Article (refereed) - postprint

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**Mesophilic mineral-weathering bacteria inhabit the critical-zone of a perennially cold basaltic environment**

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**Abstract**

The weathering of silicate in the world's critical-zone (rock-soil interface) is a natural mechanism providing a feedback on atmospheric $CO_2$ concentrations through the carbonate-silicate cycle. We examined culturable bacterial communities from a critical-zone in western Iceland to determine the optimum growth temperature, ability to solubilise phosphate-containing minerals, which are abundant within the critical-zone area examined here. The majority of isolated bacteria were able to solubilize mineral-state phosphate. Almost all bacterial isolates were mesophilic (growth optima of 20-45°C), despite critical-zone temperatures that were continuously below 15°C, although all isolates could grow at temperatures associated with the critical-zone (-2.8 – 13.1°C). Only three isolates were shown to have thermal optima for growth that were within temperatures experienced at the critical-zone. These findings show that the bacteria that inhabit the western Icelandic critical-zone have temperature growth optima suboptimally adapted to their environment, implying that other adaptations may be more important for their long-term persistance in this environment. Moreover, our study showed that the cold basaltic critical-zone is a region of active phosphate mineral-weathering.

## Introduction

The critical-zone is a heterogeneous region in which many globally significant biogeochemical processes occur. The combination of rock, soil and water make this environment a complex yet important region, as it is in this zone that the weathering of rock occurs (Chorover et al. 2007). Recent studies of weathering have concentrated on silicate substrates (Bennett et al. 2001, Hilley and Porder 2008, Millot et al. 2002, Parker 2009, Turner et al. 2010) as through weathering of this material, nutrients, metals and minerals are released to the environment and made available to the biosphere. Simultaneously atmospheric $CO_2$ is sequestered during weathering in the the carbonate-silicate cycle.and stored in the form of carbonates for geological time periods (Berner 1999, Berner et al. 1983, Urey and Korff 1952). This process has been reported as being responsible for sequestration of between 1.5 and 2.6 x $10^8$ tons C $y^{-1}$ of atmospheric $CO_2$ (Arthur 2000, Hilley and Porder 2008, Varekamp et al. 1992).

Rates of silicate weathering are affected by a number of factors. For example, temperature has a positive effect on silicate weathering (Velbel 1993, White et al. 1999), as predicted by the Arrhenius equation which predict that higher temperatures increase chemical reaction rates (Turner, White et al. 2010). Meteorological conditions, such as rainfall, may also influence rates of silicate weathering through hydrolysis of the substrate. Water produces carbonic acid by dissolving atmospheric $CO_2$, lowering the pH of substrate and enhancing the dissolution of silicate materials. For example, the mean pH of Icelandic precipitation (pH = 5.4 ± 0.46) was observed as 0.2 pH units below the classification limits of acidic precipitation (pH 5.6), although this was marginal (Gislason et al. 1996). In addition to these abiotic factors, biota, such as

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lichens, weather and scar the rock face that they inhabit through chemical and physical methods of weathering (Andrews and Williams 2000, Banfield et al. 1999, Brady et al. 1999). More recent studies have shown that prokaryotes can significantly increase bedrock denudation rates (Smeaton 2009) through enhanced proton liberation and elemental release from substrates (Bonneville et al. 2009, Collignon et al. 2011, Štyriaková et al. 2012, Uroz et al. 2007, Uroz et al. 2011), the textural alteration of substrates, visualised by microscopy (Herrera et al. 2008, Mailloux et al. 2009) or the measured reduction of substrate mass (Abdulla 2009).

Microbial communities are known to weather their mineral environments through phenotypic expression of physiologies such as mineral phosphate solubilisation (MPS) (Kim et al. 1997, Richardson 2001, Rodríguez and Fraga 1999). Additionally, growth rates and phenotypic traits such as mineral solubilisation are closely controlled or limited by a range of environmental factors (O'Brien and Lindow 1989), including temperature, a major determinant of bacterial growth rates and activity (Pietikåinen et al. 2005). Therfore, the temperature experienced at the rock-soil interface would be expected to exert an important influence on critical-zone weathering, with the seasonal fluctuations caused by annual variations in ambient temperature.

Other key factors which are hypothesised to be rate limiting to bacterial weathering concern the material liberated into the ecosystem during the weathering process. Moulton and Berner (1998) suggested that there are differential metal availabilities caused by different weathering rates. Such availabilities could secondarily impact biotic weathering rates through, for example the toxicity effects of liberated heavy metals on bacterial growth  (see Giller et al. 2009 for a review, Leita et al. 1995).

The effects of land coverage on weathering rates has been studied (Moulton and Berner 1998), as well as the effects that land usage have on the microbial communities (Hartmann et al. 2009, Summers et al. 2013). However, the physiology and potential weathering activity of these bacterial communities has still not been adequately addressed and represents a knowledge gap in understanding the diversity involved in silicate weathering. Although vegetation plays a key role in structuring bacterial diversity (Estrada-de los Santos et al. 2011, Hartmann, Schmid et al. 2009, Miniaci et al. 2007), at the critical-zone, secondary regulation may also be occuring due to the action of temperature regime and weathering rates themselves.

While soil bacterial communities in general have received significant attention (see Janssen 2006 for a review) there has only recently been a sustained effort to understand sub-surface critical-zone microbial communities (Akob and Küsel 2011); as well as understanding what role they have in the weathering of silicates at the critical-zone. The results of biotic and abiotic factors influencing weathering are all co-correlated and as a result disentangling them is problematic *in-situ*, therefore an *in-vitro* approach was performed in this study.

Previous work by Summers, Whiteley et al. (2013) indicated differing microbial diversity at the sites studied in this paper, and thus, a key factor to be resolved is the interaction between temperature and physiology. Therefore, to understand better the physiology of critical-zone bacteria and how it correlates to environmental conditions at the critical-zone, we examined isolated bacteria from an Icelandic critical-zone, focusing on the effects of temperature and their ability to solubilise phosphate from minerals.

