TITLE: Mineral type and solution chemistry affect the structure and composition of actively growing bacterial communities as revealed by bromodeoxyuridine immunocapture and 16S rRNA pyrosequencing

AUTHORS: Kelly L.C. [1,2,a], Colin Y. [1,2,3], Turpault M-P. [3], Uroz S. [1,2,3*]

[1] INRA, UMR1136 Interactions Arbres-Microorganismes, 54280 Champenoux, France ; [2] Université de Lorraine, UMR1136 Interactions Arbres-Microorganismes, 54500 Vandoeuvre-lès-Nancy, France ; [3] INRA UR 1138 "Biogéochimie des Ecosystèmes Forestiers", Centre INRA de Nancy, 54280 Champenoux, France.

Short title: Minerals activate specific bacteria

[a] present address**:** School of Science and the Environment, Division of Biology and Conservation Ecology, Manchester Metropolitan University, M1 5GD, Manchester, UK

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*Corresponding author: Mailing address: UMR 1136 INRA Lorraine Université, Interactions Arbres Micro-organismes, 54280 Champenoux, France. Phone: +33 (0)3 83 39 40 81, Fax: +33 (0)3 83 39 40 69. E-mail: uroz@nancy.inra.fr

ABSTRACT

Understanding how minerals affect bacterial communities and their *in-situ* activities in relation to environmental conditions are central issues in soil microbial ecology, as minerals represent essential reservoirs of inorganic nutrients for the biosphere. To determine the impact of mineral type and solution chemistry on soil bacterial communities, we compared the diversity, composition and functional abilities of a soil bacterial community incubated in presence/absence of different mineral types (apatite, biotite, obsidian). Microcosms were prepared containing different liquid culture media devoid of particular essential nutrients, the nutrients provided only in the introduced minerals and therefore only available to the microbial community through mineral dissolution by biotic and/or abiotic processes. By combining functional screening of bacterial isolates, and community analysis by bromodeoxyuridine DNA immunocapture and 16S rRNA gene pyrosequencing, we demonstrated that bacterial communities were mainly impacted by the solution chemistry at the taxonomic level, and by the mineral type at the functional level. Metabolically active bacterial communities varied with solution chemistry and mineral type. *Burkholderia* were significantly enriched in the obsidian treatment compared to the biotite treatment and were the most effective isolates at solubilizing phosphorous or mobilizing iron, in all the treatments. A detailed analysis revealed that the 16S rRNA gene sequences of the OTUs or isolated strains assigned as *Burkholderia* in our study showed high homology with effective mineral weathering bacteria previously recovered from the same experimental site.

INTRODUCTION

Rocks and minerals represent physical support and essential sources of nutritive elements for the long-lasting development of the biosphere. This is especially the case in the critical zone, which encompasses from the top of the vegetation canopy to the base of the weathered bedrock (U.S. Natl. Res. Council Comm. Basic Res. Oppor. Earth Sci. 2001). Indeed, soil is composed of mosaics of rocks and minerals varying in their type, chemical composition, size, reactivity and weatherability [1]. Besides nutrients from atmospheric deposits, and the recycling of nutrients contained in organic matter, minerals represent an important reservoir of soil inorganic nutrients. However, these nutrients are not directly accessible to plants and microorganisms due to the inertness of rocks and minerals to environmental conditions. Certain minerals such as apatite are weathered quickly in acidic conditions, while minerals such as phyllosilicates remain comparatively recalcitrant [2]. In this context, all these rocks and minerals differentially impact the biosphere due to their different inherent properties.

For decades, it is well established that mineral weathering results from a combination of abiotic and biotic processes contributing to the release of the chemical elements entrapped in their crystal structure [64]. Abiotic processes such as water circulation, alternative freeze-thaw events and the acidification of their surrounding environment are known to impact mineral stability [3]. At the same time, plants, fungi, lichens and bacteria contribute to the dissolution of rocks and minerals [4-9]. Through the force applied by roots or hyphae [10] on mineral particles, plants and fungi are known to physically disrupt them, releasing nutrients and generating new weatherable surfaces. Lichens, which may colonize the same rock surface for centuries, are known to produce acidifying and chelating metabolites that contribute to mineral weathering and lichen nutrition [4]. Indeed, the production of protons, organic acids and siderophores have been evidenced for plants and lichens, as indeed for fungi and bacteria [5,9,11]. The role of mycorrhizal fungi in mineral weathering is now well established [7,8,12]. Their role in the formation of tunnels into mineral particles was even proposed [13]. Through the symbiotic association formed with their plant partner, mycorrhizal fungi scavenge the soil environment and transfer inorganic

71 nutrients to the plant in exchange of photosynthates. The contribution of soil bacterial communities in
72 mineral weathering, nutrient cycling and plant nutrition has also been demonstrated [9,14-16]. Several
73 studies reported the presence and even the enrichment of effective mineral weathering bacteria in the
74 rhizosphere of different plants, suggesting an active role in nutrient mobilization and plant nutrition [15-
75 17]. Notably, using a microcosm experiment, it was demonstrated that the introduction on an effective
76 mineral weathering bacterial strain increased significantly mineral dissolution, tree nutrition and growth
77 comparatively to non-inoculated controls [16].
78 Where the role of microorganisms in mineral weathering processes was evidenced, it was primarily
79 from a plant nutrition perspective, focusing mainly on the root environment and its extensions (i.e.
80 rhizosphere or mycorrhizosphere). All these studies clearly highlighted that the rhizosphere is a reactive
81 interface where nutrients are intensively released, and a microbial habitat where microorganisms
82 capable of weathering minerals are enriched [17-18]. However other reactive interfaces and microbial
83 habitats may exist. Among them, rocks and minerals represent a poorly investigated interface [1].
84 Indeed, rocks and minerals are usually considered as inert substrata for soil microbiologists, although
85 they contain essential nutrients and trace elements. Nevertheless, several studies in terrestrial and
86 aquatic environments have now evidenced that rocks and minerals support the development of complex
87 microbial communities, and even select specific microorganisms according to their physicochemical
88 properties [19-24]. Comparing different types of mineral inclusions present in granite, Gleeson et al. [19-
89 20] revealed that both bacterial and fungal communities were structured according to the chemical
90 composition of minerals, while Icelandic bacterial communities were found to differ on volcanic rocks of
91 different chemical composition [23-24].
92 Together, these data show that microorganisms are capable of contributing to mineral weathering,
93 nutrient cycling and plant nutrition, but also that their distribution and diversity is strongly determined by
94 the physicochemical properties of rocks and minerals. However, we can wonder how minerals impact
95 the functioning of soil microbial communities. A few studies performed on model fungal or bacterial

