

*Mineralosphere : a microbial habitat ?*

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**The Mineralosphere concept: mineralogical control of the distribution and function of the mineral-associated bacterial communities**

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## Highlights

- Minerals can be considered as a real microbial habitat, the mineralosphere
- Mineralogy drives the taxonomic and functional distribution of bacteria
- Mineral-associated bacteria are capable of weathering minerals
- Minerals can induce or repress bacterial functions

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29 **Abstract**

30 Soil is composed of a mosaic of different rocks and minerals, usually considered as inert substrata for  
31 microbial colonization. However, recent findings suggest that minerals, in soils and elsewhere, favour  
32 the development of specific microbial communities according to their mineralogy, nutritive content and  
33 weatherability. Based upon recent studies, we highlight how bacterial communities are distributed on  
34 the surface of and in close proximity to minerals. We also consider the potential role of the mineral-  
35 associated bacterial communities in mineral weathering and nutrient cycling in soils, with a specific  
36 focus on nutrient-poor and acidic forest ecosystems. We propose to define this microbial habitat as the  
37 mineralosphere, where key drivers of the microbial communities are the physico-chemical properties of  
38 the minerals.

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41 **Key words:** microbial habitat, bacteria, mineral weathering, mineral chemistry, mineral weatherability,  
42 nutrient cycling, forest soil

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46 **1. Rocks and Minerals: support of life**

47 Earth conditions allow the continuous genesis of igneous, metamorphic, and sedimentary rocks. Such  
48 processes continue to shape landscapes, generating new rock material as a result of tectonics,  
49 sedimentation of organic and inorganic particles, or conversely rock erosion over geological timescales.  
50 Our planet can therefore be considered as a mosaic of different rocks and minerals (**Figure 1**),  
51 subjected to various degrees of environmental pressure and characterized by different physicochemical  
52 characteristics. Minerals are also differentiated according to their weatherability. Indeed, certain classes  
53 of mineral are easily weathered in acidic conditions, while others are quite recalcitrant [1]. Due to their  
54 nutritive content and their variable dissolution rates, mineral surfaces can be considered as reactive  
55 interfaces where nutritive cations are potentially accessible to the biosphere.

56 Whatever their location and their physical and chemical form, minerals play a central role in our  
57 environment. Regardless of their location and origins, from atmospheric dust, aquatic, terrestrial, deep-  
58 biosphere minerals or even human teeth, all mineral and rock environments have the potential for  
59 microbial colonization. Rocks and minerals serve as physical supports for attachment of  
60 microorganisms (bacteria, fungi) and plants, and as nutritive reserves participating in nutrient cycling,  
61 soil fertility and water quality. From an evolutionary perspective, microbial habitation of minerals seems  
62 to be an ancient strategy [2-4]. Interestingly, life on Earth itself may have originated within a mineral  
63 habitat [5]. Analyses of microbial interactions in the critical-zone have revealed the exceptional abilities  
64 of microorganisms, both prokaryotes and eukaryotes, to successfully colonize and interact with a  
65 diverse array of rocks and minerals [6-21].

66 Do these minerals drive the establishment and development of specific microorganisms adapted to  
67 colonize such environments? Or conversely, are these minerals merely inert supports for microbial  
68 opportunists? Answering this question is of major importance because nutrient-poor, rocky mineral  
69 environments represent one of the main sources of nutritive cations for ecosystem functioning. One way  
70 to answer this question is to determine whether mineral colonization is a random process, or controlled

71 by environmental conditions (extrinsic factors) and/or mineral characteristics (intrinsic factors). In this  
72 opinion paper, we argue that minerals represent specific microbial habitats, the intrinsic characteristics  
73 of which control microbial community establishment. We propose to call such a mineral-influenced  
74 habitats the mineralosphere [22]. We have chosen to focus on heterotrophic bacterial communities, the  
75 role of which in mineral weathering remains poorly understood compared to fungal communities.  
76 Analysing mainly *in situ* examples from aquatic and terrestrial environments, we will consider the  
77 extrinsic and intrinsic factors which make minerals suitable habitats for microbial colonisation. We will  
78 also discuss the links between mineral chemistry and weatherability, together with the composition,  
79 diversity and functional potentials of the mineral-inhabiting bacterial communities. A more thorough  
80 understanding of the factors driving mineral colonization by bacteria will allow enhanced appreciation of  
81 the potential roles of bacteria in mineral weathering, soil formation and nutrient cycling.