**Materials and methods**

**Sample locations.** Cultured bacterial isolates likely to be responsible for weathering and also harbouring tolerances to the environmental conditions experienced at the critical-zone were required for this study. In order to achieve this, environmental soil samples containing representatives of the bacterial community from the Icelandic critical-zone ($n$ = 18) were collected from two sites located near Skorradalur lake, Iceland: Tree covered – TCS (64°30.577N, 21°23.497W); Lichen and moss covered – LMS (64°29.279N, 21°31.660W). Soil samples were collected from the critical-zone at a depth of *c.* 30 cm below any visible root systems that contained rock and mineral fragments that did not exhibit significant evidence of weathering. These sites have been investigated previously and it was shown that the rates of weathering significantly differed in sites with different vegetation coverage, although the sites had a broadly similar lithology (Moulton and Berner 1998, Moulton et al. 2000). Vegetation present at sample sites consisted of higher order plants such as birch and conifer trees at the TCS site, whereas lichens and mosses partially covered the LMS site. Samples were collected aseptically in June 2009 and stored in sterile bags (Whirlpak, Fisher Scientific, Loughborough, UK) at ambient outdoor temperature (*c.* 11°C) before being stored at 4°C on return to the laboratory. Storage was no longer than 2 weeks prior to culture.

**Physicochemical characteristics of sample sites.**

*Temperatures in the critical zone*

To determine the temperature range experienced at the critical-zone, U12-008 dataloggers (Tempcon instrumentation, Arundel, UK) linked to TMC6 HB external thermistors were left *in-*

*situ* for 23 months from June 2009 to May 2011. Thermisters were positioned to give temperature readings for the critical-zone and the air temperature directly above the sample sites. The loggers were set to record temperature data every hour.

Metal concentrations

In order to characterise the geochemical environment from which our isolates were obtained, the concentrations of total and soluble metals were measured ($n = 18$).

**1 - Total metals:** The major elements within soil samples were determined by X-ray fluorescence (XRF) spectrometry. Samples were prepared by fusing one part powdered soil sample with five parts FluXana flux (20% lithium tetraborate w/w, 80% lithium metaborate w/w) at 1000 °C. Samples were then poured into a mould and cooled to room temperature, forming a glass disc. These glass discs were analysed using an ARL 8420+ dual goniometer wavelength dispersive XRF spectrometer. Data analysis was conducted as previously described (Ramsey et al. 1995, Watson 1996).

**2 - Bioavailable metals:** Each sample was analysed in triplicate for concentrations of bioavailable Cu, Zn, Fe and Mn by diethylenetriaminepentaacetic acid (DTPA) extraction of metal from the soil matrix as described by Liang and Karamanos (1993). Following extraction of soluble metals, all solutions were stored in 5% $HNO_3$ acid solution at -80°C. Subsequently, DTPA extracted solutions were diluted ($10^{-1}$) with 5% $HNO_3$ solution and ICP-AES (Leemna, Prodigy spec, NH, USA) analysis was carried out according to Yang et al. (2008) using the following standards and wavelengths: Cu (324.754 nm), Fe (239.563 nm), Zn (213.856 nm), Mn (259.372 nm).

*Carbon availability*

Soil organic carbon was measured by oven-drying all samples at 100 °C overnight to obtain dry weight. Soil organic carbon–free weight was obtained following 1 h at 500 °C as described by Heiri et al. (2001). Soil organic carbon was calculated by subtracting soil organic carbon–free weight from the air-dried weight.

**Bacterial isolations.** To isolate organisms present at the critical-zone, low organic solidified growth media (LOM) was prepared. This consisted of, tryptic soy broth (BD Difco, Oxford, UK), supplemented with agar (Oxoid, Basingstoke, UK; Bacterial Agar No1) in a 1:9 vol/vol ratio and diluted to a final concentration of 1.5% and containing 0.5 g of crushed soil, which had been dried, ground to powder and autoclaved. Sterile crushed soil was added to provide a source of any organics present at the critical-zone, in an attempt to increase the diversity of the isolates obtained.

Soil bacteria were isolated from soil slurries generated by adding 1 g (wet weight) of soil sample to sterile dionised water (sdH$_2$O) in a 1:1 vol/vol mixture. Samples were vortexed to homogenise the slurries. Media plates were inoculated with 30 µL of soil solution and incubated for up to 120 hours. In a further attempt to increase the diversity of culturable bacteria, different aerobic conditions and temperatures were employed in several treatments (Table 1). A proportion of plates were incubated at 20°C in a static condition (Model 305, cooled incubator; LMS LTD, Kent, UK); all other plates were incubated at 10°C in a static condition, in order to promote psychrotolerant and psychrophilic species that were expected to inhabit Icelandic critical-zone soil samples. Isolates grown anaerobically were cultured using sealed chambers with anaerobic

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environment generation pouches (AN0035, Oxoid, Hampshire, UK); the resulting environment was <1% $O_2$ and between 9-13% $CO_2$. No hydrogen was generated during incubations.

Individual colonies, from all incubation methods, were subcultured (multiple times if necessary) onto streak plates to obtain pure isolates which were then maintained in aerobic conditions at 10°C. In this study we chose to study obligate or facultatively aerobic organisms to simplify cultivation procedures. Organisms from the pool of isolates obtained under anaerobic conditions were therefore those that were also capable of growing aerobically. Thirty five culturable bacterial candidates were selected for further downstream physiological experiments. For long term storage of isolates, all organisms were aseptically transferred to a 50% lysogeny broth (LB) with 50% glycerol solution in $sdH_2O$ (1:1 vol/vol) and stored at -80°C until analysis.

**Mineral phosphate solubilisation (MPS).** To measure the mineral weathering efficacy of each isolate, MPS plates were prepared as previously described by Mailloux, Alexandrova et al. (2009) with tricalcium phosphate (TCP; VWR international, Lutterworth, UK) used as a solid state mineral phosphate source. The use of MPS plates permitted the identification of MPS phenotypes associated with isolated strains through observation of halos around bacterial colonies following incubation on opaque plates.

