TOWARDS REVEALING THE HABITABILITY, PRODUCTIVITY, AND MICROBIAL DIVERSITY OF ICELANDIC LAVA FIELDS: AN INTERDISCIPLINARY APPROACH

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Report Contributors
Elena S. Amador
Morgan L. Cable
Edward Schwieterman

Table of Contents

About Us

Our team was an international collaboration consisting mostly of graduate students and early career post-docs stemming from a variety of scientific backgrounds. This project began as a series of field exercises at the NASA-Nordic Summer School "Water, Ice, and the Origin of Life" in 2012. During the summer school, students formulated scientific questions and designed sampling strategies for ATP bioluminescence assay measurements of basaltic rocks, weathered rhyolite and glacial ice. Elena Amador is a graduate student at the University of Washington earning a Ph.D. in Earth and Space Sciences and Astrobiology. Edward Schwieterman is also a graduate student at the University of Washington working towards a Ph.D. in Astronomy and Astrobiology. Morgan Cable recently finished a NASA Postdoctoral Fellowship and is currently a Research Scientist at the Jet Propulsion Laboratory.

Other contributors to this study not supported by the Lewis & Clark Exploration Fund include: Nosheen Chaundry (Cranfield University), David Cullen (Cranfield University), Thomas Cullen, Diana Gentry (Stanford University), Wolf Geppert (Stockholm University), Malene Jacobsen (University of Copenhagen), Gayathri Murukesan (University of Turku), Adam Stevens (The Open University), Amanda Stockton (JPL/Caltech), and Chang Yin (Stockholm University).

1. Introduction: Iceland as a Terrestrial Analog for Mars Habitability Experiments

The goal of our expedition was to evaluate in-situ life detection and characterization methods in Iceland as a Mars mission analog and consisted of two major components. The first component was to evaluate our field lab techniques and sampling protocols as a simulated planetary exploration mission. Our field lab operated simultaneously with our sampling campaign, which afforded us the opportunity to quickly revise our sampling strategies to obtain additional information and correct any deficiencies in the original scheme. The second, scientifically driven component, was to quantify the differences in gross microbial productivity and diversity over varying spatial scales at our sites. The microbial productivity was quantified with an adenosine triphosphate (ATP) bioluminescence assay and validated with fluorescence microscopy of identical subsamples, while domain-level diversity was explored with qPCR.

Out of several possibilities, and after an initial survey, we chose two primary sampling locations: the Eldfell volcanic lava field on the island of Heimaey (1973) and the recent Fimmvörðuháls lava field (2010). Icelandic lava fields are of astrobiological interest because they contain biologically extreme conditions such as desiccation, low nutrient availability, and high temperatures (e.g. around fumaroles). While these environments can be unforgiving, microbial communities nonetheless establish themselves and proliferate [1]. Icelandic lava fields in particular have been considered viable Mars

analog sites [2,3]. Additionally, by examining one of the youngest accessible lava sites, Fimmvörðuháls, we can observe the initial process of colonization by microorganisms in detail. Furthermore, given the remoteness of Iceland's lava fields, sites tend to be isolated from high levels of anthropogenic contamination and manipulation.

There are several challenges associated with robotic in-situ sampling. Consumables such as reagents limit the number of samples that can be taken. Processing constraints required by extraction techniques are dependent on a prior mission design and cannot be altered in the field. Many interesting sites may be inaccessible to robotic explorers. Assuming that life is even present in the general vicinity of a lander mission, its spatial and environmental variation may rise and drop below detection thresholds. With limited samples and a fixed protocol, it is then crucial to choose a representative set of sampling locations to maximize the possibility of success. One important question that can be explored at analog sites on Earth is the relationship between visible (i.e., color, morphology, geology) homogeneity of an area and the biological productivity and diversity that will modulate the strength of a life detection signal. It is important to quantify this factor before we explore the effects of differing environmental parameters.

Our initial sample sites were chosen such that a remote observer, or surface explorer, would interpret them as visually homogeneous with respect to color, grain size, roughness, and temperature. This kind of study is crucial to set a baseline control for campaigns that will sample over other environmental gradients, such as in temperature, pH, oxidation state, etc.

In the following report we will: (1) detail the selection of our field sites in Iceland, (2) describe our sampling methods and analysis techniques, (3) present our preliminary results and interpretations, and (4) discuss our tentative conclusions.