strains, have demonstrated that specific functions were up- or down-regulated when mineral particles were introduced in the culture medium [25-26]. The possibility that introduction of minerals into the culture medium imposes upon to bacteria a stress was even proposed by Bryce *et al*. [55]. In this context, our aim in this study was to determine whether minerals select and stimulate specific bacterial communities. We hypothesized that minerals, according to their physicochemical properties and the nutrient availability of their surrounding environment, impact the diversity, composition, and function of the soil bacterial communities. To test this hypothesis, microcosm experiments were developed to test how a same bacterial community is structured by the presence or absence of different mineral types under similar culture conditions. Two culture media were adapted to create nutrient deficiency complementary to the nutrients contained in these minerals. In a first condition, iron-carrying minerals were incubated in a culture medium lacking iron, while in a second condition, phosphorous and calcium-carrying minerals were incubated in a culture medium lacking calcium and phosphorous. Concentrations of the nutrients released from the introduced minerals were determined in each condition after incubation. To assess the total and metabolically active bacterial communities, we combined 16S rRNA gene amplicon pyrosequencing with a bromodeoxyuridine (BrdU) immunocapture method. Bromodeoxyuridine is incorporated into the DNA of replicating cells as a thymidine analogue, labeling the metabolically active bacterial communities [27-31]. Total and BrdU-labelled DNA were used to determine the diversity and composition of the total and metabolically active bacterial communities respectively. Finally, to assess the mineral weathering potentials of bacteria in these different conditions, a collection of bacterial isolates coming from the different treatments with or without minerals was screened for the ability to solubilize phosphorus and mobilize iron.

MATERIALS AND METHODS

Soil sampling and inoculum preparation

On 15th October 2012, soil samples were taken from the Breuil-Chenue experimental forest site in the Morvan (France, 47°18'N, 4°5'E). The soil of the Breuil-Chenue experimental forest site is an Alocrisol, developed on the "Pierre qui Vire" granite. The bulk soil is sandy-loam textured (60% sands and <20% clays) and acidic (pH ca 4) [65]. Soil cores (20x20x20 cm) were sampled in the coppice with standards stand, after removing leaf litter. Soil samples were transported back to the lab, stored overnight at 4 °C, after which the mineral horizon (5-15 cm) was separated. After homogenization, a total of 75 g of soil was mixed with 500 mL of sterile water, and a slurry was made. Briefly, soil and sterile water were homogenized three times for 1 min in a Waring Blender (Waring, Torrington, CT, USA), cooling on ice for 5 min between homogenizations. Bacterial cells were then extracted using Nycodenz (Axis Shield, Oslo, Norway) density gradient (1.3 $g.mL^{-1}$) centrifugation, according to Lindahl and Bakken [32]. Sterile Nalgene tubes (Thermo Scientific, Waltham, MA, USA) (50 mL) were filled with 20 mL of slurry and 11.6 mL of Nycodenz solution. Following centrifugation at 10,000 xg and 15 °C for 90 min, both the upper aqueous layer and the interface were recovered together. Bacterial cells were washed twice with sterile water and suspended in 5 mL sterile water. This extract was used as inoculum for the all the microcosm experiments described below. Part of this suspension was also cryopreserved in 20% glycerol. Bacterial concentration was estimated at $1.63x10^5$ CFU mL^{-1} by plate counting on nutrient-poor culture medium (1/10 diluted Tryptic Soy Agar (TSA)).

Mineral selection

Minerals commonly found in the environment were selected according to their contrasting chemistry and weatherability. Biotite, apatite and obsidian were chosen as mineral substrata for microcosm experiments. The chemical composition of these minerals in $g.kg^{-1}$ was as follows: i) apatite (Morocco): SiO_2, 8.1; Al_2O_3, 1; Fe_2O_3, 0.95; MnO, 0.34; MgO, 0.16; CaO, 526.35 ; Na_2O, 2.32; K_2O, < below detection level (bdl) ; TiO_2, <bdl; P_2O_5, 412.6. ii) biotite (Canada): SiO_2, 390.5; Al_2O_3, 110.6; Fe_2O_3, 197.7; MnO, 7.1; MgO, 145; CaO, 0 ; Na_2O, 0; K_2O, 93.4; TiO_2, 20.9; P_2O_5, 0. iii) Obsidian (Mexico)

146 SiO_2, 763.8; Al_2O_3, 125.97; Fe_2O_3, 11.87; MnO, 0.56; MgO, 0.75; CaO, 6.23 ; Na_2O, 39.46; K_2O, 48.18;
147 TiO_2, 1.32; P_2O_5, < bdl. Their chemical composition was determined with ICP-AES (Inductively Coupled
148 Plasma – Atomic Emission Spectroscopy), after alkaline fusion with $LiBO_2$ and dissolution in HNO_3.
149 These minerals were ground, treated ultrasonically, washed three times with distilled water, calibrated
150 (mesh size 200-400 µm) and dried.
151
152 ***Microcosm design***
153 The microcosms consisted of 100 mL capped Erlenmeyer flasks filled with 40 mL of sterile culture
154 medium devoid of specific nutritive elements (see below), amended or not with 2 g of autoclaved
155 mineral particles. The microcosm experiment was separated into two conditions (Figure 1). The first
156 condition consisted of flasks filled with a modified Bushnell and Haas medium [33] (BHm) devoid of iron,
157 together with 2 g of size-calibrated biotite or obsidian. The BHm composition was as follows ($g.L^{-1}$): KCl,
158 0.020, $MgSO_4.7H_2O$, 0.150; $NaH_2PO_4.2H_2O$, 0.080; $Na_2HPO_4.2H_2O$, 0.090; $(NH_4)_2.SO_4$, 0.065; KNO_3,
159 0.100; $CaCl_2$, 0.020, buffered at pH 6.5 and supplemented with glucose (2 $g.L^{-1}$). The second condition
160 consisted of flasks containing a modified National Botanical Research Institute's phosphate growth
161 medium [34] (NBRIPm) lacking calcium and phosphorous, together with 2 g of size calibrated apatite or
162 a (1:1) mix of apatite and biotite. The apatite:biotite mix was used as a treatment to determine the
163 impact of decreasing apatite quantity, with biotite added to preserve the same total weight of mineral
164 particles in the culture medium. The NBRIPm composition was as follows ($g.L^{-1}$): $MgCl_2.6H_2O$, 5;
165 $MgSO_4.7H_2O$, 0.25; KCl, 0.20; $(NH_4)_2,SO_4$, 0.10, KNO_3, 0.100, adjusted to pH 6.5 and supplemented
166 with glucose (2 $g.L^{-1}$). Each experimental condition included additional inoculated flasks containing
167 culture medium but without mineral particles, to determine the relative effect of mineral addition in our
168 experimental conditions on the total and metabolically active bacterial communities. The culture media
169 and the carbon source were selected to focus on the heterotrophic bacterial communities due to their