Bacterial isolates ($n = 35$) were inoculated onto the MPS plate following standardisation to equal optical densities (OD = 0.5) by dilution with 1x PBS and incubated at 20°C for 60 hours. Halos surrounding bacterial colonies were identified and digital images taken. The area of individual halos was determined using ImageJ software package (v1.45, National Institutes of Health, USA) with radii of plates (90 mm) used as a datum. Positive controls using a weak HCl acid (5% v/v)

and *Pseudomonas aeruginosa* were included to confirm media solubilisation both chemically and biologically as previously described (Goldstein et al. 1999).


**Growth measurements of isolates under environmental stress.** Bacterial isolates were resuspended in 20 µL sdH$_2$O then added to 350 µL of 10% LB. These inocula were used in growth trials examining environmental temperatures and their effect on bacterial growth, following standardisation by dilution to equal optical densities (OD = 0.5).

Colony inocula ($n = 35$) were incubated at four experimental temperatures. (1, 5, 10 and 22°C ($\pm$ 0.1°C)) using a Bioscreen C reader (Labsystems Oy, Helsinki, Finland). The temperatures were selected to emulate environmental temperatures as well as temperatures higher than would normally be experienced *in-situ*.

Optical density (OD) absorbance readings were measured at 600 nm every 15 minutes, following 15 seconds of medium oscillation shaking of the microplate. Incubation and OD measurements continued until all isolates had reached stationary growth phase. Growth rates ($\mu$) were calculated as follows:

$$\mu = \frac{2.303(logN_x - logN_o)}{(t_x - t_o)}$$

Where $N_o$ is the initial OD at time $t_o$ and $N_x$ is the OD at time $t_x$. The log phase of growth curves were converted to a common log by multiplying by 2.303. Time was in hours therefore $\mu$ was expressed as h$^{-1}$. $t_o$ and $t_x$ were required to be within the exponential growth phase in order for

this calculation to be accurate; these times were individually selected for each isolate and therefore the final formulae were unique to each isolate's individual growth curve.

**Isolate identification.** Taxonomic identification of the bacterial isolates used in this study was conducted by whole cell colony PCR of isolates using the following universal primers; 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') (Lane 1991) and 1541r (5'-AAG GAG GTG ATC CAG CCG CA-3') (Embley 1991) to amplify the 16S rRNA gene (V1-V9 regions) of cultured isolates, prior to Sanger sequencing from the 5' end. Amplification was performed in a 50 µL reaction containing: 300 nM of each primer; 5 µL of 10X *Taq* buffer; 2 mM $MgCl_2$; 0.2 mM of each of the deoxynucleoside triphosphates (dNTP); with 2.5 U of *Taq* (New England Biolabs, MA, USA).

PCR conditions consisted of: an initial denaturation for 10 minutes at 94°C to promote cell lysis; followed by 30 cycles of 1 minute at 94°C; annealing for 40 seconds at 58°C; elongation for 40 seconds at 72°C with a final elongation step for 10 minutes at 72°C. All PCR products were verified by gel electrophoresis and products were stored at -20°C.

Six isolates studied failed to amplify using primers 27f and 1541r. These six isolates were amplified using domain Bacteria universal primers 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') (Marchesi et al. 1998) and 530r (5'-GTA TTA CCG CGG CTG CTG-3') (Muyzer et al. 1993) to partially amplify the 16S rRNA gene (V1 – V3 regions). Amplification was carried out using 1 µL of template DNA in a 50 µL reaction containing: 250 nM of each primer; 5 µL of 10X Taq buffer; 2 mM $MgCl_2$; 0.1 mM of each dNTP; 5 µg bovine serum albumin (BSA; New England Biolabs, MA, USA) and 1.75 U of *Taq* (New England Biolabs, MA, USA). PCR

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conditions consisted of: initial denaturation for 90 seconds at 94°C followed by 35 cycles of 45 seconds at 94°C; annealing for 60 seconds at 55°C; elongation for 3 minutes at 72°C and a final elongation step for 10 minutes at 72°C.

All PCR products were purified using an Illustra[TM] GFX[TM] PCR clean up kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instruction. Sanger termination sequencing was conducted directly on PCR amplifications using the Big Dye terminator and X-terminator kits (Applied Biosystems, CA, USA) with a 3730 DNA sequencer (Applied Biosystems, CA, USA) as per the manufacturer's instructions.

Subsequent phylogenetic analysis of all sequences was conducted using MOTHUR software (Schloss et al. 2009) to trim sequences. Taxonomic classification of sequences was performed using the Naïve Bayesian rRNA Classifier v1.0 analysis tool within MOTHUR; taxonomic training database ver 9 downloaded from Ribosomal Database Project II website (Michigan State University) (http://rdp.cme.msu.edu/). Classifications with a confidence threshold of 95% were based on the taxonomical hierarchy proposed in Bergey's Manual of Systematic Bacteriology, release 6.0.

All 16S rRNA gene sequences obtained in this study were submitted to Genbank under accession numbers KC164992 – KC165026.


**Statistical analysis.** To determine if individual factors such as initial cultivation temperature, aerobic state of initial incubations or sample site resulted in differences in MPS efficacy, a Mann Whitney U test was conducted. Overall growth treatments, and individual variables nested

within, were examined using a Kruskal Wallis test of difference to determine if there was any difference between overall growth treatments and the measured MPS efficacy.

Differences in bacterial growth at the selected temperatures were tested using a one-way ANOVA following log transformation and an Anderson Darling test of normality. In addition, a linear regression was conducted to determine any relationship between incubation temperature and specific growth rate.

## Results

**Soil physicochemical characteristics.** Recorded temperatures from LMS and TCS sites, spanning a 23 month period are shown in Figure 1 and display a clear pattern of seasonal variation. Air temperatures in the summer months reached a high of 32.1°C and a low of -14.7°C. The variability in temperature was greater for air temperature readings than those taken at critical-zones; overall there were smaller diurnal fluctuations and a reduced range of temperatures over the measurement period at the critical-zone than for air temperature readings. Temperatures at the TCS critical-zone ranged from 13.1°C to 1.0°C with a mean temperature over the measurement period of 6.2°C. As temperatures never reached freezing point, liquid water would have been available, throughout both winters, to the bacterial community inhabiting the critical-zone. Critical-zone temperatures at the LMS site ranged from 11.2°C to -2.8°C having a mean temperature over the measurement period of 3.4°C. At this site the critical-zone temperature achieved freezing point at each winter for *c.* 4 months.