1.1 Field Site Selection

Three field sites, Fimmvörðuháls (63^{o} 38' N, 19^{o} 26' W), Eldfell (63^{o} 25' N, 20^{o} 14' W), and Laugahraun (63^{o} 59' N, 19^{o} 05' W), were initially evaluated upon arrival to Iceland during the week of July 22, 2013 (see Figure 1 and Table 1). The sites were judged for their relevance to our scientific and exploration goals, as well as potential for anthropogenic contamination and future repeatability.

Fimmvörðuháls and Eldfell were ultimately selected as our primary sampling sites for the 2013 expedition given that (a) their eruption dates are relatively similar – 2010 and 1973, respectively; (b) they had limited vegetation; and (c) they had very similar sediment types: basaltic tephra. Given the similar geology, these two field sites would also allow for a temporal parameter in our analyses. The Laugahraun lava field, though geologically interesting, proved to be far too vegetated to collect homogeneous samples and was crippled by the fact that it has high tourist numbers coming through and certainly contaminating potential sampling sites.

2. Field Sampling Methods and Laboratory Protocols

Sampling sites within our field areas were selected based on their appeared homogeneity with evenly distributed basaltic tephra. A triplicate sample set was collected at each site, with each sample spaced 1 m apart. Subsequent sample sets were collected at distances of 10 m and 100 m (see Figures 2 and 3). Each sample was taken from approximately 5 cm below the surface using a rock hammer wiped with ethanol before each collection. Each sample collection was placed into a sterile 50 mL centrifuge tube to be returned to the field lab. During sample collection, team members wore gloves cleaned with ethanol and face-masks, and approached the pristine sampling site from a downwind direction to minimize contamination. Caution was taken to avoid stepping on any potential sampling site.

Samples were collected from Fimmvörðuháls on 27 and 29 July 2013, and from Eldfell on 28 July 2013 (see Figures 4 and 5). Soil samples were stored at room temperature prior to analysis. Soil samples with large grain sizes (>2 mm) were crushed using a sterilized hammer or table vise grip. All samples were analyzed within 4 days of collection.

2.1 Fluorescence microscopy

Sample extraction and preparation: A 1 mL portion of crushed sample was loaded in a microcentrifuge tube with the addition of 0.75 mL of PBS/Tween buffer and mixed via inversion and vortexing. The mixture was sonicated for 5 minutes and centrifuged for 5 minutes at 600 g. The supernatant was removed into a separate sterile tube. This process was repeated with an additional aliquot of PBS/Tween buffer; both aliquots of supernatant were combined. The supernatant (extract) was loaded into a 2 mL syringe, filtered and flushed twice with sterile water and twice with air. The filter was then removed and placed on a slide for staining. The sample filter was stained with 100 μL of SYBR Gold Nucleic Acid Gel Stain (Invitrogen) spread evenly over the center of the filter. The cover slip was applied and the sample was incubated for at least 15 minutes before imaging.

Imaging/Cell Counts: Cell counting was performed using a Partec Cycscope with 455 nm (RB) "royal blue" emission light source and 500 nm (DM) "yellow/green" dichroic mirror long pass filter. The 100X (oil) objective was used in conjunction with the imager to record digital micrographs of sample fields. Each slide (corresponding to an extracted and filtered subsample) was scanned in five randomly chosen locations. Cell count determinations were made based on the recorded images.

2.2 Quantitative PCR protocol

DNA extraction and purification was performed on 0.25 g of soil from each sample site using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.). Soil gathered outside of the field laboratory in Hvolsvöllur was used as a positive control; the negative

control was sterile deionized water. PCR amplification was performed in duplicate on a 1:10 dilution of purified DNA extract. Primers were selected to cover as wide of a range as possible, and included bacteria, fungi and Archaea (see Table 2). As this work was performed in a field lab with limited reagents, primer selection was also limited by extension temperature.

2.3 ATP bioluminescence assay

ATP was quantified using the ATP Bioluminescence Assay Kit HS II (Roche Diagnostics, Ltd.) and reader (Merck HyLiTE II) in triplicate for two aliquots of each soil sample. Briefly, 0.5 cm^3 of soil sample was extracted using 1 mL of TE extraction buffer (100 mM Tris, 4 mM EDTA, pH 7.4) with vortexing (3 s). The sample was heated to 100 °C for 5 min in a water bath, followed by cooling at room temperature for 3-5 min. Samples were then vortexed (3 s) and centrifuged (7200 g, 5 min). A 50 µL aliquot of supernatant was added to 50 µL of ATP bioluminescence reagent; this was vortexed (3 s) and immediately measured with the ATP bioluminescence reader. A standard curve was performed daily to calibrate measured ATP levels. This standard curve was generated using aliquots of ATP supplied with the assay kit over a concentration range of 0.316 pM to 1.00 nM.