ability to weather minerals in these experimental conditions, as evidenced on the experimental site of Breuil-Chenue [17].

For each condition (BHm or NBRIPm), six flasks were prepared for each of the above mineral-containing or mineral-free treatments. A thymidine analog, bromodeoxyuridine (BrdU; Roche®), was added at a final concentration of 30 μM (+BrdU) into three flasks each. The remaining three flasks remained BrdU-free (-BrdU). Microcosms were inoculated using 100 μL of inoculum, which corresponded to 4.06 $x10^2$ $CFU.mL^{-1}$. Uninoculated microcosms additional to those described above were included as controls, serving to check for contamination of media, minerals and BrdU.

Flasks were incubated at 22 °C with agitation at 140 rpm in an orbital shaker (INFORS/ MINITRON). Based on preliminary experiments and to allow sufficient time for mineral dissolution, production of bacterial biomass and BrdU labeling, and limiting the possibility of BrdU incorporation further in the trophic chain, microcosms were incubated for 8 d. After this period, a total of 16.5 mL of supernatant was removed. A volume of 15 mL of this supernatant was centrifuged at 8,000 xg to recover bacterial communities for DNA analyses and the remaining supernatant was filtered through 0.22 μm pore size filters to remove biomass and mineral particles before chemical analyses. Additionally, 1.5 mL was stored in 20% (v/v) glycerol at -80 °C for subsequent culturing.

Microcosms supernatant analyses and mineral particles observation

The 15 mL supernatants were analyzed for nutrients devoid in the initial solution and only present in the introduced mineral particles. The concentrations of Al and Fe in the BHm condition and Ca and P in the NBRIPm condition were determined by inductively coupled plasma-atomic emission spectrometry (700 Series ICP-OES, AGILENT TECHNOLOGIES). The pH was measured with a pH-meter (DL70 ES, METTLER). Chemical analyses were performed in all the independent replicates, except in the non-inoculated treatments for which technical replicates were analyzed. Additionally, the mineral particles

were collected and a subsample was observed using a Hitachi S2500 scanning electron microscope (SEM).

Bacterial collections and taxonomic characterization

For each inoculated Erlenmeyer flask, whatever the treatment, a volume of 0.2 mL of the glycerol-preserved samples was used to perform serial dilutions in sterile distilled water. Dilutions were spread onto a 1/10 diluted Tryptic Soy Agar (TSA) medium (Tryptic Soy Broth from Difco, 3 $g.L^{-1}$ and agar, 15 $g.L^{-1}$). Colonies were counted after a 5 d incubation period at 25°C and expressed as colony forming units (CFU) mL^{-1}. Bacterial colonies were collected at random and were purified by three successive platings on 1/10 diluted TSA. Up to ten colonies were collected at random from each of the biological replicates for each treatment, giving up to 60 isolates per treatment (BHm alone without mineral, BHm+biotite, BHm+obsidian; NBRIP alone without mineral, NBRIP+apatite, NBRIP+ apatite+biotite). A total of 377 bacteria were isolated and tested for their ability to mobilize inorganic nutrients using functional assays to permit to determine. All the isolates used in this study were cryopreserved at -80 °C in 20% glycerol and then cultivated on 1/10 diluted TSA at 25 °C. Taxonomic characterization was performed on a subsample of 141 bacterial isolates (ca 20 isolates per condition, randomly selected) from this collection, using the universal set of 16S RNA primers pA (5'-AGAGTTT- GATCCTGGCTCAG-3') and 907r (5'-CCGTCAATTCMTTTGAGTTT- 3') [35-36]. Polymerase chain reactions were performed in a total reaction volume of 50 µL containing 20 µL PCR Mastermix (5-PRIME), 2 µL of primers (10 µM) and 4 µL of cell extract. Cell extract was prepared by adding one isolate colony into 100 µL of sterile water. The following temperature cycle was used: an initial denaturation step of 4 min at 94 °C followed by 30 cycles of 30 s denaturation at 94 °C, 1 min annealing at 53 °C and 1 min 30 s extension at 72 °C, and a final extension for 10 min at 72 °C. PCR products were purified and concentrated using the QiaQuick PCR purification kit (Qiagen). The Sanger sequencing reactions were performed by MWG Eurofin Operon. The sequencing primer used was pA [35]. The partial 16S rRNA gene sequences were

compared with the sequences in the GenBank databases (www.ncbi.mlm.nih.gov.BLAST), using the BLAST program [37] and taxonomically assigned.