Concentrations of total metals recorded from both TCS and LMS sites indicated that there were significantly higher concentration of Fe and Mn at the TCS site. While concentrations of total Cu

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and Zn were also higher at the TCS site, these were not statistically significant (Table 2). The mean concentration of bioavailable metal ions at each site was calculated. There was found to be no significant difference in the bioavailability of metal ions at each site, with the exception of Cu.

**Bacteria taxonomic identification.** All isolates were sequenced to provide taxonomic identification down to genus level (Table 3). Overall the diversity of isolates was low as >50% of isolates were represented by either *Pseudomonas* or *Stenotrophomonas* spp. Other taxa obtained from the critical-zone area examined here include *Alcaligenes*, *Arthrobacter, Bacillus*, *Brevundimonas, Leucobacter, Ochrobactrum*, *Paenibacillus* and *Staphylococcus* spp, though at low abundances.

**Mineral phosphate solubilisation cultures.** Phenotypic tests for phosphate solubilisation of recovered isolates revealed the presence of halos around most of the bacterial strains inoculated onto the MPS plates. Thirty two of the 35 bacterial isolates tested showed evidence of biotically-induced phosphate weathering, the three isolates that did not exhibit the MPS phenotype originated from the LMS site.

The measurements of halo area provided a quantifiable measure of the weathering capability of each isolate (Table 3). Specifically, it was observed that there was no significant difference in weathering capability of isolates between sites (Figure 2a; Mann Whitney U, $U = 153$, $p = 0.7456$) nor dominance of a particular taxa amongst the most efficient MPS bacteria (Figure 3; Kruskal-Wallis test, $df = 9$, $p = 0.67$). Furthermore, neither the initial culturing treatments

(Figure 4a; see Table 1 for details) nor isolation temperature were found to have a significant effect on the weathering capability of bacterial isolates tested (Figure 2b; Kruskal-Wallis test, df = 5, $p$ = 0.8; Mann Whitney U, $U$ = 188, $p$ = 0.239, respectively). Finally, the weathering capability of bacterial isolates was not significantly different between initial aerobic and anaerobic culturing environments (Figure 4b; Mann Whitney U, $U$ = 93, $p$ = 0.249). In summary, it was revealed that the isolates' physiology in relation to weathering capability was not associated with any facet of location, isolation strategy, phylogenetic identity or aerobic status.

**Bacterial growth at variable temperatures.** Most isolates showed optimal rates of growth when exposed to temperatures of 22°C (Table S1). However, most isolates were also capable of growth at a temperature of 1°C. The only exceptions to this were isolates S1 24 and S1 20 (*Arthrobacter* and *Stenotrophomonas* spp, respectively) which displayed a growth optima at 10°C and isolates S1 24, AW3 13 and AW1 11 (*Arthrobacter, Bacillus* and *Paenibacillus* spp, respectively) which showed no evidence of growth at 1°C. Moreover, the observed effect of temperature on mean growth did result in a significant difference in growth rates with regards to temperature (Figure 5; ANOVA, $F_{1-3}$ = 358.4, $p$ <0.001). The relationship between increased temperature and specific growth rate was confirmed by linear regression (R = 0.706, $p$ <0.001).

**Discussion**

Understanding how bacterial physiology correlates to environmental conditions at the critical-zone is an essential task for understanding how organisms might contribute to critical-zone

processes such as rock weathering. However, the physiological requirements of bacteria at the critical-zone are not known.

Both sites studied here (TCS and LMS sites) exhibited large temperature ranges throughout the year. At the critical-zone the temperature ranges were smaller with fewer fluctuations compared to air temperatures both seasonally and diurnally. The air and critical-zone temperatures at site LMS were lower than at site TCS despite the fact that temperatures were measured at equal depths. In addition, the temperature ranges at the critical-zone were greater at LMS sites than at the TCS site (14.4°C and 12.1°C respectively). Temperatures experienced at the critical-zone never exceeded 15°C

Despite these annual temperature data, the most striking observation was that all isolates except two grew optimally at 22°C, of the four temperatures studied. Isolate S1 24 (*Arthrobacter* sp.) and isolate S1 20 (*Stenotrophomonas* sp) exhibited optimal growth at 10°C. Therefore, the majority of the isolates can be described as psychrotolerant (Morita 1975). These data suggest that there could be seasonal variations in the activity of critical-zone bacterial weathering, with higher temperatures during the summer favouring mesophilic inhabitants of the critical-zone (Collignon et al., 2011).

There are a variety of possible explanations for why the isolated organisms do not have growth optima matched to critical-zone environmental conditions. Bacteria might exhibit more than one optimal temperature for growth, with the optima being dependant on a particular metabolic process involved in the utilization of a specific energy source (Oppenheimer and Drost-Hansen 1960).

Tang et al. (1997) presented evidence that the optimum growth temperature for cyanobacteria in polar regions was greater than the environmental temperatures, similar to our study. They propose that these organisms succeed by slow persistent growth and that other competitive advantages, such as adaptation to desiccation and freeze-thaw cycles, might account for their dominance. Similarly, Cockell et al. (2010) studied bacterial isolates (primarily *Arthrobacter*) living in gypsum in the Canadian High Arctic. They found no psychrophiles and suggested desiccation and freeze-tolerance might similarly be important for bacteria living in High Arctic soils and salts. In the critical-zone studied here, periodic desiccation cannot be discounted as the LMS site experienced freezing temperatures during the period of measurement.

We cannot rule out the possibility that our cultivation media selected for mesophiles or that those that are most easily cultivated in the laboratory are predominantly mesophilic, although the isolation of two bacteria with optimal growth temperatures lower than 15°C suggests that the media allowed for the growth of low-temperature adapted organisms. Even if this was the case, our data show that a diversity of mineral-weathering mesophilic organisms are potentially active in the cold critical-zone.