3. Results and Preliminary Interpretations

Samples were successfully collected and analyzed from two field locations, Fimmvörðuháls and Eldfell. All samples collected appeared to be homogeneous based on our initially determined parameters (color, grain size, and roughness). Something that became apparent over the course of this field campaign was the concept of "field of view". Depending on the distance from the observer, sample sites could appear more or less homogeneous, from a geologic standpoint. For example, when choosing our sampling sites for Fimmvörðuháls, we first observed the field area from atop the scoria cone Magni and our chosen sampling sites appeared completely homogeneous. Once we approached these sites on foot, there were small variations in the moisture of the tephra (most likely to due to melting of snow cover) and oxidation states as determined by grain colors.

Another important point to note is that sampling at the Eldfell lava field on July 28 was disturbed by a sudden downpour of rain as is typical in the Westman Island (and Iceland in general) during this time of year. This may have ultimately caused unexpected contamination of the samples. Tephra samples collected at Fimmvörðuháls may have initially come as pyroclastic material from the Eyjafjallajökull eruption rather than the associated Fimmvörðuháls flows. This is less of a concern given that they are both sourced from the same magma chamber and erupted within days of one another.

All samples were analyzed using fluorescence microscopy. Figure 6 provides an example of positive and negative controls and a typical sample micrograph. Auto-fluorescing sources tended to be red while cells appeared yellow/green. Cell count variation did not exhibit any observable trends between sample sites on any scale (see Figure 7). This may be due to the fact that cell counts were low for most samples, or that this technique is more subject to the influence of human error. Quantitation of cells using fluorescence microscopy also proved difficult in the field overall. Filters were challenging to use and transfer. Prior to any subsequent expeditions, the fluorescence microscopy protocol should be refined to correct for these challenges.

DNA was successfully extracted from all samples, and the quantitative PCR field protocol was found to be effective for all primers used (see Table 2). Data analysis is ongoing, so only results from the Fimmvörðuháls site will be discussed here. Bacteria, fungi and Archaea were detected in all samples; levels of bacteria were typically greater than or equal to those of Archaea (normalized to the positive control). Minimal fungi populations were present in all samples with the exception of one: the 'red' location FIM-1C-1 (believed to be red due to oxidized iron). This particular location also had the highest levels of bacteria of any site, indicating significant biodiversity. Other sites with high biodiversity included FIM-1A-1, FIM-1B-2 and FIM-3-1. Though typically sites on the 1-meter scale had comparable biodiversity levels, this was not always the case. Aside from the 'red' site (FIM-1C-1), there was no discernable correlation between physical characteristics and microorganism biodiversity. Two adjacent sites, separated by only 1 m and visually homogeneous (with respect to color, grain size, roughness, and temperature), could have markedly different biodiversity levels.

ATP was detected in all samples analyzed, indicating actively metabolizing microorganisms in every sample site of both locations. Interestingly, the degree of variation of this parameter differed between the two sites. At the Eldfell location, the amount of variation of ATP content increased with distance (see Figure 8). For example, samples from two sites 100 m apart had a much larger variation in ATP content than two sites only 10 m apart. The Fimmvörðuháls sites, however, exhibited very little change in variation over distance. The difference in ATP content between two samples was close to the same, whether they were 1 m, 10 m or 100 m apart from one another. This is consistent with lava field age, in that the older lava field (Eldfell, 1973) has had more time to establish a complex microbial community, whereas the younger lava field (Fimmvörðuháls, 2010) most likely is still in the initial stages of inhabiting this new environment. The preliminary qPCR results also support this hypothesis, in that the deviation in bacterial, fungal and Archaeal populations do not exhibit an obvious trend with distance for Fimmvörðuháls (see Figure 9).

The proposed age effect can be summarized as follows. In the more established site the sources of heterogeneity may have varied as a function of nutrient access, angle of the sun, microclimate, and other factors. While we did not sample at the Laugahraun site, we observed substantial heterogeneity in the visible vegetation, possibly because certain localities were more favorable for these reasons. This is the limit of a highly evolved site. In the limit of a new site (e.g. the ash has just fallen), everything is sterile and therefore homogeneous. Perhaps for the first wave of organisms in this new site every locality has

approximately equal viability. Factors such as the angle of the sun may not be as relevant, because the organisms are nutrient limited, not photon limited, and the nutrient availability doesn't vary, because the substrate is all the same. If true, this makes the considering the variability between localities that appear similar very important from a planetary exploration standpoint, because any site a life detection mission could conceivably visit will be extremely old.

