

# A Study of the Microbial Community at the Interface between Granite Bedrock and Soil Using a Culture-Independent and Culture-Dependent Approach

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## Abstract

The dissolution of minerals plays an important role in the formation of soils and sediments. In nutrient limiting soils, minerals constitute a major reservoir of bio-essential cations. Of particular interest is granite as it is the major rock type of the continental land mass. Although certain bacteria have been shown to enhance weathering of granite-forming minerals, little is known about the dissolution of granite, at the whole rock scale, and the microbial community involved. In this study, both culture-independent and culture-dependent approaches were used to study the bacterial community at the interface between granite bedrock and nutrient limiting soil in Dartmoor National Park, United Kingdom. High throughput sequencing demonstrated that over 70% of the bacterial population consisted of the bacterial classes Bacilli, Beta-proteobacteria and Gamma-proteobacteria. Bacteria belonging to the genera *Serratia*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Chromobacterium* and *Burkholderia* were isolated from the sample site. All of the isolates were able to grow in a minimal growth medium, which contained glucose and ammonium chloride, with granite as the sole source of bio-essential elements. Sixty six percent of the isolates significantly enhanced basalt dissolution ( $p < 0.05$ ). Dissolution of Si, K, Ca and Mg correlated with production of oxalic acid and acidification. The results of this study suggest that microorganisms in nutrient limiting soils can enhance the rate of granite dissolution, which is an important part of the biogeo-

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chemical cycle.

## Keywords

Mineral Weathering, Soil Community

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## 1. Introduction

The silicates account for over 90% of the Earth's crust. The dissolution of silicate minerals results in the formation of soil and sediment, and is an important process in long-term carbon cycling. In nutrient limiting soils, such as acidic soils, silicate minerals also constitute a major reservoir of bio-essential cations, for example, calcium, potassium and magnesium [1]. Microorganisms, such as bacteria and fungi are known to enhance the dissolution rates of silicates (for reviews on the role of fungi and bacteria see [2] and [3], respectively). Bacteria are able to accelerate the rate of elemental release from minerals either directly through the acquisition of limiting nutrients, or indirectly through the production of metabolic by-products that lower the pH or change the saturation state of the mineral [3]-[12].

Silicate minerals are the principle component of igneous rocks, such as granite, which is the most common rock type of the continental land mass. Microbe-granite interactions may therefore, play an important role in biogeochemical cycling of bio-essential elements. However, there is relatively little known about microbe-granite interactions and the role of microorganisms in granite dissolution. Laboratory-based experiments, with granite-forming minerals, have suggested that microorganisms produce organic acids, siderophores and extracellular polysaccharides that enhance granite dissolution [13] [14]. Yet these studies have predominately focused on single minerals rather than the whole rock scale, where the rate of dissolution and reaction pathways would be different [13]-[17]. Previous experiments have also mainly focused on commercial bacteria rather than bacteria isolated from a granitic environment.

In nutrient limiting soils, a small number of studies have investigated the role of microorganisms in granite-forming minerals using *in-situ* mesocosm experiments. The presence of minerals has been shown to influence the diversity of the microbial communities [18]-[23]. Long-term experiments have demonstrated a correlation between mineral dissolution and community abundance, in nutrient limiting acidic soils [3] [24]. Yet, it is unclear if the correlation is due to increased bioavailability of inorganic nutrients due to weathering in the surrounding soil or to the active role of microorganisms in weathering [3] [24]. To address the role of bacteria, a parallel culture-independent and culture-dependent approach is required.

In this paper, we investigated the potential role that bacteria perform in granite weathering in nutrient limiting soils. The site for this study was an area within Dartmoor National Park, United Kingdom, which was selected due to the prevalence of granite outcrops (tors), many of which are overlain by shallow soil layers. Insight into the microbial community was obtained using a high throughput sequencing approach. In parallel, the weathering ability of microorganisms was determined by isolating members of the community and carrying out dissolution experiments in batch culture. This investigation is important for understanding the role of microorganisms in bio-geochemical cycling in nutrient limiting soil environments.

## 2. Material and Methods

### 2.1. Sample Collection

The sample sites for this study were within Dartmoor National Park, United Kingdom. Soil samples were collected in triplicate from four locations within the national park (Site 1, 53°36.218N, 3°1.781W; Site 2, 53°3.538N, 3°1.7152W; Site 3, 53°3.544N, 3°1.7066W), each at the base of a granite outcrop at the rock/soil interface. The overlying soil was easily accessible, shallow (approximately 10 cm in depth) and was characterised as acidic (Princetown series). The local vegetation at all sites was dominated by *Nardus* and *Moliniagrasses*. Samples were collected aseptically in March 2013 using corers and stored as previously described [25]. X-ray diffraction (XRD) and X-ray fluorescence (XRF) analysis confirmed the composition of the bedrock was granite. Anorthoclase, quartz, biotite and kaolinite were identified as the major phases from XRD analysis and the major ele-

ments were identified with XRF, as shown in [Table 1](#).