The presence of metals can also affect microbial growth. Previous chemical analysis of soils from these study sites using X-ray fluorescence (XRF) analysis indicated that several metals such as Al and Fe were present at TCS and LMS sites in differing concentrations (Summers, Whiteley et al. 2013). As these were total metal concentrations present in the soils it was not a measure of what was available to the bacterial community. This was overcome in this paper by using ICP-AES to measure soluble metal concentrations. Only Cu exhibited any significant difference in concentration between samples sites.

Some volcanic regions are oligotrophic (Cockell et al. 2011, Jorquera et al. 2008), caused by a limitation in bioavailable phosphate which is made available from mineral weathering. Many bacterial species are known to grow in phosphate-limited environments by weathering rocks and releasing insoluble phosphate into the environment (Goldstein, Braverman et al. 1999, Hamdali et al. 2012, Jorquera, Hernandez et al. 2008, Perez et al. 2007, Richardson 2001, Vera et al. 2008). Most of the bacteria isolated in this study were able to solubilise mineral phosphate although no taxa exhibited a greater weathering efficacy over any other..

Although no individual taxa was able to be determined as a key weathering agent, future studies should concentrate on the functions used to catayze silicate weathering. One such mechanism by which bacterially-induced weathering occurs could include the production of organic acids, such as gluconic acid, which lowers the pH of the immediate area around the bacterium (Uroz, Calvaruso et al. 2007).

In conclusion, this study shows that bacteria in the cold basaltic critical-zone grow at sub-optimum growth temperatures. Despite environmental temperatures lower than the growth optima for many of the isolates studied here, and potentially deleterious effects of environmental concentrations of Cu for bacterial growth, we showed that many organisms at the critical-zone can actively engage in phosphate-mineral weathering. Our study indicates that organisms at the critical-zone are not merely passive inhabitants of this region, but likely play an active role in critical-zone biogeochemistry, particularly rock weathering.

**Acknowledgements**

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**Figures and tables:**

**Table 1:** Summery of the initial growth conditions used to isolate various bacterial strains from critical-zone soil samples.

| Treatment | Incubation temperature (°C) | Aerobic/anaerobic environment | Sample site |
|---|---|---|---|
| $Aero^{10}_{LMS}$ | 10 | Aerobic | LMS |
| $Aero^{20}_{LMS}$ | 20 | Aerobic | LMS |
| $Anaero^{20}_{LMS}$ | 20 | Anaerobic | LMS |
| $Aero^{10}_{TCS}$ | 10 | Aerobic | TCS |
| $Aero^{20}_{TCS}$ | 20 | Aerobic | TCS |
| $Anaero^{20}_{TCS}$ | 20 | Anaerobic | TCS |

**Table 2:** Mean metal concentrations of total and bioavailable metals in TCS and LMS critical-zone samples ($n = 18$). Significant differences between sample sites are highlighted and analysed using a Mann Whitney U test. Test statistic ($U$) is provided.

| | Cu | | Fe | | Mn | | Zn | |
|---|---|---|---|---|---|---|---|---|
| | TCS | LMS | TCS | LMS | TCS | LMS | TCS | LMS |
| Total metal concentrations | | | | | | | | |
| Mean (mM) | 2.08 | 2.00 | 2811.35 | 2651 | 1.85 | 1.80 | 41.87 | 38.2 |
| SD | 0.15 | 0.10 | 57.30 | 94.90 | 0.07 | 0.15 | 1.82 | 1.82 |
| | | | | | | | | |
| *p* value | **0.**344 | | **0.002** | | **0.005** | | 0.674 | |
| U | 22.5 | | 5 | | 5 | | 27.5 | |
| Bioavailable metal concentrations | | | | | | | | |
| Mean (mM) | 1.13 | 0.74 | 44.19 | 42.82 | 1.28 | 1.57 | 0.26 | 0.22 |
| SD | 0.41 | 0.22 | 27.33 | 15.81 | 0.69 | 0.53 | 0.18 | 0.21 |
| | | | | | | | | |
| *p* value | **0.024** | | 0.734 | | 0.489 | | 0.135 | |
| U | 66 | | 45 | | 32 | | 58 | |

**Table 3:** Weathering capability of individual isolates grown on MPS plates. Displayed are area of halo and original isolation treatments for each isolate.

| Isolate # | Area ($mm^2$) | Isolation Method | Genus |
|---|---|---|---|
| AW1 10 | 91.6 | $\text{Anaero}^{20}_{\text{LMS}}$ | *Brevundimonas* sp |
| AW1 11 | 0 | $\text{Anaero}^{20}_{\text{LMS}}$ | *Paenibacillus* sp |
| AW1 4 | 99.8 | $\text{Anaero}^{20}_{\text{LMS}}$ | *Brevundimonas* sp |
| AW1 5 | 69.3 | $\text{Anaero}^{20}_{\text{LMS}}$ | *Pseudomonas* sp |
| AW1 7 | 96.2 | $\text{Anaero}^{20}_{\text{LMS}}$ | *Stenotrophomonas* sp |
| AW1 8 | 65 | $\text{Anaero}^{20}_{\text{LMS}}$ | *Stenotrophomonas* sp |
| AW1 9 | 61.9 | $\text{Anaero}^{20}_{\text{LMS}}$ | *Stenotrophomonas* sp |
| AW3 12 | 94.3 | $\text{Anaero}^{20}_{\text{TCS}}$ | *Paenibacillus* sp |
| AW3 13 | 46.9 | $\text{Anaero}^{20}_{\text{TCS}}$ | *Bacillus* sp |
| AW3 2 | 87.2 | $\text{Anaero}^{20}_{\text{TCS}}$ | *Stenotrophomonas* sp |
| S1 11 | 110.5 | $\text{Aero}^{20}_{\text{LMS}}$ | *Leucobacter* sp |
| S1 13 | 47.8 | $\text{Aero}^{20}_{\text{LMS}}$ | *Leucobacter* sp |
| S1 20 | 34.4 | $\text{Aero}^{20}_{\text{LMS}}$ | *Stenotrophomonas* sp |
| S1 21 | 29.5 | $\text{Aero}^{20}_{\text{LMS}}$ | *Stenotrophomonas* sp |
| S1 24 | 68.3 | $\text{Aero}^{20}_{\text{LMS}}$ | *Arthrobacter* sp |
| S1 27 | 101 | $\text{Aero}^{10}_{\text{LMS}}$ | *Leucobacter* sp |
| S1 28 | 36.9 | $\text{Aero}^{10}_{\text{LMS}}$ | *Pseudomonas* sp |
| S1 3 | 82.3 | $\text{Aero}^{20}_{\text{LMS}}$ | *Stenotrophomonas* sp |
| S1 30 | 80.6 | $\text{Aero}^{10}_{\text{LMS}}$ | *Pseudomonas* sp |

