These conclusions also apply to the search for relict biomarkers. Indeed, the variation in microbial diversity even at single sample level suggests the conjectural possibility of a rare organism producing a preservable biomarker that was present in one sample, but not another. In Hawai’ian basalts, lipid biomarkers have been shown to vary between samples, illustrating how heterogeneity in microbial diversity also translates into heterogeneity in preservable biomarkers (Brady et al. 2017). Different molecular biomarkers have also been shown to vary in distribution even within the same samples over centimetre scales (Gentry et al. 2017).

6 More Hydrological Activity Does Not Imply Greater Microbial Biomass or Diversity—Select Samples in a Non-discriminatory Way

An implicit assumption in Mars sample selection is the notion that where there is more water or more sustained water, there is likely to be more life. This paradigm has been encouraged by the maxim, ‘follow the water’ (Hubbard et al. 2002). Although the notion that longer-lived hydrothermal systems or lakes are more likely to sustain long-lived habitable conditions and therefore higher biomass and more diverse microbial communities makes intuitive sense, countervailing results can be observed in many volcanic environments (Fig. 3).

The results of the NASA BASALT study in Hawai’i showed several significant results with respect to Mars sample collection. The hypothesis that active fumaroles (locations where meteoric or magmatic water circulates through active geothermal regions) would be sites of enhanced microbial diversity (since high water availability and the presence of diverse elements in solution would be conducive to life) was not supported (Cockell et al. 2019). Of all sample types, active fumaroles had the lowest diversity. One hypothesis to explain the relatively low diversity of bacterial communities within active fumaroles is that their high temperatures might prohibit colonisation by certain taxa. High temperatures in geothermal settings have previously been found to restrict microbial diversity compared to more mesophilic environments. Sharp et al. (2014) found that in geothermal environments, peak diversity occurred at 24 °C. Thus, merely picking sites that appeared to have hosted active hydrothermal systems may not a priori guarantee samples with the highest biological potential. Similarly low diversities are observed in other volcanic Mars-analogue sites where physical and chemical extremes, for example in volcanic lakes, limit life (Hynek et al.
Both the abundance and diversity of microorganisms do not necessarily correlate directly with the hydrological cycle. Investigation of different volcanic materials in Hawai‘i by the NASA BASALT project. The materials are: Active fumaroles (material exposed to active venting within a fumarole), active intermediate (material from the periphery of one of the active fumaroles), intermediate fumarole (material associated with fumaroles showing low levels of activity and/or transient activity, with temperatures between 30–70 °C), relict fumarole (material associated with an inactive fumarole), unaltered (material associated with unweathered basalt), syn-emplacement (material associated with basalt altered during its emplacement primarily by magmatically associated water and gas or meteoric water associated with the heated environment around the lava). The data in (A) show that microbial abundance is highly variable and not correlated with material type and its exposure to water. Image B shows microbial diversity in the different materials at phylum level. Image C shows the microbial diversity of the materials as three different measures of diversity (Observed, Chao 1 and Shannon diversity). Note that relict fumaroles have higher overall bacterial diversity than active fumaroles, but this trend was not obvious for archaeal communities, with one phylum, Crenarchaeota, only occurring in the active fumaroles (D). Data from Cockell et al. (2019). The NASA BASALT study found that as fumaroles transition to a relict state, average alpha diversity increases. This could be explained by the development of less extreme temperature conditions, even though the potential flux of bioavailable nutrients in fumarolic water is reduced.

Despite these findings, fumarolic environments can harbour a diversity of extremophiles in extreme deserts where water flow is enhanced compared to persistently dry environments that surround them (Costello et al. 2009; Soo et al. 2009; Wall et al. 2015) and although extreme physical conditions can limit diversity, fumaroles are nevertheless high priority sites for sample collection and for biological material that may yield insights into deep-branching thermophilic lineages.
The complexity of sample selection is exacerbated by the fact that the most diverse biota may not always be the most likely to produce long-lasting biosignatures. For example, although active fumaroles studied by NASA BASALT had the lowest overall bacterial diversity, they contained the most diverse populations of archaea (Fig. 3; Cockell et al. 2019). Ether-bound intact polar lipids found in archaea are believed to be more stable than ester-bound bacterial lipids and thus expected to survive longer over geological timescales (e.g. Harvey et al. 1986; Logemann et al. 2011). A preferential survival of archaeal lipids could theoretically mean that the active fumaroles would leave a more detectable signature of past life than other locations. The possibility for a lack of correlation between microbial biomass, diversity and preservation potential emphasises the need for multiple samples from diverse hydrological regimens.

From the point of view of Mars sample collection, however, the lesson is clear. Apparently intuitive ideas may not be borne out by microbial ecology and therefore sample collection on Mars should be informed by terrestrial ecology.