The age of the sites is only one possibility that could explain the difference in the degree of variation in ATP content as a function of distance. Other possible variables include the relative isolation of the sites from sources of contamination, such as the transport of material by wind from more vegetated or inhabited areas, and the extent of human visitation. The Fimmvörðuháls lava field, for example, is farther from continuously populated locations and vegetated areas than the Eldfell field, which is itself close to the fishing village on Heimaey and the ocean. To accurately control for these parameters, a more systematic study of many sites of varying age would be necessary.

4. Conclusions

The 2013 Iceland field campaign provided an excellent test bed for testing in situ life detection techniques in the context of a simulated planetary exploration mission. The two weeks spent exploring Icelandic terrains, developing sample protocols and laboratory techniques has allowed us to further the study of a) the level of metabolic activity present in volcanic tephra at two sites, and b) the variation of this parameter at different spatial scales at the same site.

Our major conclusions can be subdivided into two parts, one regarding our development of planetary exploration protocols and the other our scientifically driven question(s). Our field protocol proved to be a successful method of determining what sampling protocol a future robotic or human scientist might employ when collecting samples from a field site. The field laboratory was essential, in that we could collect samples, analyze them the next day, and use that information to inform our decisions regarding subsequent sampling. We were continually building on the data we were gathering while testing our analytical techniques in the lab. The ATP and qPCR experiments proved to be reliable experimental methods, while the microscopy experiments were quite technically challenging in the quick-paced field environment. Further development of this technique in a field lab environment will be necessary before use in another expedition.

We also came to some broad conclusions regarding colonization of volcanic lava fields that will inform future fieldwork. Our results are consistent with the interpretation that biological variability in geologically homogeneous sample sites varies with lava field age. Younger lava fields tend to show the same variation in biodiversity and habitability regardless of sampling proximity, whereas older lava fields are increasingly heterogeneous at larger spatial scales. We attribute this to an initial homogeneous colonization of microbes early in the age of a lava field given a homogeneous geology. Over time as specific environmental factors change, biodiversity can increase. These factors may include, but are not limited to, distance to nutrient sources, solar insolation,

and proximity to hydrothermal input. Such factors may cause microenvironments to form where biomass production is more favorable, leading to changes the size and complexity of microbial communities.

This work has implications for field sampling and analysis of volcanic regions such as those on Mars. Given the ages of lava flows on this and other planetary bodies, we anticipate a high variation in biodiversity between geologically homogeneous areas, should life exist. We therefore recommend a robotic sampling strategy with as many spatially separated sampling sites as practical, even in areas that appear to be homogeneous.

5. References

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6. Figure

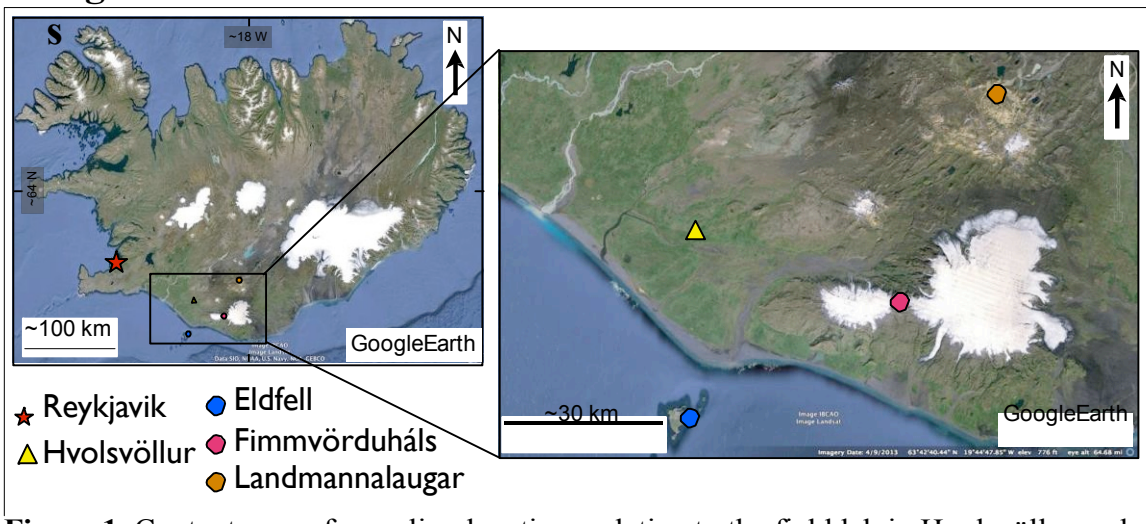

Figure 1. Context map of sampling locations relative to the field lab in Hvolsvöllur and the nearby capital city of Reykjavik.