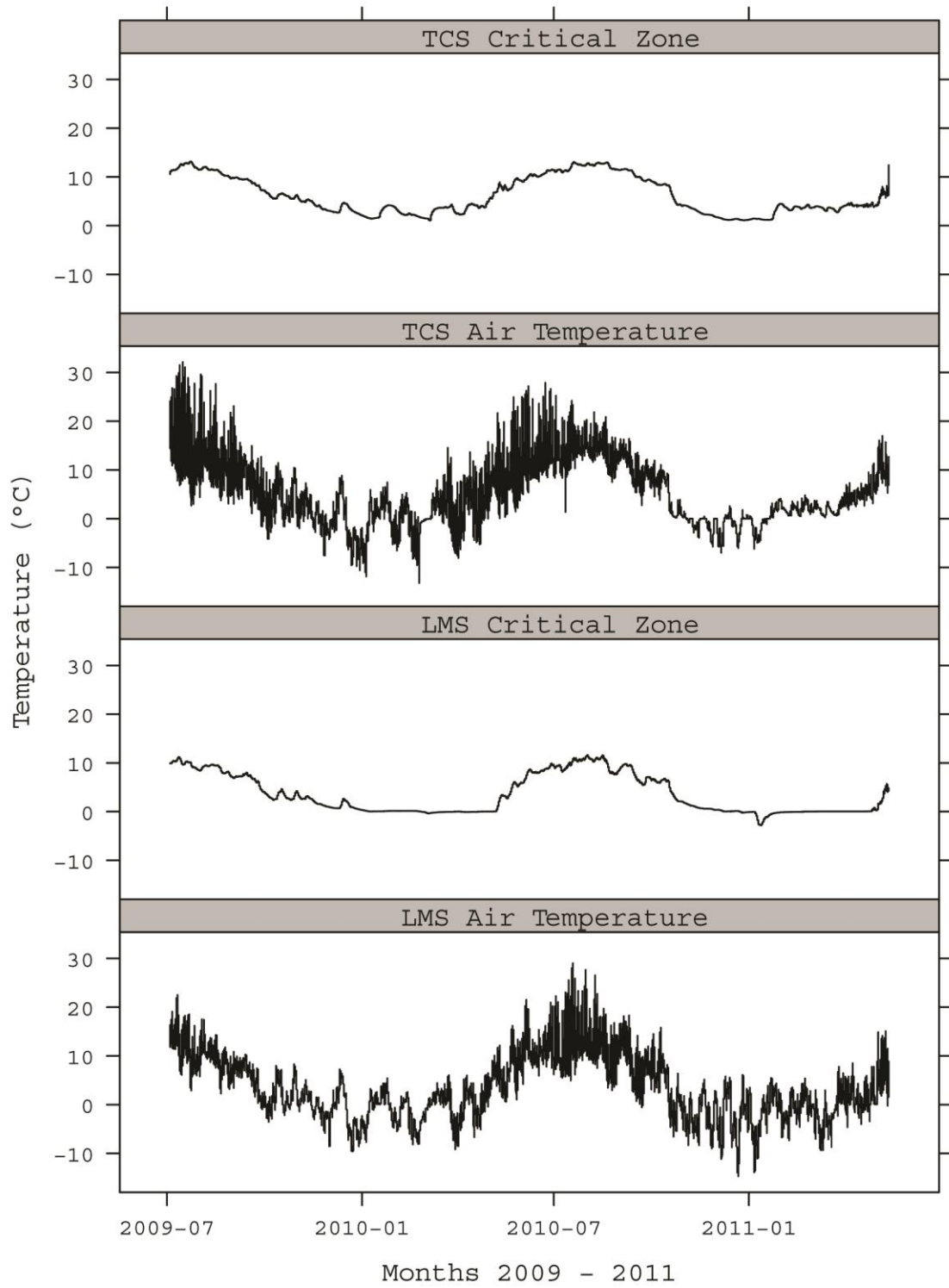| | | | |
|---|---|---|---|
| S1 31 | 67.1 | Aero$^{10}_{LMS}$ | *Stenotrophomonas* sp |
| S1 32 | 0 | Aero$^{10}_{LMS}$ | *Leucobacter* sp |
| S1 33 | 0 | Aero$^{10}_{LMS}$ | *Pseudomonas* sp |
| S1 34 | 63.1 | Aero$^{10}_{LMS}$ | *Leucobacter* sp |
| S1 35 | 111 | Aero$^{10}_{LMS}$ | *Stenotrophomonas* sp |
| S1 37 | 33.3 | Aero$^{10}_{LMS}$ | *Brevundimonas* sp |
| S3 13 | 76.4 | Aero$^{20}_{TCS}$ | *Stenotrophomonas* sp |
| S3 18 | 74.3 | Aero$^{20}_{TCS}$ | *Stenotrophomonas* sp |
| S3 27b | 61.1 | Aero$^{10}_{TCS}$ | *Staphylococcus* sp |
| S3 27c1 | 46.7 | Aero$^{10}_{TCS}$ | *Pseudomonas* sp |
| S3 27c2 | 75.3 | Aero$^{10}_{TCS}$ | *Stenotrophomonas* sp |
| S3 28d | 85.3 | Aero$^{10}_{TCS}$ | *Alcaligenes* sp |
| S3 29b | 42.6 | Aero$^{10}_{TCS}$ | *Pseudomonas* sp |
| S3 32 | 82.4 | Aero$^{10}_{TCS}$ | *Brevundimonas* sp |
| S3 38 | 10.5 | Aero$^{10}_{TCS}$ | *Pseudomonas* sp |
| S3 6 | 79.6 | Aero$^{20}_{TCS}$ | *Ochrobactrum* sp |