## 2.2. Community Analysis

DNA was extracted from each of the soil samples using the bead-beating phenol extraction protocol previously described [26]. The DNA was PCR amplified using a set of primers specific to the V3-V6 hypervariable region of the bacterial 16S rRNA gene [27], which was carried out according to Sogin *et al.* [28]. Sequencing was carried out using an Ion Torrent Personal Genome Machine (Life Technologies, Paisley UK) with a 316 chip.

## 2.3. Bioinformatic Analysis

The MOTHUR's Costello pipeline was used to process and analyse the raw sequence data [29]. Reads were removed from further analysis if one of the following criteria were met: 1) the read length was shorter than 150 bp, (2) number of ambiguous bases was greater than zero. The sequences were processed as previously described [25]. Rarefaction curves were generated using MG-RAST [30] and all sequences obtained in this study were submitted to MG-RAST [30], under identification numbers 4548120.3, 4548121.3, 4548122.3 and 4548123.3.

## 2.4. Isolation of Bacteria

Aerobic heterotrophs were isolated from the sample site using agar plates. One gram of soil was scattered onto an agar plate, which contained 0.5 g of powdered granite (fraction size <100 µm), 65 of NH<sub>4</sub>Cl mg·L<sup>-1</sup> and 15 g L<sup>-1</sup> of Bacto Agar. The plates were incubated at room temperature, in the dark, for four weeks. Pure cultures were obtained by streaking individual colonies onto fresh plates. Routine growth was maintained in a minimal growth medium that contained the following (mg·L<sup>-1</sup>): 10 of FeCl<sub>3</sub>, 150 of MgSO<sub>4</sub>·6H<sub>2</sub>O, 20 of CaCl<sub>2</sub>, 20 of KCl, 65 of NH<sub>4</sub>Cl, 100 of NaNO<sub>3</sub>, 70 of K<sub>2</sub>HPO<sub>4</sub>, 60 of KH<sub>2</sub>PO<sub>4</sub>, 20 of glucose and the pH was adjusted to pH 6.5 with 100 mM HCl.

The bacteria were identified based on near-full length 16S rRNA gene sequences. Total nucleic acids were extracted from the isolates using the Phe: Chl: Iaa bead beating protocol described by [26]. The 16S rRNA gene was amplified using two sets of primers: 27f-Com2 and Com1-1541r, as previously described [25]. BioEdit software (v.7.1.3.0) was used to align the sequences and the resulting contigs were approximately 1500 bp in length. The nearest sequences were identified in the GenBank database using the BLASTN program [31]. All contiguous sequences were deposited into Genbank, as shown in [Table 2](#).

**Table 1.** Major oxide composition (weight (%)) of the granite, Dartmoor national park, United Kingdom.

Oxides	Weight (%)			
	Site 1	Site 2	Site 3	Site 4
SiO <sub>2</sub>	75.18	74.94	75.01	75.95
TiO <sub>2</sub>	0.15	0.06	0.08	0.12
Al <sub>2</sub> O <sub>3</sub>	12.66	13.62	11.65	12.52
Fe <sub>2</sub> O <sub>3</sub>	1.91	0.95	1.95	1.02
MnO	0.07	0.05	0.06	0.02
MgO	0.22	0.07	0.14	0.15
CaO	0.42	0.25	0.35	0.39
Na <sub>2</sub> O	3.05	3.04	3.11	3.15
K <sub>2</sub> O	4.73	5.23	5.54	4.87
P <sub>2</sub> O <sub>5</sub>	0.19	0.201	0.19	0.22
LOI	0.68	1.18	1.01	0.99
Total	99.28	99.58	99.03	99.38

LOI is Loss on ignition.

**Table 2.** Identification of the bacteria isolated from the interface between the granitic bedrock and nutrient limiting soil.