Functional bioassays

The ability of bacterial isolates to solubilise tricalcium orthophosphate (TCP) was assessed following the modified protocol of Lepleux et al. [38], which allows the detection of organic acids and protons. Their ability to mobilize iron was assessed using a chrome azurol S (CAS) assay, which allows the detection of chelating compounds (organic acids and siderophores)[39]. These two bioassays are commonly used to determine the mineral weathering potential of bacterial strains [9,40-41]. In this study we used a 1/10 diluted TCP medium [38] composed of the following ($g.L^{-1}$) in distilled water: 0.5 g NH_4Cl, 0.1 g NaCl, 0.1 g $MgSO_4$, $7H_2O$, 1 g glucose, 0.4 g $Ca_3(PO_4)_2$ and 15 g agar. Each 1 L of CAS medium contained 800 mL of Solution 1 (34.36 g of Pipe Na_2 buffer (Sigma), 0.3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 0.246 g $MgSO_4$,$7H_2O$, 0.0147 g $CaCl_2$,$2H_2O$ and 15 g agar in 800 ml H_2O; pH 6.8), 100 mL of Solution 2 (4 g glucose in 100 mL H_2O) and 100 mLof Solution 3 (mix of 0.0905 g chrome azurol S in 75 mL, 0.0024 g $FeCl_3$ in 15 mL, 0.1640 g hexadecyl trimethyl ammonium bromide in 60 mL).

For each bioassay, each bacterial isolate was first grown on 1/10 diluted TSA agar medium at 25 °C for 72 h, then subcultured for 72 h in Luria Bertani (LB) broth at 25 °C. Luria Bertani (LB) broth was here used to generate higher bacterial biomass. Biomass was then collected, washed three times in sterile water and suspended in 2 mL sterile water to obtain a suspension at λ595 nm = 0.8 (ca. 10^9 cell. mL^{-1}). Ten microliters of this suspension was then dropped in triplicate onto one plate of each TCP and CAS medium. After incubation at 25 °C for 7 d, the diameter of each colony and the diameter of discoloration zone were measured to determine the ability of each bacterial isolate to mobilize iron (CAS) and to solubilize inorganic phosphorous (TCP). The diameter of the discoloration zones was used to determine i) the relative efficacy of each bacterial isolate in each bioassay and ii) their distribution into two classes of efficacy based on the presence or absence of discoloration zones on the different media.

244

245 ***Microcosm DNA extraction and recovery of BrdU-labeled DNA***

246 Pellets obtained after centrifugation of the 15 mL of microcosm supernatants were used to extract total
247 DNA, from all microcosms. This strategy was used as no bacterial development was observed on
248 mineral particles by epifluorescence microscopy. Total DNA was extracted from pellets using the
249 PowerSoil DNA isolation kit (MO-BIO Laboratories Inc., Carlsbad, CA, USA) after minor modifications of
250 the protocol as follows. The samples were treated according to the manufacturer's instructions, except
251 that pre-treatments with lysozyme (1 $mg.mL^{-1}$ final concentration) and proteinase K (0.5 $mg.mL^{-1}$ final
252 concentration) were added. Total DNA was similarly extracted from the inoculum, hereafter referred to
253 as 'Inoculum'. The quantity and quality of the total DNA were evaluated by agarose gel electrophoresis
254 and with a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

255 DNA immunocapture was used to recover BrdU-labeled DNA from total DNA according to the protocol
256 of McMahon et al. [30]. The protocol was applied to all total DNA extracts described above, regardless
257 of the presence/absence of BrdU in the source microcosm. Briefly, 20 μL of total DNA extract was
258 denatured at 100 °C and cooled rapidly, then added to a herring sperm-anti-BrdU antibody mix and
259 incubated at room temperature (30 min). Following incubation, anti-BrdU magnetic beads (Dynabeads;
260 Invitrogen) washed with phosphate buffered saline were added and the samples hybridized for 40 min
261 at room temperature. Following hybridization, tubes were placed in a magnetic stand and beads washed
262 eight times to remove non-BrdU-labeled DNA. To recover the labeled DNA, 1.7 mM anti-BrdU was
263 added to the tubes and incubated at room temperature for 40 min. The liquid phase recovered following
264 this incubation containing, in theory, only the labeled DNA.

265

266 ***16S rRNA gene amplicon pyrosequencing and bioinformatics processing***

267 All total and BrdU DNA samples were subjected to amplification of 16S rRNA genes under the
268 following conditions: 94 °C for 5 min, followed by thirty cycles of 94 °C for 1 min, 52 °C for 1 min and 72

°C for 1 min, with a final extension at 72 °C for 10 min. Each reaction contained, per 50 μL reaction volume: 1.5 μL template DNA, 1.5 μL $MgCl_2$ (25 mM; Promega), 1.0 μL dNTP mix (10 mM; Promega); 1.5 μL each primer (10μm) and 2.5 U Taq polymerase (Taq PCR Core Kit, Qiagen). Primers used were 787r (xxxx-attagataccytgtagtcc) [42] and 1073f (xxxx-acgagctgacgacarccatg) [42], where xxxxx represents a 5 base unique barcode tag for each sample. Amplifications were replicated six times for each sample, products pooled and purified using a QiaQuick PCR purification kit (Qiagen) and subsequently visualized on a 1% agarose gel stained with ethidium bromide. The amplicon length and concentration were estimated by agarose gel electrophoresis and with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). An equimolar mix of all amplicon libraries was used for pyrosequencing on the Genome Sequencer (GS) FLX 454 Titanium platform (Roche) at Beckman Coulter Genomics (Danvers, MA, USA). Pyrosequencing resulted in 648,337 reads, of which 543,652 passed length (>300bp) and quality criteria (quality score(mean) ≥25)[44]. MOTHUR was used to trim, denoise and align the reads and to generate the operational taxonomic units (OTUs; 97 % sequence similarity) [45]. From each of the samples, 2,244 sequences (corresponding to the smaller set of sequences after MOTHUR processing) encompassing the V5 and V6 hypervariable regions of the 16S rRNA gene were randomly selected, yielding a total of 78,540 high-quality FASTA-formatted sequences. Sequences were classified using the Mothur Bayesian classifier (80% confidence) utilizing the Ribosomal Database Project (RDP) training set (v.9).