Predicting how biomass and diversity would be influenced by a series of diverse hydrological regimes in any given local environment is problematic. Although the paradigm of collecting from more hydrologically active environments is merited, the results from the NASA BASALT project show that ideally a large number of samples from different hydrological regimens should be collected to maximise the chance of finding rock materials that could have potentially hosted the highest biomass and diversity.

These observations on microbial diversity in volcanic environments on Earth suggest that rather than ‘follow the water’ the overall strategy should be the more cumbrous, but more accurate: ‘follow the water to large-scale environments that have been exposed to long-lived hydrological cycles, but collect across all hydrological gradients within that area’.

7 Short-Lived or Recently Formed Habitats May Contain Low Biomass and Diversity

There are very few studies that examine the microbial communities associated with newly formed volcanic materials that can be considered analogous to young materials on Mars or volcanic materials that experienced transient interactions with liquid water. In general, microbial biomass and diversity increases over time in the first years after colonisation of a lava flow, but most studies have focused on lava flows many tens or even hundreds of years old. Over these time periods, it is known that volcanic terrains on Earth make felicitous places for microbial colonisation. It has been known for a long time, particularly from studies before the use of molecular biology, that volcanic materials are colonised early by oxygenic photosynthesisers such as algae and cyanobacteria (Kristinsson 1970, 1974; Schwabe 1970; Schwabe and Behre 1972; Brock 1973; Englund 1976; Henriksson and Henriksson 1982; Adamo and Violante 1991; Adamo et al. 1993; Carson and Brown 1978; Fermani et al. 2007; Stefansdottir et al. 2014). Other studies have also shown lithotrophs as early components of volcanic communities, such as organisms that oxidise volcanically-derived CO and H₂ (King 2003; Dunfield and King 2005; Nanba et al. 2004). Dunfield and King (2005) showed that the diversity of CO-oxidisers (based on amplification of coxL genes) was low in a recent (42 years old) lava flow in Hawai‘i, but lava between 108 and 300 years old had similar diversity. In volcanic environments where CO and sulphur gases (e.g., H₂S, SO₂) are actively emitted, chemoaotrophic primary production in newly formed volcanic rocks may ultimately generate organic carbon sufficient to sustain heterotrophs.
Fig. 4 Newly formed materials and those exposed to transient habitability contain highly heterogeneous, low-diversity populations. The newly formed lava flows of the Fimmvörðuháls lava flow, Eyjafjallajökull, Iceland in 2010 were colonised within 3 to 5 months by a low diversity biota. (A) Location of lava flows in Iceland (arrow), (B) Map of lava flows showing sample sites 1, 5 and 9 from which diversity analysis was undertaken, (C) Microbial abundance measured as direct cell counts at sample sites (error bars are standard deviation) taken on July 5 and August 31, 2010 showing heterogeneity. (D) Microbial diversity at phylum level shows communities dominated by Proteobacteria (specifically Betaproteobacteria). ‘J’ samples are samples collected in July; ‘A’ samples are samples collected in August. ‘Weathered’ samples are samples from the basaltic Hnausahraun lava flow, erupted circa 150–300 A.D. and is given for comparison. ‘Others’ phylum refers to other phyla, each represented by a maximum of two clones in any Fimmvörðuháls library (data from Kelly et al. 2014)

Where studies on the early (less than a year old) colonisation of volcanic terrains have been undertaken, they have shown the extremely low diversity that is often sustained by new volcanic materials, even on Earth, where atmospheric inoculation occurs and microbially-accessible organic carbon is available. A study of the early colonisation of Icelandic lava flows showed that within three months after the eruption of Eyjafjallajökull in 2010, colonisation had occurred (Fig. 4) (Kelly et al. 2014). Biomass in some sites was low, but at others exceeded $10^6$ cells/g dry weight (Fig. 4) in samples of $\sim50$ cm$^3$. Nevertheless, the diversity was low and was dominated by Betaproteobacteria, in contrast to older lava flows that typically contain an order of magnitude more biomass and have higher abundance of Acidobacteria and Actinobacteria (Kelly et al. 2010, 2011; Byloos et al. 2018). Although some of the organisms on the fresh lava may have used organic material atmospherically delivered to the surface of the lava flows, the presence of chemoautotrophic members of the Thiobacillus genus suggested that some of the population may have used iron and sulphur from the primary volcanic materials (Kelly et al. 2014).