Isolate	Genebankno°	Closest GenBank relative	Sequence identify (%)	Class	Siderophore ( $\mu\text{mol}\cdot\text{L}^{-1}$ EDTA equivalent)
G_01	KU245735	<i>Serratiasp.</i>	100%	<i>Gamma-proteobacteria</i>	$0.00 \pm 0.00$
G_05	KU245737	<i>Pseudomonas sp.</i>	100%	<i>Gamma-proteobacteria</i>	$2.05 \pm 0.20^{**}$
G_02	KU245738	<i>Pseudomonas sp.</i>	99%	<i>Gamma-proteobacteria</i>	$0.92 \pm 0.15^{**}$
G_10	KU245734	<i>Paenibacillus sp.</i>	97%	<i>Bacilli</i>	$1.15 \pm 0.22^{**}$
G_12	KU245742	<i>Bacillus sp.</i>	100%	<i>Bacilli</i>	$0.64 \pm 0.23^{**}$
G_14	KU245736	<i>Bacillus sp.</i>	99%	<i>Bacilli</i>	$0.85 \pm 0.05^{**}$
G_20	KU245739	<i>Burkholderia</i>	99%	<i>Beta-proteobacteria</i>	$1.35 \pm 0.22^{**}$
G_22	KU245740	<i>Burkholderia</i>	99%	<i>Beta-proteobacteria</i>	$0.78 \pm 0.15^{**}$
G_23	KU245741	<i>Chromobacterium</i>	99%	<i>Beta-proteobacteria</i>	$1.94 \pm 0.09^{**}$

## 2.5. Siderophore Production

To determine the ability of the isolates to produce siderophores the chromeazurol S liquid assay (CAS) was used [32]. The detection of siderophores was quantified and defined according to Payne [33]. The isolates were grown in the minimal medium, without iron (iron-limited) and siderophore production was measured in stationary stage cells. As a control we used *Cupriavidus metallidurans* CH34, which has previously been shown to produce siderophores under iron limiting conditions [34].

## 2.6. Granite Dissolution Experiment

The dissolution experiments were carried out in batch culture with granite as the sole source of bio-essential elements. The granite was prepared as previously described [34]. The specific surface area of the ground rock was measured using multi-point BET (Brunauer, Emmett and Teller, at Imperial College London) with  $\text{N}_2$  and yielded a surface area of  $1.26 \text{ m}^2\cdot\text{g}^{-1}$ . The growth medium for the dissolution experiment contained the following ( $\text{g}\cdot\text{L}^{-1}$ ): 0.2 of glucose, 0.06 of  $\text{NH}_4\text{Cl}$  and 20 of granite. Two grams of granite, which had been prepared by sonication as previously described [34], was placed in an acid-washed 125 mL glass Erlenmeyer culture flask and autoclaved at  $121^\circ\text{C}$  for 15 mins. One hundred mL of liquid medium was added to the flask and the pH was adjusted to pH 7.0 with 10 mM NaOH. Prior to inoculation, the isolates were grown in the modified minimal medium for 5 days. The cells were harvested by centrifugation at  $4,000 \times g$ , for 5 min, and the pellet was washed and resuspended (final cell density of  $10^7$  to  $10^8 \text{ cell mL}^{-1}$ ) in 50 mM Tris buffer (pH 7.0). A 0.5% inoculum was used to inoculate the flasks. Abiotic controls were prepared in an identical manner to the biological flasks and each treatment was prepared in triplicate.

## 2.7. Measuring Microbial Growth

To monitor microbial growth and pH, 1 mL aliquots were aseptically removed after 1, 4, 7, 14, 21, 28 days. Cells were stained with the nucleic acid-binding dye SYBR Green I DNA (0.1% w/v stock; Life Technologies, Paisley, UK). One mL of culture was filtered through a  $0.2 \mu\text{m}$  black polycarbonate filter and then washed with 100  $\mu\text{L}$  of  $\text{ddH}_2\text{O}$ . Cells were enumerated using a Leica DMRP microscope equipped with epifluorescence, as previously described [35]. The growth rate constant ( $k$ ) for the log phase of growth was determined, as previously described [36]. The pH was measured using an Orion 3-Star Thermo Scientific bench top meter.

## 2.8. Chemical Analyses

ICP-MS (Agilent 7500s ICP-MS with New Wave 213 laser system) was used to measure the total concentration of dissolved elements. The initial rate of elemental dissolution was calculated as previously described [16]. Glucose concentration was measured using the Amplex red glucose kit (Invitrogen, Paisley, UK). The absorbance was measured at 595 nm and compared with a calibration curve of known glucose concentrations. Oxalate was measured using an oxalate oxidase assay (Trinity Biotech), at 590 nm, as per manufacturer's instructions.

## 2.9. Statistical Analysis

Statistical analysis was carried out using Open source R stat version 3.0.3 (R Development Core Team, 2010), using the Vegan Package [37]). Overall differences in taxa, at the four sample sites, were compared using an ANOSIM test of difference [38]. Confirmation of the significance of any differences was identified using independent *t*-test. The ANOVA test in Microsoft Excel was used to determine a relationship between chemical dissolution and specific growth rates.