82

## 83 **2. Is mineral colonization random or specific?**

84 If the establishment, succession and/or persistence of mineral-colonizing microbial communities on  
85 minerals are driven by the minerals themselves, the factors driving this colonization are expected to  
86 vary among environments. Here, we examine potential extrinsic and intrinsic factors to offer a wider  
87 perspective of the subject.

88

### 89 **2.1. Extrinsic factors: environmental factors**

90 Surface and subsurface minerals are subjected to very different conditions that may influence  
91 microbial colonization on minerals. Surface minerals are exposed to ambient air conditions and the  
92 presence of more or less complex biofilms due to atmospheric deposition can be easily observed on  
93 any stone or monument. Notably, several studies have reported that microbial colonisation of mineral  
94 surfaces was strongly affected by the temperature, pH, light, UV irradiation, oxygen, water and nutrient  
95 availability, and aeolian erosion [7,15,23]. For example, Wierzbos et al. [15] reported that the microbial

96 colonization of gypsum crust in the Atacama Desert was controlled by moisture, exhibiting higher  
97 colonization in a wet environment contrary to the drier one. In acid mine drainage where very acidic  
98 conditions (pH 2) occur, specific microorganisms such as *Thiobacillus* are enriched on pyrite surfaces,  
99 from which they use the ferric iron as electron acceptor [24]. These and other studies suggest that  
100 mineral colonization is strongly determined by environmental factors, especially in environments  
101 characterized by extreme temperatures, pH and water content, such as deserts. One may explain the  
102 presence of microorganisms in such locations by an adaptive strategy, where bacteria seek protection  
103 from the extrinsic parameters (i.e. irradiation, temperature, water content), or as the result of a passive  
104 accumulation [7,23,33,34]. Comparatively, subsurface minerals are exposed to quite different  
105 environmental conditions, whether it is in the critical-zone or aquatic environments, and few studies  
106 have evaluated *in situ* the environmental factors determining microbial colonization of such minerals  
107 [25-26]. Comparing minerals incubated in a petroleum-contaminated aquifer in three geochemically  
108 zones, Mauck and Roberts revealed that mineral colonization by microbes was strongly dependent on  
109 the carbon and oxygen availability [25]. In a batch experiment, Scholl et al [26] reported increased  
110 bacterial cell attachment on microcline and quartz surfaces according to the pH and ionic strength of the  
111 environment, highlighting the importance of the surface binding forces in the attachment of microbes to  
112 mineral surfaces. Although no direct demonstration of the effects of soil pH, organic matter, nutrient  
113 availability or land cover on mineral colonization have been shown on subsurface minerals or rocks *in*  
114 *situ*, these extrinsic factors are known to impact the composition and diversity of soil microbial  
115 communities at a larger scale [27-32]. Consequently, we expect that these factors will also impact the  
116 colonization as well as the functioning of the mineral-associated microbial communities at smaller  
117 scales.