It is a significant point that arguments about the evolutionary likelihood of particular metabolisms on Mars (i.e. whether it is relevant to discuss searching for biomarkers of the relatively biochemically and evolutionarily advanced oxygenic photosynthesis compared to chemolithotrophic metabolisms) are irrelevant to the points made in this paper. All metabolic
and taxonomic groups of microorganisms examined on early lava flows on Earth have shown a heterogeneous distribution with a low biomass and diversity. These observations emphasise the points in Sects. 4–6. If volcanic materials have not been exposed to long-lived hydrological regimens and mature microbial communities have not been established, then the heterogeneous distribution of biomass and diversity may be exacerbated by overall low biomass, emphasising the need for multiple samples and suitable sample size.

8 Is It Possible for Samples to Be Habitable, but Devoid of Life?

Is there a possibility of collecting samples on Mars that fully satisfy the conditions for habitability but are devoid of a biota? If Mars never hosted an origin of life or a transfer of life to that planet then all environments that have energy sources, CHNOPS elements and liquid water and are habitable by terrestrial standards would be devoid of life. However, even if Mars has hosted life, transient habitats such as near-surface water melted by impacts could be devoid of life if that habitat remained disconnected from inhabited regions (Cockell 2011). If sample collection is undertaken within a confined area (for example, a single impact crater or outflow channel), the possibility of sampling a once habitable, but never inhabited palaeoenvironment is conceivable. As we cannot quantify this likelihood in the absence of a knowledge of life on Mars, this theoretical possibility would suggest that collecting samples from different environments, not merely within the same local environment but across much wider areas, should be a priority to maximise the chances of collecting samples containing life (Cockell and McMahon 2019). It is important to note that if Mars was lifeless, this strategy will also strengthen the conclusion that the planet was lifeless by multiplying the number of sampled habitable environments that can be shown to be devoid of life.

An important distinction is whether uncolonised regions in a sample are devoid of life because they lack some fundamental requirement for life making them uninhabitable, or whether they are habitable zones that can support life, but they have not yet been colonised. For example, uncolonised regions of obsidian studied by Herrera et al. (2008) are likely uncolonised because of the lack of freely available iron. In other words, they may be uninhabitable.

On Earth, habitable samples devoid of life at the centimetre scale do seem to be rare. As organisms reproduce, fluid flow tends to carry them into new habitable spaces so that at micron-scales, some part of that habitable space is colonised. Although not all habitable space will be colonised, the movement of microbes does lead to the ecological reality that when habitable samples are examined at centimetre scales, they usually contain life.

Real examples on Earth of pervasive centimetre-scale habitable samples devoid of life are recently formed lava flows in which hours to days after cooling to below the upper temperature limit for life, microbes are so heterogeneous and sparse that at centimetre scales some rocks remain uncolonised, particularly those beneath the surface of the flow that have not been subject to atmospheric colonisation (Cockell 2014).

These ecological considerations lead to relevant observations. In general, where life has accessed an environment, fluid flow ensures that even though space can be habitable but unoccupied, at macroscopic centimetre scales samples generally contain examples of life. In environments where habitable conditions were extremely transient, this assumption may break down and habitable samples devoid of life at centimetre scales may be prevalent. In the specific case of Mars, if the planet was lifeless or the biomass extremely localised to
certain regions, entire regions or environments may be habitable but devoid of life. Large samples suites are required to test these hypotheses.

An important question is how many samples are minimally required to determine that a given material is devoid of life in all examples of that material. On the Earth, a high level of biological confidence might be obtained by showing that life was not present in three sets of three triplicates of such a material across one location with this sample set repeated across three geographically separated regions of that material, whether on the surface or subsurface.

It may never be possible to demonstrate conclusively that Mars has always been lifeless, since short-lived, localised pockets of life could have left scant or undetectable evidence, or no evidence at all. However, if the sampling regimen described in the previous paragraph was deployed across the geological records of diverse palaeohabitats with good potential to preserve biosignatures (e.g., lake sediments, evaporitic deposits, and hydrothermal sinter deposits) and no evidence of life was found, the balance of evidence would shift in favour of the hypothesis that Mars has never been inhabited. In principle, a Bayesian approach could be developed to model this shift; these statistical issues merit greater attention.

9 A Strategy for Sample Collection on Mars

In this section, we summarise six recommendations for sample collection on Mars based on observations of the microbial ecology of volcanic materials on Earth. We provide a brief rationale for each. The strategy does not take into account cost, mass, or space restrictions associated with specific missions such as robotic Mars sample return missions (Sherwood et al. 2003; MEPAG 2008; iMOST 2018), but rather provides an insight into a biologically ideal sample suite, whether it be collected by robot or human explorers.