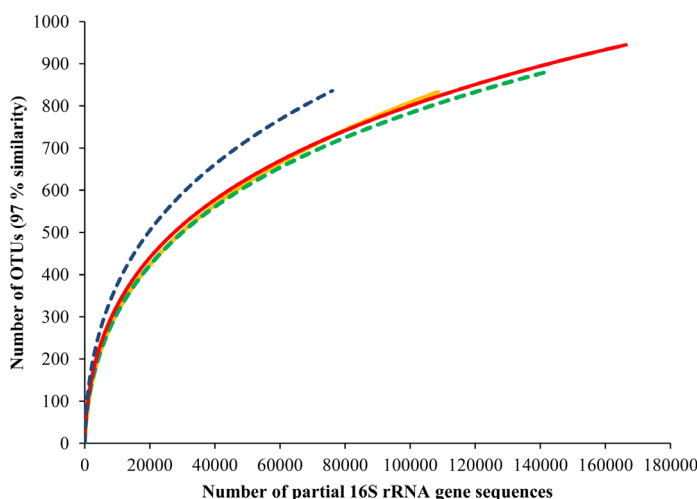
## 3. Results

### 3.1. Composition of the Bacterial Community

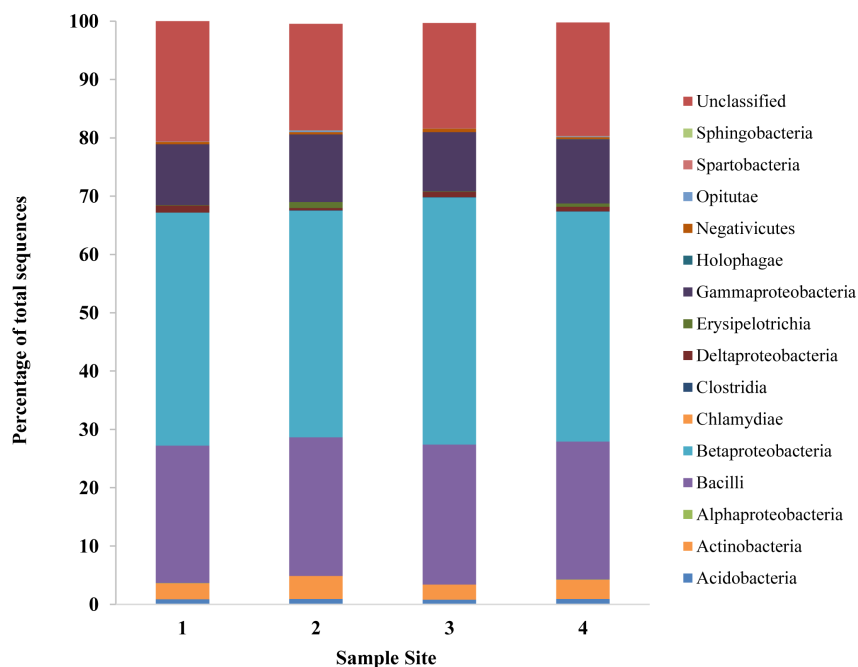
Ion torrent was used to identify the major bacterial classes at the interface between the outcrop and the soil. Each of the sample sites were represented by 74934 (Site 1), 76631 (Site 2), 72941 (Site 3) and 51703 (Site 4) bacterial sequences. Based on the rarefaction, the qualitative OTU (97% sequence similarity) richness, for each sample site, was high and did not appear to reach asymptote (Figure 1). Taxonomical assignment of the sequences demonstrated that over 85% of the microbial community was dominated by three bacterial classes (Figure 2). The most abundant class was the Beta-proteobacteria (Site 1, 39.98%; Site 2, 38.90%; Site 3, 42.38%; Site 4, 39.44%), which represented an overall relative abundance of  $40.17\% \pm 1.33$ . This was followed by Bacilli ( $23.73\% \pm 0.166\%$ ) and Gamma-proteobacteria ( $10.78\% \pm 0.56\%$ ). The dataset consisted of  $19.10\% \pm 1.04\%$  of unclassified sequences, overall.

### 3.2. Microbial Isolates

From the powdered granite plates eight isolates were obtained, which belonged to the bacterial classes, Bacilli, Gamma-proteobacteria and Beta-proteobacteria, as shown in Table 2. The Gamma-proteobacteria isolates were related to the genus *Pseudomonas* (G\_02, and G\_05) and *Serratia* (G\_01). The majority of the Bacilli isolates showed similar identities to cultivated strains of the genus *Bacillus* ranging from 99% to 100% (G\_02 and G\_14). The Beta-proteobacteria showed similar identities to *Burkholderia* (G\_20 and G\_22) and *Chromobacterium* (G\_23). Each of the isolates listed in Table 2 were screened for siderophore production using the Chrome Azurol S assay. In the minimal medium, without iron, seven of the isolates produced siderophores ( $p < 0.05$ ), as shown in Table 2. However, in the glucose-NH<sub>4</sub> medium with granite we were unable to detect any siderophore production (data not shown).