**Figure 1:** Critical-zone and air temperature data from sites LMS and TCS. Data collected over a 23 month period from June 2009.
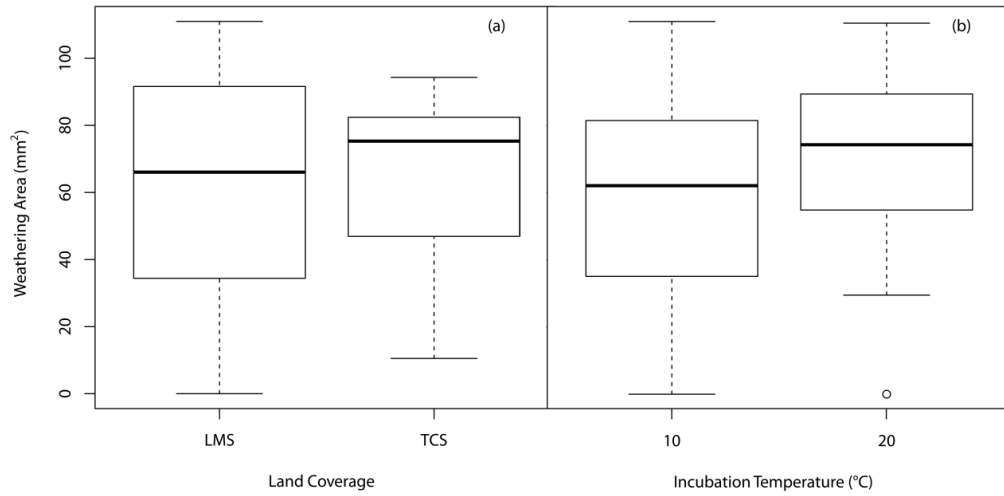
**Figure 2:** The weathering efficacy of bacterial isolates subcultured from environments containing (a) dissimilar land vegetation and (b) under controlled temperature conditions. Boxplots present the median MPS potential with 95% CI included.
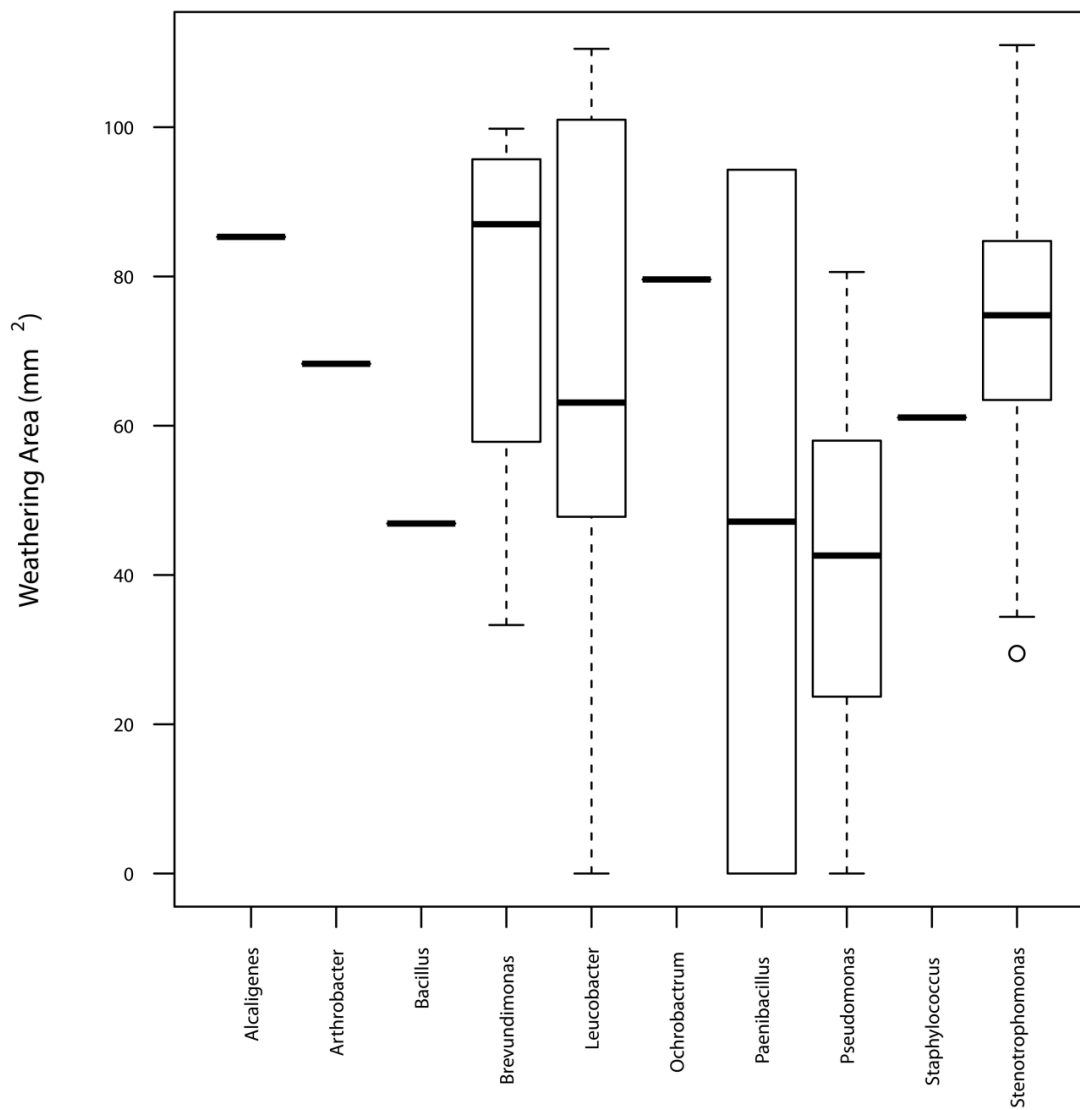
**Figure 3:** The differences in phosphate weathering efficacy of isolates from differing bacterial taxa. Boxplots present the median MPS potential with 95% CI included.
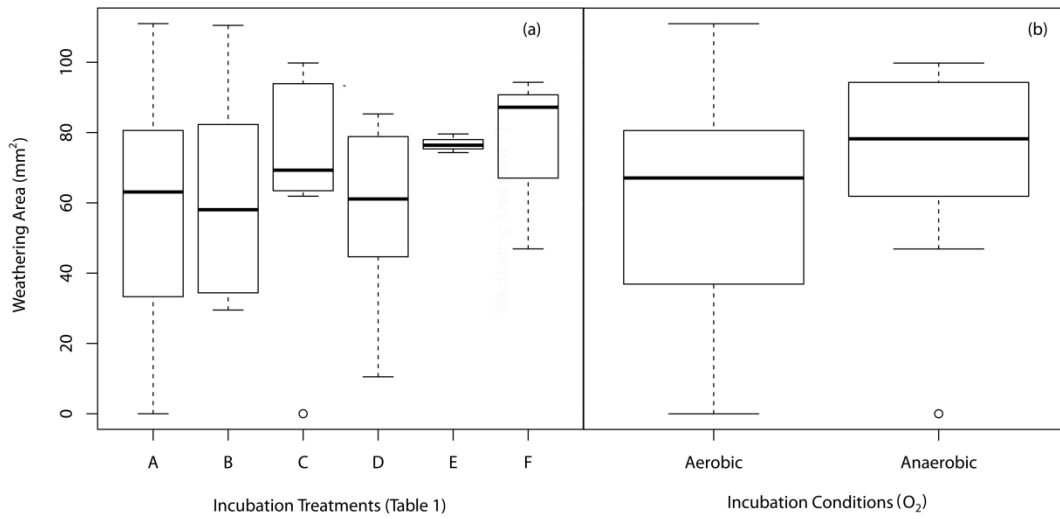