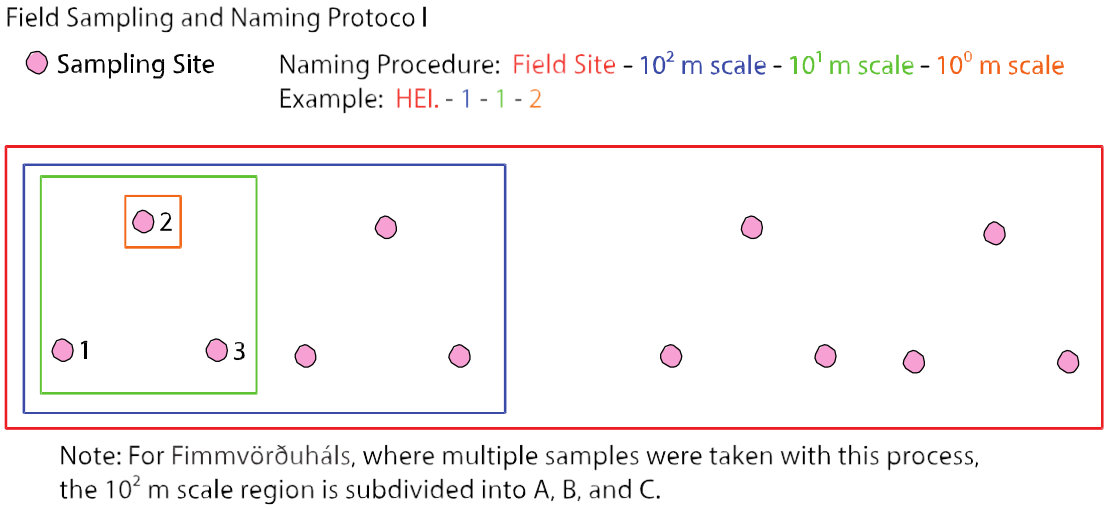

Figure 2. Field sampling strategy. Samples were collected at distances of 1 m, 10 m and 100 m to determine variation of biodiversity over orders of magnitude.