**Figure 1.** Rarefaction curves of the partial bacterial 16S rRNA gene sequences obtained from the interface between granite bedrock and nutrient limiting soil, using Ion Torrent. The curves show the relationship between the increased number of bacterial OTUs (97% sequence similarity) and the number of randomly samples sequences from each sample site (Site 1 (----); Site 2 (—); Site 3 (---) and Site 4 (----)).



**Figure 2.** The relative abundance of the 16 most common bacterial classes identified at the interface between the granitic bedrock and the nutrient limiting soil, at the four sample sites in Dartmoor, National Park, UK. Each sample was analysed in triplicate and the sequences for each sample site were pooled together.

### 3.3. Granite Weathering Experiment

The isolates were able to grow in glucose- $\text{NH}_4$  medium with granite, but not in the absence of granite. As demonstrated in **Figure 3**, growth varied between each of the isolates. For example, at 7 days G\_05 was in stationary phase (**Figure 3(b)**); whilst G\_10 was in early exponential phase (**Figure 3(h)**). For each of the isolates, the pH of the growth media decreased during growth, dropping during exponential growth to between 3.0 and 3.5, and then increasing to a steady-state equilibrium of between pH 4.0 and 6.0 (**Figure 3**). The bacteria consumed the glucose as a carbon source, which was demonstrated by a decrease in glucose concentration during growth (**Figure 4(a)**). As the glucose was consumed oxalate was produced (**Figure 4(b)**). The concentration of oxalate produced varied between the isolates, for example at day 28 the amount of oxalate produced by G\_01 was 150  $\mu\text{M}$  compared to 30  $\mu\text{M}$  (G\_05).

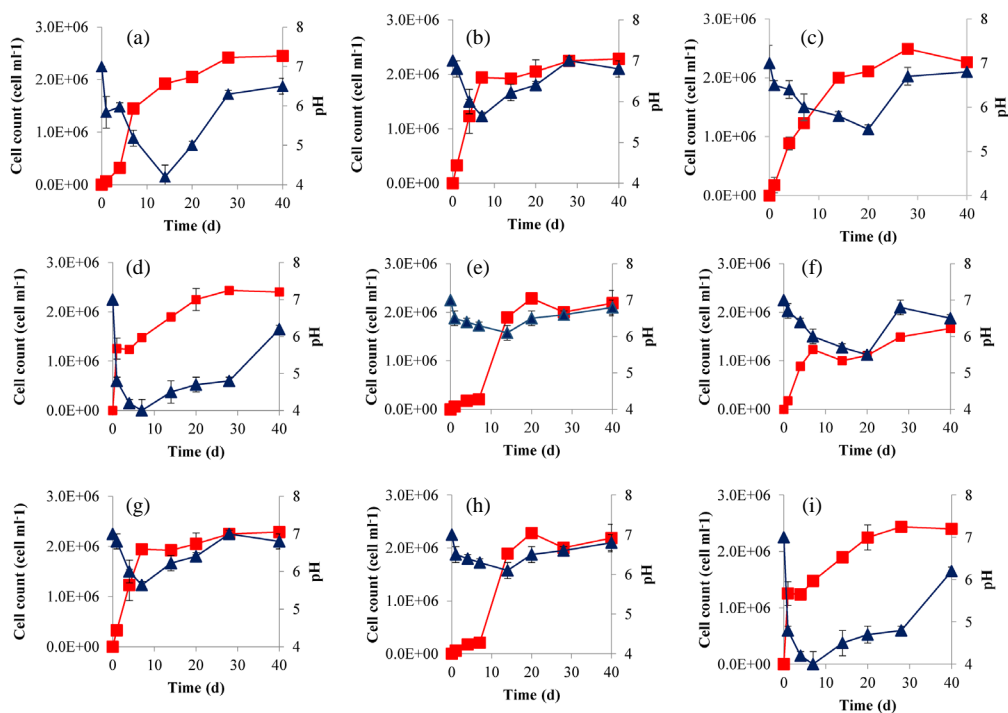
### 3.4. Elemental Release

Granite dissolution was measured through the release of key elements (Si, K, Ca and Mg) into the growth medium. The linear elemental release rates ( $R_i^l$ ) from the initial part of the experiment (days 1 to 7) were calculated, as shown in **Table 3**. The  $R_i^l$  values varied between the isolates, for example, the  $R_i^l$  value for K ranged from  $1.11 \pm 0.14 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  (G\_10) to  $3.87 \pm 0.05 \times 10^{-12} \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  (G\_01). Whereas, the  $R_i^l$  value in the abiotic control for K was  $1.53 \pm 0.03 \times 10^{-12} \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . Six of the isolates significantly enhanced the rate of Si, K, Ca and Mg dissolution ( $p < 0.05$ ), as shown in **Table 3**.