**Figure 4:** Weathering efficacy of bacterial isolates measured under various experimental growth conditions: (a) Incubation treatments as shown in Table 1 and (b) anaerobic and aerobic incubation. Boxplots present the median MPS potential with 95% CI included.
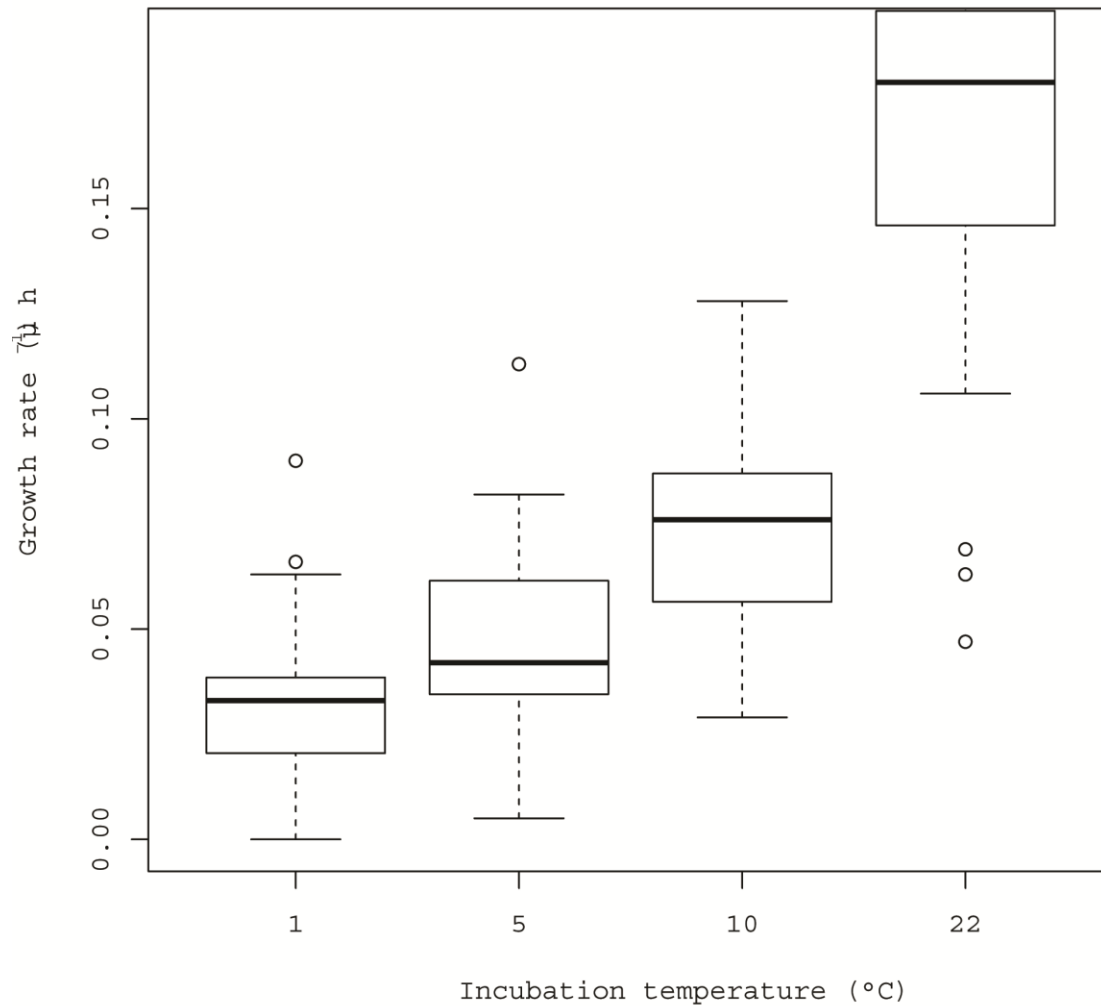
**Figure 5:** The pooled growth rates of all isolates used in this study ($n = 35$). The effect of temperature was observed as having a significant influence on specific growth rates ($p < 0.001$) as growth increased with temperature.