Statistical analyses

The effect of the conditions (BHm vs NBRIPm) and treatments (with and without minerals) on the solution chemistry was determined by analysis of variance (two factor ANOVA; factor 1= inoculation, and factor2= minerals). Culturable bacteria counts were compared as Log CFU number between the different conditions and treatments through ANOVA. For the 16S rRNA gene sequence data set, analysis of variance (ANOVA, $p < 0.05$) and multivariate analyses were done. For the functional assays

294 (phosphorous solubilisation and iron mobilisation), the effect of the treatments (with or without mineral)
295 on the functional ability was determined by one factor ANOVA at a threshold level of 0.05. Two classes
296 of bacterial isolates were discriminated according to their ability to produce or not a discoloration zone
297 on the TCP and CAS assays: 'non effective', no halo; 'effective', presence of a halo. The proportions of
298 isolates per class were compared using a Chi2 test ($P < 0.05$) associated to a Monte Carlo test with
299 5000 simulations and a Marascuilo procedure. Analyses of similarity (ANOSIM) based on Bray-Curtis
300 distances were performed on 16S rDNA pyrosequence data using the ANOSIM function of the R Vegan
301 package [46].
302
303 ***Accession numbers***
304 The 454 pyrosequencing data generated for this study were submitted to the Sequence Read Archive
305 (SRA) and are available under the Bioproject ID: PRJNA169429 and submission number SUB1187487.
306 The partial 16S rRNA gene sequences of 141 bacterial isolates from the different microcosm conditions
307 have been deposited into GenBank under the accession numbers KU060006 to KU060146.
308
309 **RESULTS**
310 ***Chemical analyses of the solution and bacterial population sizes***
311 Variations of pH were observed between the different treatments of both BHm and NBRIPm media
312 (Table 1; ANOVA 2 factor, $P<0.02$ for the BHm medium and $P<0.0001$ for the NBRIPm medium) after 8
313 d incubation. In the BHm condition, significant acidification was observed only for the mineral-free
314 inoculated treatment ($P<0.027$; pH inoculated mineral-free=4.7, pH 6.5 and 6.3 for the inoculated
315 treatments containing biotite and obsidian, respectively). In the NBRIPm condition, acidification was
316 observed for all the inoculated treatments, reaching an average pH of 3.75 compared to the non-
317 inoculated treatments ($P<0.0001$). No significant difference of pH was observed between the inoculated
318 treatments with or without minerals ($P=0.98$). Although the chemical analyses were done only on the
319 culture supernatants without integration of the nutrient content of bacterial cells, measures in both

media revealed significant differences (Table 1). In the BHm condition, the amounts of iron and aluminum measured in solution were very low. For aluminium a weak decrease was observed between uninoculated and inoculated treatments ($P<0.0001$), only significant for the obsidian treatment ($P<0.0001$). For iron, no significant differences were observed between inoculated and uninoculated mineral treatments ($P=0.95$). In the NBRIPm condition, significantly more calcium and phosphorous were detected in the treatments containing minerals and inoculum than in other treatments, including uninoculated mineral treatments ($P<0.0001$). The densities of total culturable bacteria differed significantly between the two conditions ($P<0.0001$), with an average of 7.53×10^7 CFU.mL^{-1} in the BHm condition and 6.07×10^3 CFU.mL^{-1} in the NBRIPm condition. For each condition, no difference in density was observed between the different treatments.

Analysis of total and active bacterial communities based on 16S rRNA gene amplicon pyrosequencing

Pyrosequencing analysis revealed a substantial decrease in diversity and change in composition between the inoculum, which corresponds to the initial diversity observed in the forest soil of Breuil-Chenue, and the BHm and NBRIPm conditions. Considering the same number of sequences for each sample (n=2,244), the inoculum harbored higher Shannon (5.1) and Simpson (0.97) indices and a higher number of observed OTUs (612)(Table S1). Considering total DNA in all microcosms, the BHm and NBRIPm conditions showed lower Shannon (0.9 to 1.76) and Simpson (0.42 to 0.765) indices, and lower numbers of observed OTUs (20 to 48). Although the diversity was strongly reduced in the microcosms compared to the inoculum, the rarefaction curves generated for the microcosms did not reach a plateau (Table S1). In the BHm condition, the mineral-free treatment harbored lower diversity and a lower number of observed OTUs than obsidian and biotite treatments (mineral-free < obsidian < biotite; $P>0.05$). On the contrary, in the NBRIPm condition, the mineral-free treatment harbored higher

diversity and a higher number of observed OTUs than apatite and apatite-biotite treatments (apatite +biotite< apatite< medium alone; *P*>0.05).

Compared to the total communities described above, the metabolically active fraction of the communities exhibited higher Shannon (0.6 to 2.2) and Simpson (0.31 to 0.757) indices and observed OTUs (17 to 81) for some treatments. For all the diversity proxies used, the biotite treatment harbored lower diversity than the obsidian (obsidian >; *P*=0.03). In the NBRIPm condition, active communities from the mineral-free treatment harbored higher diversity than the treatments with minerals (mineral-free > apatite + biotite > apatite; *P*>0.05).

At the taxonomic level, the inoculum was also strongly differentiated from the communities observed after 8 d incubation in microcosms. Indeed, the inoculum was characterized by a high proportion of phyla such as Acidobacteria (40%), Proteobacteria (24%), Verrucomicrobia (2.5%), and Bacteroidetes (2.1%) (Figure 2). In the microcosms, Acidobacteria remained undetected in total and active community fractions. Comparison of the total communities of the BHm and NBRIPm conditions revealed an important differentiation between these two conditions as evidenced by multivariate analysis (Figure 3A and B) and ANOSIM analysis (R=0.81, *P*=0.001). The total community of the BHm condition appeared dominated by representatives of Beta- (74% average) and Gammaproteobacteria (24%), corresponding to OTUs assigned to *Burkholderia* and *Dyella*. For the NBRIPm condition, the total community appeared dominated by representatives of Gammaproteobacteria (64%), Actinobacteria (29%) and Betaproteobacteria (5%) (Figures 2 and 3), corresponding to OTUs assigned as unclassified Enterobacteriaceae, *Streptacidiphilus* and *Burkholderia*. A detailed analysis of the relative distribution of the 16S rRNA gene sequences revealed that the total communities of the BHm condition with minerals were enriched in representatives of Gammaproteobacteria (36% with biotite vs 10% without mineral; *P*=0.068) and a decrease in representatives of Betaproteobacteria (85% without mineral vs 68% with minerals; *P*>0.05) and Bacteroidetes (4.3% without mineral vs 0.4% with minerals; *P*>0.05). In the NBRIPm condition, a non-significant decrease in the abundance of representatives of Beta- and

Gammaproteobacteria was observed in the presence of apatite or apatite+biotite compared to the mineral-free treatment. On the contrary, Actinobacteria mainly assigned to *Streptacidiphilus* appeared enriched in the mineral treatments, compared to the mineral-free treatment.