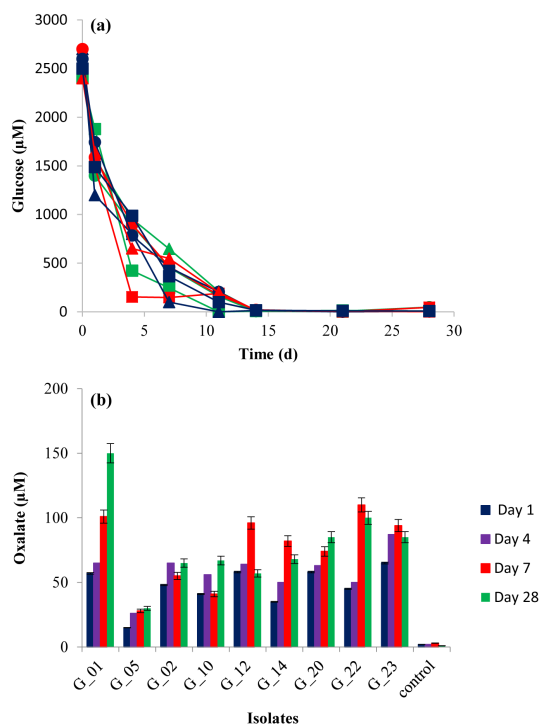
**Figure 5** demonstrates a relationship between Mg, Ca, Si and K release and pH. A Pearson's product-moment correlation shows a clear correlation between the  $R_i^l$  values for Mg ( $p < 0.001$ ), Ca ( $p < 0.001$ ), Si ( $p < 0.001$ ) and K ( $p < 0.001$ ) and the maximum decrease in pH ( $-\Delta\text{pH}$ ). A clear relationship was also demonstrated between the concentration of oxalate in the growth media and Mg, Ca, Si and K release, as demonstrated in **Figure 6**.

## 4. Discussion

The aim of this study was to further our understanding of the role that bacteria play in rock weathering, in nutrient



**Figure 3.** Cell counts (■) and change in pH (▲) of the medium over time for each of the isolates: G\_22 (A), G\_05 (B), G\_23 (C), G\_20 (D), G\_14 (E), G\_02 (F), G\_23 (G), G\_10 (H), G\_01 (I). The values reported are the means of three independent experiments, and the standard error associated with these determinations is shown.



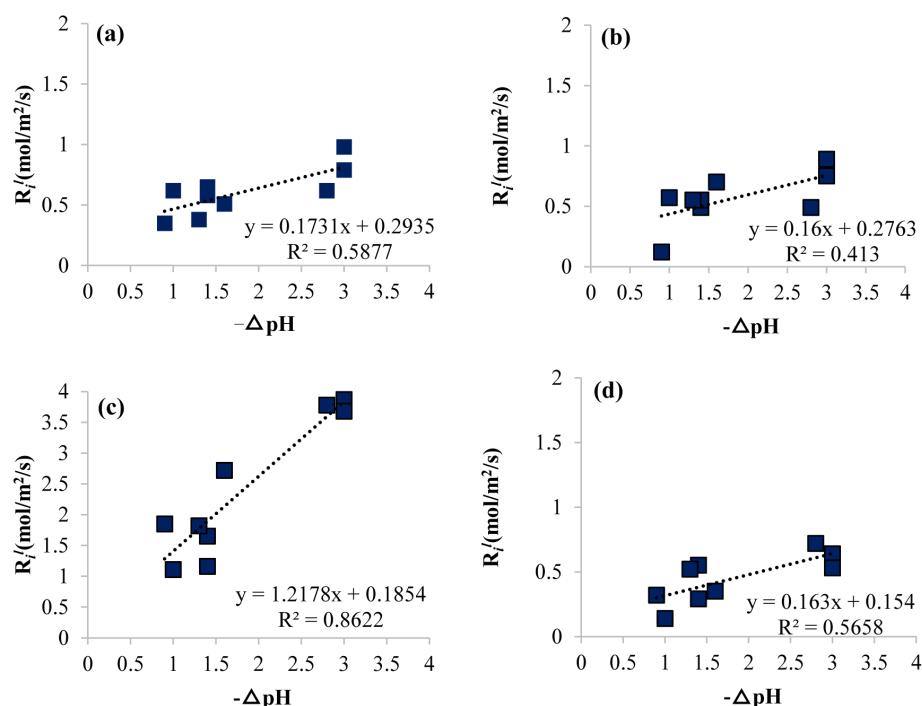
**Figure 4.** (a) Concentration of glucose in the growth medium for each of the isolates G\_22 (■); G\_05 (■); G\_23 (■); G\_20 (▲); G\_14 (▲); G\_02 (▲); G\_23 (●); G\_10 (●); G\_01 (●). Concentration of oxalic acid in the growth medium after 1, 4, 7 and 28 days. The values reported are the means of three independent experiments, and the standard error associated with these determinations is shown.