118

## 119 **2.2. Intrinsic factors: Do mineral characteristics influence bacterial colonization?**

### 120 2.2.1. Physical attributes

121 By adopting an endolithic lifestyle, the presence of cracks, fissures, cavities and pores in minerals are  
122 expected to provide microbial protection against the extrinsic factors described previously [7,23,33,35].  
123 Analysing the apparent heterogeneity of colonization of granites, sandstones and other minerals,  
124 several studies reported preferential microbial development according to porosity and size of the  
125 mineral particles, or on the edges formed during mineral dissolution [7,23,33,35]. Considering granitic  
126 rock fragments, Abdulla [33] demonstrated an increased Actinomycetes colonization of minerals with  
127 higher porosity. Analysing various size fractions of soil sandstones, Certini et al. [35] showed that the  
128 smallest sandstone fractions were more colonized than the largest fractions. Incubating model Gram  
129 negative and Gram positive bacterial strains in the presence of biotite and plagioclase feldspar, Barker  
130 et al. [36] showed that bacteria colonized all mineral surfaces, with preferential accumulation along the  
131 cleavage steps and edges of mineral particles. Altogether these *in situ* and *in labo* observations suggest  
132 that mineral colonization is not random but determined by the physical attributes of minerals. Among  
133 them, the preferential colonization of the smaller size fractions may be explained by the reactivity of  
134 mineral surfaces. Indeed, the smaller mineral fractions are characterized by higher reactive surfaces,  
135 giving them more available for microbial colonization. Due to their negative charge, bacterial cells could  
136 establish electrostatic interactions with the positive charges of these specific sites, interactions that may  
137 be reversible according to localised pH conditions [26, 39]. This preferential colonization of microbes on  
138 mineral surfaces, due to chemotactic processes can allow to the establishment of complex biofilms [40].  
139 Such biofilms have been observed *in situ* on the surface of various stone monuments, aquatic or aquifer  
140 environments [40], *in labo* using pure-culture based experiments [41-42], but rarely *in situ* on soil  
141 minerals. Consequently, experiments analysing microbial colonization on soil mineral surfaces varying  
142 in size, porosity or charge are necessary to confirm the relative importance of these physical attributes.

143

144 2.2.2. Mineral chemistry and weatherability

145 Minerals are categorized according to their chemical composition and complexity [42]. Primary  
146 minerals (e.g. silicates) are formed during rock genesis, while secondary minerals (e.g. oxides, clay  
147 minerals) are precipitates of chemicals released from primary minerals during weathering, or formed  
148 during hydrothermal events. Although few contain carbon or nitrogen, rocks and minerals can contain  
149 key nutritive or toxic elements. Consequently, the chemical composition and dissolution rates of  
150 minerals may be considered as important drivers of the microbial communities.

151

152 a) Impact of mineral chemistry

153 Analysis of natural mineral inclusions such as muscovite, plagioclase, K-feldspar and quartz, extracted  
154 *in situ* from various rocks, revealed that bacterial community structure varied in relation to the inclusion  
155 type, with some phylotypes present only on certain inclusions [10, 43-46]. A detailed analysis revealed  
156 positive and negative correlations between the abundance of some phylotypes and the elemental  
157 composition of the inclusion. Surprisingly, elements such as sodium and silica were identified as key  
158 structuring elements. Similarly, Hutchens et al. [10] revealed a relative specificity of certain phylotypes  
159 only detected on quartz (SiO<sub>2</sub>), considered as a nutrient-poor mineral. The impact of host rock chemistry  
160 was also investigated in cave environments. Comparing two distinct areas in Carlsbad Cavern, Barton  
161 et al. [47] revealed that the first area, composed mainly of CaCO<sub>3</sub>, harboured a higher cell density but a  
162 lower bacterial diversity compared to the second area characterized by a more complex mineral  
163 composition (volcanic and metamorphic minerals). Based on a 16S rDNA cloning-sequencing approach,  
164 the authors showed an enrichment of *Actinobacteria* in the CaCO<sub>3</sub> environment and, on the contrary, a  
165 dominance of alpha-, beta- and gamma-proteobacteria on the complex minerals [47].