As the total communities present in the BHm and NBRIPm conditions were different, the structure and composition of the metabolically active communities were assessed independently for each condition, including the treatments without BrdU (Figure 4). After immunocapture, either no 16S rRNA PCR amplification or weak signals were obtained for treatments in which no BrdU was added. For those giving a weak PCR signal, 16S rRNA gene amplicon pyrosequencing analysis revealed a low diversity, dominated by OTUs absent or very rare in the BrdU-treated samples, supported by few sequences, and assigned as *Serratia*, *Variovorax* or unclassified bacteria. For each condition (BHm or NBRIPm), total and metabolically active bacterial communities were differentiated by multivariate analysis, with a stronger effect in the BHm condition than in the NBRIPm condition (Figure S1A,B and S2A,B). This trend was confirmed by an ANOSIM analysis, which was only significant for the BHm condition (R=0.45, *P*=0.002) and not for the NBRIPm condition (R=-0.105, *P*=0.73). In both conditions, Betaproteobacteria appeared reduced and Gammaproteobacteria increased in active communities compared to total communities (Figure 2). However, this trend was only significant in the BHm condition, in the mineral-free and biotite treatments (Betaproteobacteria, P=0.0002; Gammaproteobacteria, *P*=0.007). A specific focus on the metabolically active communities in each condition revealed contrasting patterns. In the BHm condition, metabolically active communities were significantly enriched in Betaproteobacteria in the obsidian treatment (58% of sequences) compared to other treatments (24%, without minerals and 4%, with biotite; *P*=0.01). Gammaproteobacteria appeared significantly enriched in the biotite treatment (79%) compared to the obsidian treatment (30.5%; *P*=0.041). A detailed analysis at the genus or OTU level revealed that these differences were explained by significant variations in the proportions of *Burkholderia* (obsidian > biotite; *P*=0.03), unclassified *Enterobacteriaceae* (biotite >obsidian; *P*=0.047), *Pseudomonas* (obsidian>biotite; *P*=0.012) and *Arthrobacter* (obsidian > biotite; *P*=0.04). This

differentiation between the active communities from the different BHm treatments was confirmed by multivariate analysis, at a variety of phylogenetic levels (phylum to OTU; Figure 4C). On the contrary, in the NBRIPm condition, no significant differences were observed between the treatments for the metabolically active communities at any phylogenetic level considered. Although non-significant, the mineral treatments harbored higher proportions of Gammaproteobacteria (99% apatite; 84% apatite +biotite) than in the mineral-free treatment (54%; *P*=0.38). This absence of differentiation was also confirmed by multivariate analysis (Figure 4D).

Functional and taxonomic characterization of culturable bacterial isolates from the microcosms

The functional screening of the 377 bacterial isolates obtained from the different conditions and treatments of this study revealed significant differences according to ANOVA and Chi^2 analyses (Figure 5). In the BHm condition, the most effective phosphorous solubilizing bacterial isolates were detected in the obsidian treatment (P<0.0001; Figure 5A). The most effective iron-mobilizing bacterial isolates were also obtained in the obsidian treatment (Figure 5B), but only significantly more effective than those isolated from the biotite treatment (P<0.05). Indeed, isolates from the initial inoculum or from the mineral-free treatment were as effective as the other treatments. The frequency of effective iron-mobilizing bacterial isolates was significantly higher in the mineral-free treatment compared to the obsidian treatment (P=0.006; mineral-free treatment > obsidian; Figure 6A). No difference was observed concerning the frequency of effective phosphorous solubilizing bacterial isolates in the BHm condition. In the NBRIPm condition, the most effective phosphorous solubilizing isolates were detected in the apatite+biotite treatment (P<0.05; Figure 5C). No significant differences were observed for the ability to mobilize iron, although the isolates from the apatite+biotite treatment were the most effective (P=0.29; Figure 5D). The frequency of effective iron-mobilizing isolates was significantly higher in the mineral-free treatment compared to the apatite treatment (P=0.007; mineral-free > apatite+biotite; Figure 6B). The frequency of effective phosphorous solubilizing isolates was significantly higher in the mineral

419 treatments (compared to the mineral-free treatment (P=0.001; apatite=apatite+biotite > mineral-free;
420 Figure 6B).
421 For the taxonomic analysis, a subsample of 141 bacterial isolates randomly selected was
422 characterized based on the partial Sanger sequencing of their 16S rRNA gene. This analysis revealed
423 that these bacterial isolates were members of Betaproteobacteria, Gammaproteobacteria,
424 Alphaproteobacteria, Actinobacteria and Firmicutes, representing the genera *Arthrobacter,*
425 *Acinetobacter, Brachybacterium, Burkholderia, Buttiauxella, Dyella, Kluyvera, Leifsonia, Micrococcus,*
426 *Phyllobacterium, Pseudomonas, Rhodanobacter, Serratia, Silvimonas, Staphylococcus* and *Yersinia*,
427 with *Burkholderia* being the most abundantly represented genus at 83% and 50% in the BHm and
428 NBRIPm collections, respectively. A detailed analysis of the bacterial collections also highlighted that
429 most of the dominant culturable bacterial genera (*Kluyvera, Dyella, Burkholderia, Phyllobacterium,*
430 *Pseudomonas*) were also dominant in the 16S rRNA pyrosequencing data obtained from the
431 microcosms (Table S2).
432 Comparison of the functional potentials with the taxonomic affiliation of the bacterial isolates revealed
433 that those assigned as *Burkholderia* were the most effective in solubilizing phosphorous and mobilizing
434 iron in the BHm conditions. This relationship was not tested for the other genera due to their low
435 representativeness in the collection (n<3). In the NBRIPm, bacterial isolates assigned to *Kluyvera* were
436 significantly more effective in solubilizing phosphorous than those assigned to *Burkholderia* (p<0.009).
437 Concerning the ability to mobilize iron, *Burkholderia* isolates appeared non significantly more effective
438 than the *Kluyvera* (*P*=0.8). Comparing the overlapping portion (120 bases) of the 16S rRNA gene
439 sequences of the *Burkholderia* isolates obtained by Sanger sequencing and the dominant OTU
440 sequences detected in the total and active communities after pyrosequencing revealed a strong
441 homology (97 to 100%). In the NBRIPm condition, all the 15 isolates assigned as *Kluyvera*
442 (Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae) were effective in solubilizing
443 phosphorous. Notably, the overlapping portion (120 bases) between the 16S rRNA gene sequences of