**Table 3.** Biotic and abiotic mediated linear release rates of Si, K, Ca and Mg dissolution.

Isolates	Dissolution rate ( $10^{-12} \text{ mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )			
	Mg	Si	K	Ca
G_01	$0.98 \pm 0.23^{**}$	$0.64 \pm 0.10^{**}$	$3.87 \pm 0.05^{**}$	$0.99 \pm 0.11^{**}$
G_05	$0.51 \pm 0.12^*$	$0.45 \pm 0.01^*$	$2.72 \pm 0.14^{**}$	$0.50 \pm 0.04^{**}$
G_02	$0.38 \pm 0.13^*$	$0.52 \pm 0.05^*$	$1.82 \pm 0.02^*$	$0.55 \pm 0.08^*$
G_10	$0.62 \pm 0.24^*$	$0.44 \pm 0.05^*$	$1.72 \pm 0.04^*$	$0.57 \pm 0.17^*$
G_12	$0.58 \pm 0.32$	$0.29 \pm 0.12$	$1.65 \pm 0.38$	$0.55 \pm 0.09^*$
G_14	$0.35 \pm 0.21$	$0.42 \pm 0.11$	$1.85 \pm 0.01^*$	$0.30 \pm 0.11$
G_20	$0.79 \pm 0.38^*$	$0.53 \pm 0.11^*$	$3.68 \pm 0.06^{**}$	$0.89 \pm 0.11^{**}$
G_22	$0.62 \pm 0.2^*$	$0.72 \pm 0.20^{**}$	$3.78 \pm 0.12^{**}$	$0.89 \pm 0.14^{**}$
G_23	$0.65 \pm 0.11^*$	$0.55 \pm 0.16^*$	$1.16 \pm 0.01$	$0.49 \pm 0.47$
Abiotic control	$0.29 \pm 0.02$	$0.19 \pm 0.14$	$1.53 \pm 0.03$	$0.25 \pm 0.12$

<sup>\*\*</sup>The value is highly significant ( $p < 0.01$ ); <sup>\*</sup>The value is significant ( $p < 0.05$ ).

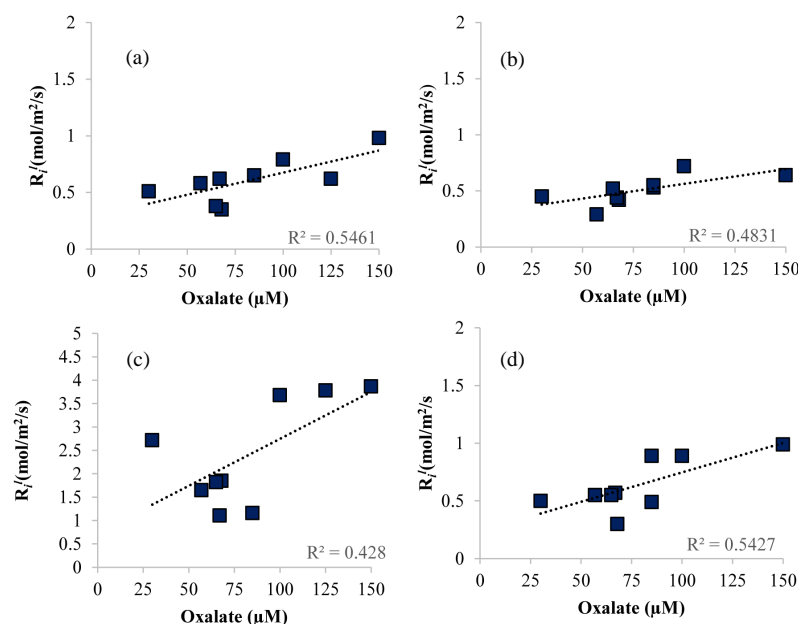


**Figure 5.**  $R_i$  values for Mg (a), Ca (b), K (c), and Si (d) versus  $-\Delta\text{pH}$  (change in pH), for each of the bacterium. The R value was determined using a Pearson's product-moment correlation.

limiting soil. For this study we focused on granite bedrock, because it is the most common rock type in the continental land mass. Samples were collected from below any visible root systems, which reduced the direct influence of the above ground vegetation and the associated rhizosphere bacterial community, which have been shown to influence mineral weathering [3] [39]–[43].