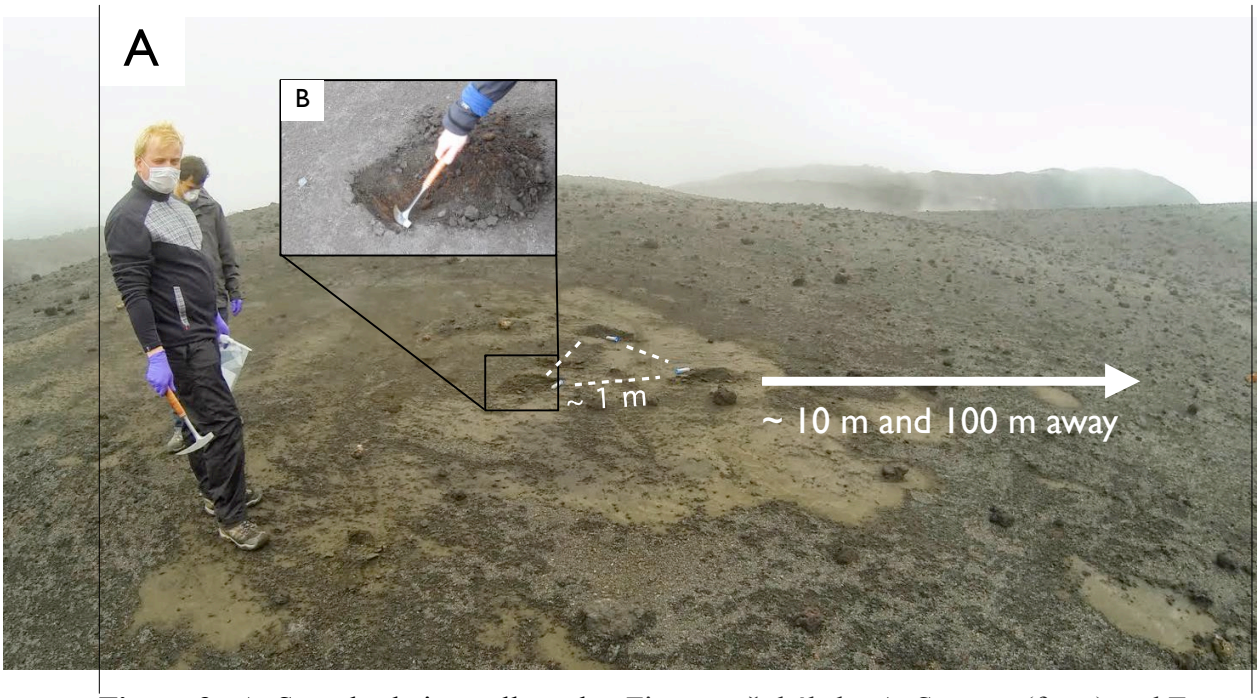

Figure 3. **A.** Samples being collected at Fimmvörðuháls by A. Stevens (front) and E. Schwieterman (back) using field sampling protocol. **B.** Zoomed-in view of rock hammer sampling method.

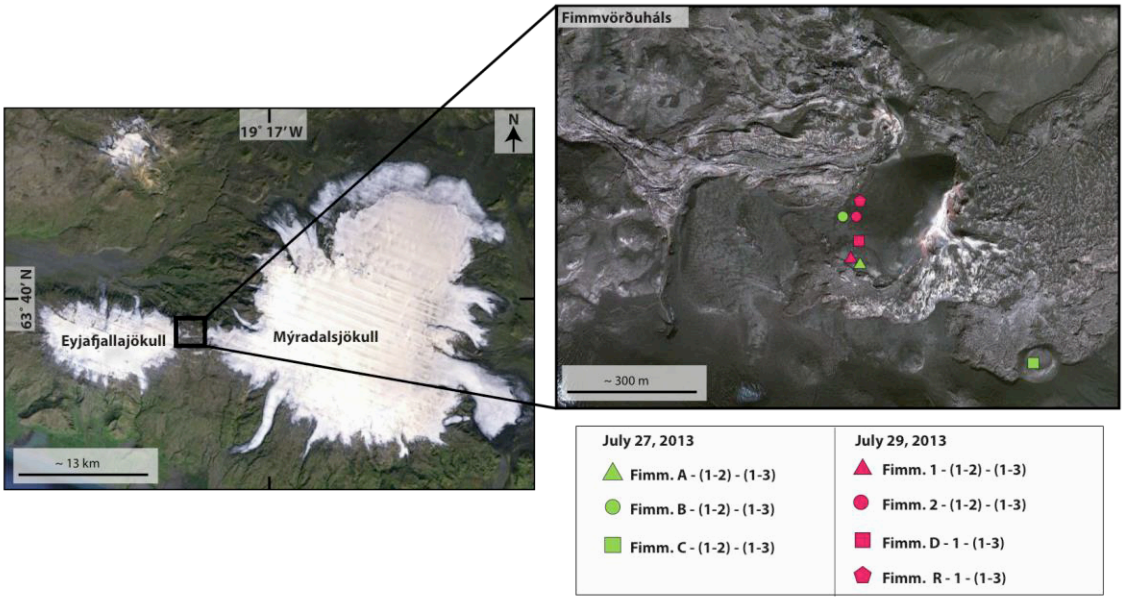

Figure 4. Map of sample sites at Fimmvörðuháls.