444 *Kluyvera* strains and the dominant OTU sequences, assigned as unclassified Enterobacteriaceae,
445 revealed a strong homology (97 to 100%).
446
447 **DISCUSSION**
448 Using combined culture-dependent and -independent methods, our study revealed that the diversity,
449 composition and function of a soil bacterial community are affected by the solution chemistry and the
450 presence of mineral particles in the solution. Notably, a combination of DNA-BrdU immunocapture and
451 16S rRNA gene amplicon pyrosequencing highlighted that different fractions of the total bacterial
452 communities are metabolically activate, depending on the mineral type present in their environment.
453 Although our experimental conditions introduced biases due to the culture conditions, this study
454 represents to our knowledge the first experiment testing the relative impact of mineral type on complex
455 bacterial communities coming from forest soil.
456 The selective effect of minerals and rocks has now been evidenced in several terrestrial and aquatic
457 environments [1, 19-24, 47-50]. Although a consensus remains difficult to obtain, these studies have
458 shown that the physico-chemical properties, nutritive or toxic content and weatherability of minerals
459 affect the diversity and composition of the microbial communities inhabiting their surface [1,47,50,55].
460 Indeed, according to the environmental conditions (i.e. pH, ionic strength, nutrient availability) minerals
461 can attract bacterial cells on their surface due to their positive surface charges, and this attraction can
462 vary with mineral type [51-54]. The importance of the chemical content of minerals was also proposed
463 as an important driver of the development and survival of microbial communities [48, 50-51,55-56]. As
464 an example, nutritive elements such phosphate, potassium, magnesium or iron entrapped in the crystal
465 structure of minerals have been shown to determine the colonization but also the composition of the
466 bacterial communities [20,48,50]. At the same time, other studies proposed that other chemical
467 elements such as aluminium may have a toxic impact on microbial communities, inhibiting the
468 development of certain microorganisms on their surface [19-20,56]. At last, Uroz et al. [1,50] proposed

469 that mineral weatherability determines the diversity and composition of mineral-associated bacterial
470 communities, showing that the most weatherable minerals were those harboring the lowest diversity
471 contrary to the recalcitrant minerals. In our study, after 8-days incubation, no evident effect of the
472 mineral type was observed in term of density, diversity, structure or composition of total bacterial
473 communities in each condition although mineral dissolution was measured in the NBRIPm condition.
474 Part of this absence of effect on the total bacterial communities may be related to the short incubation
475 time and to the absence of biofilm formation on the mineral surfaces, whatever the conditions and
476 treatments. Focusing only on the total communities, the main driver was the culture media, which vary
477 in nutrient availability, with the higher nutrient concentration in the NBRIPm medium, and in its buffering
478 capacity with presence of a weak buffer only in the BHm medium.
479 Although the total bacterial communities did not vary significantly according to the mineral type in our
480 study, the metabolically active communities analyzed using DNA-BrdU immunocapture method differed.
481 A global analysis of the 16S rRNA gene sequence distribution revealed that the metabolically active
482 communities were dominated by Gammaproteobacteria mainly assigned as unclassified
483 Enterobacteriaceae in both BHm and NBRIPm conditions, and Betaproteobacteria assigned as
484 *Burkholderia*. Although non significant, a detailed analysis at the OTU level revealed that the main
485 OTUs of unclassified Enterobacteriaceae were selectively enriched in the BHm condition in presence of
486 mineral while decreased in the NBRIPm condition, suggesting that representatives of these taxa were
487 reactive to mineral type (Figure S3). In the BHm medium, which lacks iron, diversity, structure and
488 composition of the active bacterial communities changed depending on the mineral type. Our
489 pyrosequencing data revealed that in the same condition (BHm), the treatment incubated with obisidian
490 was significantly different from the treatment incubated with biotite. A detailed analysis of the distribution
491 of the 16S rRNA gene sequences revealed that the active communities were enriched in
492 representatives of Gammaproteobacteria in the treatment with biotite while the obsidian treatment was
493 enriched in Betaproteobacteria. Comparison of the partial 16S rRNA gene sequences generated in the

494 culturable and pyrosequencing approaches revealed a good overlap, with the same genera (*Kluyvera,*
495 *Dyella, Burkholderia, Phyllobacterium, Pseudomonas*) dominating the dataset. Notably, the functional
496 screening of the bacterial strains affiliated to some these genera (ie. *Burkholderia, Kluyevera*) showed
497 they were effective to mobilize iron and phosphorous. Interestingly, the Betaproteobacteria OTU
498 sequences obtained in the pyrosequencing analysis as well as the Sanger 16S rRNA gene sequences
499 obtained for the Burkholderia isolates harbored strong homology with 16S rRNA gene sequences of
500 effective mineral-weathering *Burkholderia* strains effective previously isolated from the same
501 experimental site, suggesting that in our microcosm conditions they were participating in mineral
502 weathering [17]. In the NBRIPm condition, both total and active communities presented a chaotic
503 structure and were not significantly different, suggesting that in this condition with or without minerals,
504 members of the total communities were all metabolically actives. Altogether, these results show that
505 depending of the solution chemistry and mineral type, specific bacteria are metabolically active and may
506 contribute to mineral weathering.
507 In parallel to this cultivation-independent approach, functional screening of bacterial strains coming
508 from the different conditions and treatments were assayed for their ability to solubilize inorganic
509 phosphorous and to mobilize iron. Such approaches have been used to determine the functional
510 structure of mineral weathering bacterial communities along soil fertility gradient or to decipher the
511 potential differences existing between soil and (mycor)rhizosphere bacteria [17,38,57-59]. Here, we
512 showed that effective P-solubilizing bacteria were present in the different conditions and treatments, but
513 that significant variations of efficacy existed depending of the mineral type. If a limited effect was
514 observed in the BHm condition, with only a significant higher efficacy of the bacterial isolates coming
515 from the obsidian treatment, a higher effect was observed in the NBRIPm condition. Frequency analysis
516 revealed that P-solubilizing ability was enriched in the treatment with minerals comparatively to the
517 control without mineral, with a higher proportion in presence of apatite/biotite mix than in presence of
518 apatite. Another pattern of distribution of the isolates in class of efficacy was observed for the ability to