High throughput sequencing was used to obtain a 16S rRNA gene library large enough to be representative of the bacterial community. Although the rarefaction curve did not reach asymptote, we obtained over 276,209 sequences after quality filtering. This allowed for an in-depth analysis of the microbial diversity at the interface between the granite and soil at this location, which demonstrated that the community was dominated by Beta-proteobacteria ( $40.17\% \pm 1.33\%$ ). This is in agreement with previous work that has demonstrated that the bacterial





**Figure 6.**  $R_i$  values for Mg (a), Ca (b), K (c), and Si (d) versus oxalate, for each of the bacterium. The R value was determined using a Pearson's product-moment correlation.

community on the surface of minerals, such as plagioclase (an important component of granite) and apatite (a phosphate-bearing accessory mineral) are dominated by Beta-proteobacteria [20] [24].

A percentage of these sequences were also identified as unclassified ( $19.10\% \pm 1.04\%$ ). This was potentially due to the short-read lengths, which has made detailed phylogenetic characterisation of the data difficult. Subsequent to this study, 400 bp sequencing has become possible using the Ion Torrent Personal Genome Machine which could, to some extent, overcome this problem. However, in previous studies unclassified sequences have had substantial overlap with the so called “rare biosphere”, which are low in abundance and are poorly understood [28] [44], but they may play a crucial role in modifying their environment. For example, in our data set we identified the novel genera *Ferribacterium* and *Ferrithrix* in low abundance ( $<0.001\%$ ), both of which are involved in iron cycling [45] [46]. These novel genera may play a role in weathering iron-bearing minerals, such as biotite, which is present in granite. However, future work is required to determine the ability of these genera to weather specific silicate minerals.

Linking diversity to mineral weathering requires culturing members of the bacterial community to investigate their weathering potential [1] [47]. The isolation procedure that was used in this study selected for heterotrophic aerobes, which resulted in the isolation of members of the genera *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Serratia*, *Burkholderia* and *Chromobacterium*. Each of the isolates was able to grow using granite as a sole source of bio-essential elements. Six of the isolates *Burkholderia* (G\_20 and G\_22), *Pseudomonas* (G\_05 and G\_02), *Serratia* (G\_01) and *Paenibacillus* (G\_10) significantly enhanced the release of elements from the granite. Previous laboratory based experiments have demonstrated that members of these genera are able to enhance mineral weathering [3] [14] [42] [48]–[51]. Furthermore, in nutrient limiting forest soil, Uroz *et al.*, [20] [24] demonstrated a potential correlation between the abundance of the genus *Burkholderia* and the rate of apatite, plagioclase and phlogopite-quartz dissolution.

Under nutrient limiting conditions, bacteria have been shown to produce siderophores, which are known to enhance the rate of iron-oxide and iron-silicate dissolution by approximately one order of magnitude [52]–[55]. The production of siderophores is dependent on the lack of available soluble iron. In this paper, siderophore production was measured in the minimal growth medium, without the granite (no iron source); however, no siderophore production was detected in the presence of granite. This is in agreement with previous studies, which have been unable to detect siderophore production in the presence of basalt or granite [14] [34]. For example, Akers and Magee [56] demonstrated that volcanic ash and silicate rocks repressed the synthesis of the

siderophore rhodotourulic acid by *Rhodotoruliapilimanae*. Potentially, sufficient iron was leached from the silicate material in aqueous state ( $\text{Fe}^{2+}$ ) to repress siderophore production.

Aerobic heterotrophic bacteria are also known to increase silicate weathering through acidification either through organic acids or proton-promotion [57]–[60]. Several organic acids, such as acetic, gluconic, citric and oxalic have been shown to enhance weathering [52] [59]–[61]. The organic acids act as a ligand, which directly affects mineral dissolution by complexing metal ions at the surface and therefore assisting the release of metals through ligand-promoted dissolution [61]. In this study, a correlation between the concentration of oxalate in the growth medium and dissolution was demonstrated. Although it is difficult to distinguish between organic ligands and proton-mediated dissolution, in acidic conditions, organic acids are more likely to be protonated. Therefore the carboxyl acid is a weaker complex agent of metals, suggesting that proton-mediated dissolution is dominate [62].

## 5. Conclusion

In this study, we used a culture-dependent and culture-independent approach to study the microbial community at the interface between granite bedrock and soil in a nutrient limiting environment. The results from this study suggest that heterotrophic bacteria can enhance the rate of dissolution. Future work will focus on the mechanism bacteria employ to sequester bio-essential elements. This is important for further understanding the regulation of biogeochemical cycling in nutrient limiting environments.

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