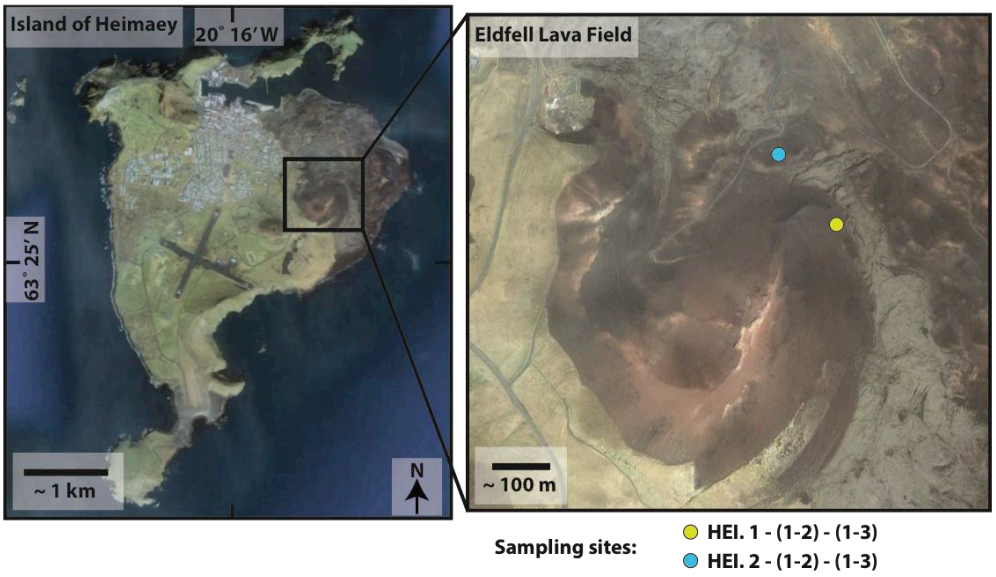

Figure 5. Map of sample sites at Eldfell.

Figure 6. Fluorescence microscopy micrographs of a typical sample and controls. **Left:** Positive control from vegetated soil. Center: Negative control from sterile extraction. **Right:** An extraction from a sample collected at the Fimmvörðuháls location.

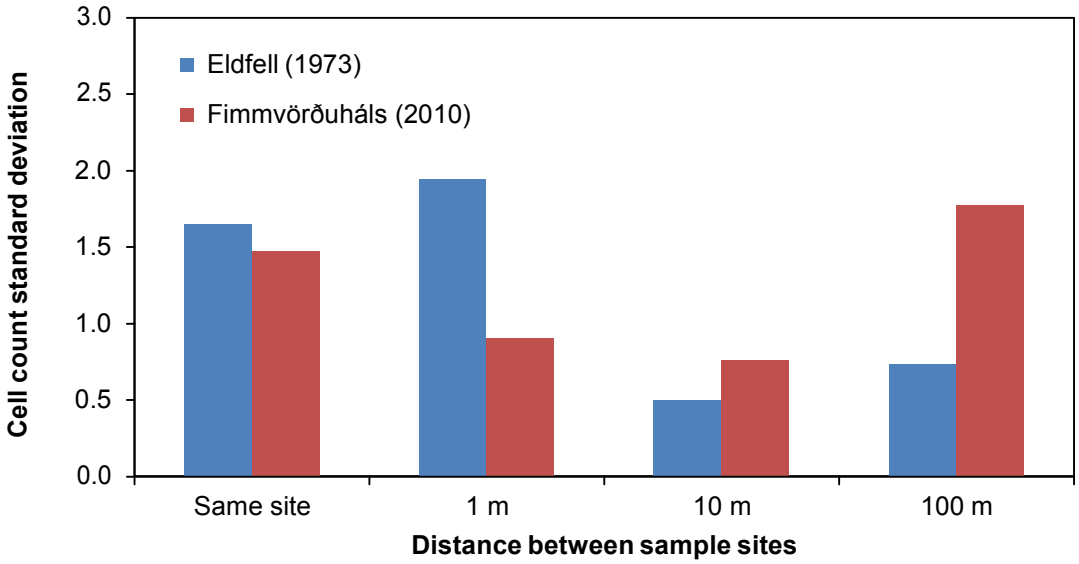

Figure 7. Variation in cell counts between samples collected at various distances. Variation does not appear to be dependent on distance for either location.