519 mobilize iron. Indeed, in both BHm and NBRIPm conditions, higher proportions of effective iron-
520 mobilizing bacterial strains were observed in the culture media devoid of minerals than in their
521 presence, suggesting that iron was accessible in the different treatments containing mineral particles.
522 Such results fit very well with our knowledge on the regulation of siderophore production in bacteria [60-
523 63]. Using DNA microarray on the model strain *Cupriavidus metallidurans* CH34, Olsson-Francis *et al*.
524 [25] highlighted a repression of the siderophore machinery in presence of basalt in the culture medium
525 comparatively to in absence. Similarly, Page and Huyer [63] showed that introduction of different
526 mineral types in the culture medium of a strain of *Azotobacter vinerlandii* allowed to a repression of
527 siderophore production for weatherable minerals and a de-repression for recalcitrant minerals. In our
528 experimental conditions, biotite is a recalcitrant mineral comparatively to obsidian and apatite, which
529 may explain why more effective iron-mobilizing bacteria were found in the treatments with biotite.
530 Whatever the functional bioassay used, strains assigned to *Burkholderia* or *Kluyvera* were the most
531 effective at mobilizing nutrients. Altogether, our cultivation-dependent data suggest a modification of the
532 functional structure of the mineral weathering bacterial communities according to the mineral type.
533
534 **Conclusion**
535 This work demonstrated that minerals, solution chemistry and pH are important drivers of the
536 taxonomic and functional structure of bacterial communities. Notably, the cultivation-dependent
537 approach revealed that in presence of mineral, effective P-solubilizing bacteria were enriched while the
538 proportion of effective Fe-mobilizing bacteria decreased. For the first time, we highlighted using a
539 combined DNA-bromodeoxyuridine immunocapture and pyrosequencing approach that in the same
540 condition, different mineral types supported the activity of different parts of a same bacterial community.
541 Although we can not exclude that introduction of minerals into the culture medium imposed upon to
542 bacteria a stress as recently proposed by Bryce *et al*. [55], our results fit very well with the newly
543 proposed concept of mineralosphere [1]. Indeed, this concept proposed that minerals select specific

544 bacterial communities according to mineral physico-chemical properties, adapted to the mineral
545 environment and actively involved in its dissolution. The physiological activation observed in our study
546 through the BrdU incorporation combined to the presence of effective mineral weathering bacteria
547 assigned as *Burkholderia* or *Kluyvera* and showing strong homology with the 16S rRNA pyrosequences
548 generated support this concept. Further studies combining environmental genomics and geochemical
549 approaches will be required to confirm these results on other minerals and in other sites, and to reveal
550 how are *in situ* regulated the mineral-associated bacterial communities and what is their relative impact
551 on mineral weathering and nutrient cycling. However, these results brings a new perspective to soil
552 microbial ecology, as our current knowledge of the taxonomic and functional diversity of microorganisms
553 in soil is based mainly on composite geologically heterogeneous samples and does pay too much
554 attention on soil minerals and rocks.
555
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562
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743 Figure legends
744
745 **Figure 1: Design of the microcosm experiment**
746
747 **Figure 2: Relative distribution of the 16S rRNA gene sequences of the major taxa detected in the**
748 **different conditions (BHm and NBRIPm) and treatments.** Treatments are presented as follow: NM,
749 no mineral; Tot, total communities; Brdu, active communities. For each treatment, the data presented
750 are the mean of 3 independent replicates, except for the inoculum (n=1).
751
752
753 **Figure 3: Multivariate analysis showing the differentiation of the total bacterial communities**
754 **according to culture conditions**. **Panel A** presents how the different treatments are distributed
755 according to F1 and F2 axis. Treatments are presented as follow: i) for the NBRIPm condition, Tot
756 means total communities without minerals (red), Apa.Tot means total communities of the treatment with
757 apatite (blue), Apa_Bio.tot means total communities of the treatment with apatite and biotite (purple).
758 And ii) for the BHm condition, Tot means total communities without minerals (red), Bio.Tot means total
759 communities of the treatment with biotite (blue), Obs.tot means total communities of the treatment with
760 Obsidian (purple). **Panel B** presents the vectorial distribution of the different taxa explaining the
761 distribution observed in panel A. In this analysis, principal component axis 1 and 2 explain most of the
762 variance in the data cumulatively (F1=29.90% and F2=22.77%).
763
764 **Figure 4: Multivariate analysis of the total and metabolically active bacterial communities in the**
765 **two BHm and NBRIPm conditions.** Panels A and B present the total communities in the BHm and
766 NBRIPm conditions. Panels C and D present the metabolically active communities the BHm and
767 NBRIPm conditions. In panel C, circles have been manually included to separate treatments. For each
768 panel, principal component axis 1 and 2 explaining most of the variance in the data cumulatively are
769 presented as F1 and F2.
770
771 **Figure 5: Functional potential of the bacterial isolates as assessed by measurement of the**
772 **diameter halo on the phosphorous and iron assays. A and C** Phosphorous solubilization assay for
773 BHm and NBRIPm conditions, respectively; **B and D**. Iron mobilization assay for BHm and NBRIPm
774 conditions, respectively. For each bioassay, the functional potential (P solubilization or Fe mobilization)
775 of bacterial strains coming from the inoculum was added. Bars correspond to average efficacy and

776 those presented with different letters (a,b,c) are significantly different according to one-factor ANOVA
777 and a Bonferroni-Dunn test (P<0.05). The error bars indicate standard deviations.
778
779 **Figure 6: Distribution of the bacterial isolates based on their origin (treatment) and their**
780 **membership in two classes of efficacy to mobilize P or Fe (Effective [black] or not effective**
781 **[white]). A.** BHm condition. **B.** NBRIPm condition. The figure presents the relative distribution (%) of the
782 bacterial isolates in the two classes of efficacy. Distribution was tested using a Chi2 analysis (P<0.05).
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