9.1 Collect Sufficiently Large Samples

Heterogeneity in microbial biomass and diversity has been observed in all volcanic materials. These effects are likely to be caused by physical, chemical and biological influences that will vary in different samples. ‘Sufficient’ size cannot be easily quantified, but observations on volcanic rocks discussed here suggest that typical sample sizes should be a minimum 10 cm³ to retain millimetre-scale vesicles, fractures and mineral grains and their associated biological targets. Defining ideal samples based on mass is less important than volume. For example, a lower density sample can contain more pore space for potential microbial colonisation than a higher density sample, counterintuitively making a lighter sample of comparable volume potentially more valuable to a biologist than a more massive sample. Less is sometimes more.

9.2 Collect Sufficiently Many Samples

Heterogeneity in samples, particularly in those where habitability is short-lived, results in high standard deviations in biomass and diversity. Statistically, to derive meaningful statements on the distribution of life, multiple samples should be collected. Three samples is a typical statistical minimum. However, microbial ecological analysis yields suggestions on preferred numbers. For example, based on sample strategies used to investigate heterogeneities in low biomass volcanic rocks (Kelly et al. 2014) sample collection numbers such as nine provide for a more comprehensive analysis, made up of three independent
sets of three triplicates from the same material at regional scale. Additional samples add to the robustness of the study, but might be limited by field time and cost. If environments are devoid of life, large sample sizes can increase the robustness of the conclusion that they were never inhabited. These sample number recommendations do not take into account separate samples used in other studies such as geology, atmospheric history and investigations for future resource use that might be collected in the limited sample sets envisaged for robotic Mars sample return (e.g., Sherwood et al. 2003; MEPAG 2008; iMOST 2018).

### 9.3 Collect Samples of the Same Material from Different Locations

Following directly from point (2) is the recognition that the heterogeneity in the biomass and diversity of life is related to large-scale biogeographical influences. It is therefore important to collect samples of the same material, but from different locations separated by ~kilometre distances. This allows for stronger statistical statements about the distribution of life over large scales. If true replication is to be achieved, avoiding the pitfall of pseudo-replication by sampling the same region, then the sample suite discussed in point (2) must be repeated in three completely geographically separated regions of the same material, yielding 27 samples in total of a given sample type. If a habitable environment was transient, this strategy maximises the chances of obtaining life-bearing rocks or being able to conclude with greater confidence that all such materials in all geographical locations are devoid of life.

### 9.4 Collect Across Varied Hydrological Regimes

‘Follow the water’ is broadly a correct view with respect to the fundamental requirement that all known life has used liquid water as a solvent. However, microbial ecology shows that locations with a high abundance of liquid water do not always harbour the most biomass and diversity. As we have discussed here, in volcanic hydrothermal regimens, high temperatures can limit life to lower biomass and diversity than drier locations at more mesophilic temperatures. It is well known that in some locations liquid water can even be uninhabitable. For example, on the Earth, magnesium chloride brines can have water activities so low as to be uninhabitable (Hallsworth et al. 2007) and such brines may exist on Mars. The lessons from volcanic environments show that sampling should be carried out across hydrological gradients and not merely in locations that appear to have hosted the most vigorous hydrological regimes. Transects capture the variation in the interaction between geochemistry and physical conditions that can influence habitability in a way independent of the quantity of liquid water.

### 9.5 Maintain Sample Integrity

Cells are distributed heterogeneously and their locations provide information on the factors that control life (e.g. presence of iron-bearing crystals, alteration rinds, fracture networks). It is therefore essential to maintain sample integrity. Where possible they should be collected with minimal damage caused by drilling, excavation etc. Sample integrity also includes the need to collect aseptically to avoid external biological contamination.

### 9.6 Minimise Post-Collection Modifications

Once collected, samples should receive minimal alteration. This includes irradiation and exposure to other physical and chemical agents. Where samples are disrupted for examination,
information on the orientation of the sample fragments and their locations should be maintained so that the geological factors that control microbial distribution within the material can be reconstructed.

10 Conclusions

Mars is a planet of volcanically derived materials. The search for life on Mars and the study of the distribution of habitable conditions is therefore greatly advanced by investigating the microbial ecology of similar environments on Earth where biomass and diversity can be directly quantified and related to environmental variables with a statistically high level of confidence. Keeping in mind environmental differences between present-day Earth and Mars, one purpose of this paper has been to provide a review of well-studied volcanic materials and their enclosed biota. We have used volcanic environments on Earth to extract general patterns of microbial growth that are applicable across many types of volcanic material. Using these patterns, we suggest principles of sample collection that, combined with appropriate taphonomic considerations, will yield maximum information for astrobiologists and planetary scientists. These principles apply to robotic exploration and to the human exploration of the planet. Ultimately, to be able to test hypotheses with high statistical confidence, sample sets with sufficient coverage and diversity, exploration across large scales, and multiple replicates are required.

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