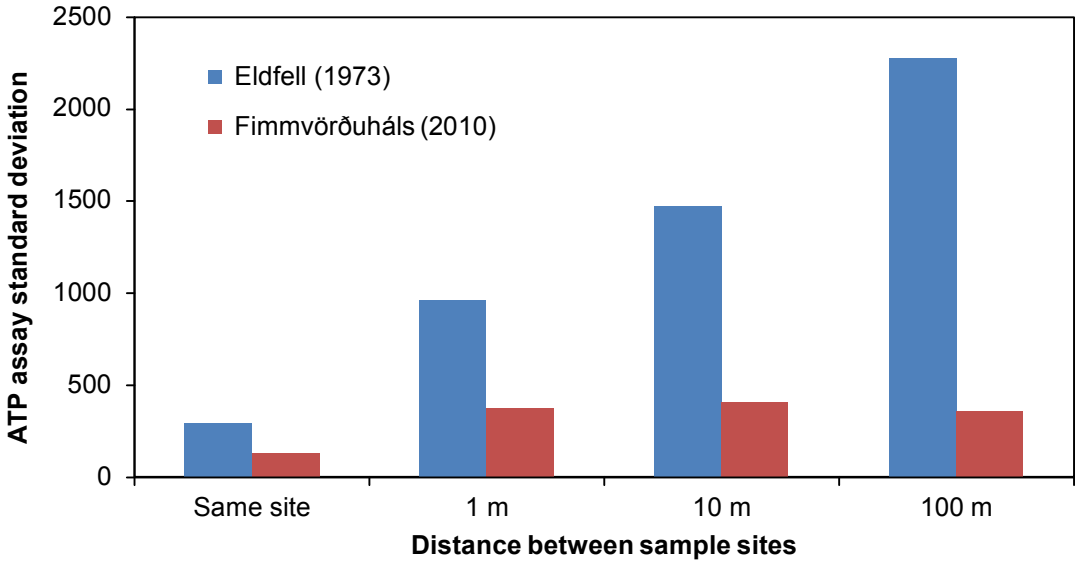

Figure 8. Variation in ATP content between samples collected at various distances. Variation increases with distance for the older location (Eldfell), and is roughly consistent for the younger lava field (Fimmvörðuháls).

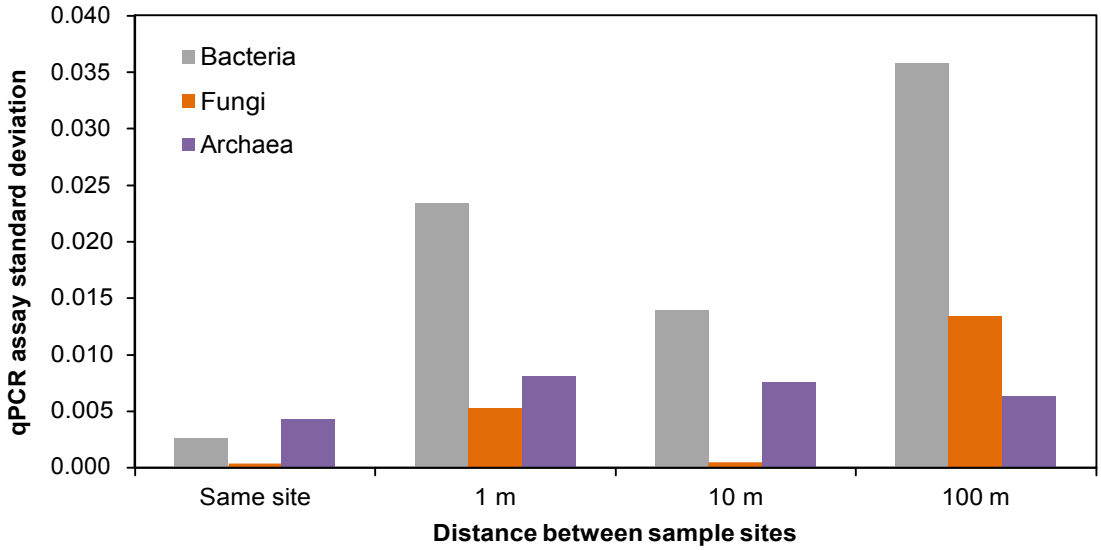

Figure 9. Variation in levels of bacteria, fungi and Archaea between samples of Fimmvörðuháls collected at various distances. There appears to be no clear trend with distance for this young lava field, consistent with ATP content.

7. Tables:

Table 1. Lava Field Descriptions

Field Site	Description
Fimmvörðuháls Lava Field	The Fimmvörðuháls lava field formed between March 20th and April 12th 2010 from a basaltic effusive eruption associated with the infamous Eyjafjallajökull eruption [4, 5].
Eldfell Lava Field	The Eldfell volcano formed in 1973 on the island of Heimaey, it is associated with the Vestmannaeyjar volcanic system [6, 7]. The Eldfell eruption had both effusive and explosive alkali basalt eruptions; samples for this study were collected from a large scoria cone associated with the eruption.
Laugahraun Lava Field at Landmannalaugar	The Laugahraun lava field is the oldest sampling site we visited, having formed in 1477. The field is characterized by very hard basaltic obsidian. Given the age of the field, the rock exposures are predominately covered by vegetation.

Table 2. PCR primers used in DNA amplification of soil extracts

Type	Gene	Forward	Reverse	Extension Temp
Bacteria	16S rRNA	E8F	E533R	54 °C
Fungi	18S rRNA	nu-SSU-0817	nu-SSU-1196	
Archaea	16S rRNA	ARC787F	ARC1059R	
Sulfate-reducing prokaryote	drsA	DSR1F	DSRR	51 °C
Aerobic methane consumption	mxaF	mxa1003F	mxa1561R	
Cyanobacteria	16S rRNA	CYA359F	CYA781R	