166 Experiments on pure minerals have been undertaken in various environments [22, 25-26, 48-52].  
167 Using a mesh bag approach (**Box 1**) in acidic forest soils, we revealed a link between mineral  
168 geochemistry and the structure of the bacterial communities [22]. Similarly, several studies performed in  
169 petroleum-contaminated aquifers reported a higher bacterial colonization of P- and Fe-rich minerals



170 compared to other minerals [16-17, 25, 45]. Authors suggested a preferential colonization of mineral  
171 surfaces containing nutritive elements that are limiting in the aquifers. These studies also reported a  
172 potential toxic effect of Al-containing minerals, resulting in reduced microbial biomass [43, 51].  
173 Furthermore, the importance of the mineral geochemistry was evidenced in extreme aquatic  
174 environments such as in a hot thermal system [53] and subglacial environments [50]. Incubating small  
175 crystals of pyrite, hematite, magnetite, olivine, calcite and quartz in the glacial meltwater stream at  
176 Robertson Glacier (Canada), Mitchell et al. [43] showed that the bacterial communities were  
177 quantitatively (higher biomass) and qualitatively (structure and composition) affected by the mineral  
178 geochemistry, especially the Fe content. The authors explained this result by a preferential colonization  
179 of mineral surfaces acting as electron donors or acceptors (pyrite, hematite, magnetite). Similarly,  
180 Phillips-Lander et al. [53] showed that bacteria preferentially colonized minerals with higher Fe and P  
181 content.

182 The impact of mineral geochemistry on microbial colonisation has been investigated further using  
183 engineered minerals. Scholl et al. [26] tested the impact of iron by comparing bacterial colonization of  
184 natural and Fe-coated minerals (quartz and muscovite). These authors demonstrated that after a short-  
185 incubation (16h), the Fe-coated minerals were more colonized than non-coated minerals. Interestingly,  
186 Rogers and Bennett [46] used artificial borosilicate glasses containing additions of apatite, goethite or a  
187 mixture at 1% final concentration. After a 9-month incubation in aquifer conditions, apatite-goethite and  
188 apatite glasses appeared more colonized than goethite glasses and unamended borosilicate glasses.

189 All the observations obtained using natural or artificially coated minerals [10, 16, 25, 43-47] show that  
190 minerals are not just inert substrata on which microbial life can expand, but that strong relationships  
191 exist between the chemistry of these minerals and the distribution of the bacterial communities. Due to  
192 their reactivity with the environmental conditions, minerals can be considered as physicochemical  
193 interfaces releasing nutrients, adsorbing compounds on their surface and forming precipitates. Notably,  
194 many of the elements (e.g. Fe, Mn, Mg, P, Ca, Na) entrapped in minerals are physiologically required by

195 bacteria as electron donors, terminal electron acceptors, co-factors or nutrients. The structuring effect of  
196 minerals and mineral chemistry presented above prompts us to propose that these factors strongly  
197 determine the distribution and functioning of the mineralosphere bacterial communities.

198

199 b) Mineral weatherability

200 As stated above, minerals vary in their recalcitrance to environmental pressures (e.g. pH), hence the  
201 nutrients contained within more recalcitrant minerals may be less accessible to biota than those of more  
202 easily weatherable minerals. Consequently, one could hypothesize that the colonization of mineral  
203 surfaces is largely determined by the weatherability of these minerals. To decipher such relationships,  
204 we used the dissolution rates presented by Palandri and Kharaka [55] for each class of mineral, to  
205 analyse the available quantitative and qualitative data from the literature related to mineral colonization  
206 by microorganisms.

207 Quantitatively, several studies performed in aquatic environments using pure minerals and/or rocks,  
208 reported a higher colonization of poorly weatherable minerals such as quartz and K/Na-feldspar [17,  
209 53]. Notably, Mitchell et al. [50] reported a greater colonization of poorly weatherable minerals such as  
210 hematite and magnetite, and conversely a comparatively reduced colonization of highly weatherable  
211 minerals such as calcite and pyrite. On the contrary, higher colonization was reported on highly  
212 weatherable minerals such as calcite [26]. Using a real-time PCR approach, Santelli et al. [56] revealed  
213 a significant correlation between the age of marine basalt and the abundance of mineral-colonizing  
214 bacteria, showing that the oldest and most weathered basalts, were the most highly colonized. In  
215 terrestrial environments, higher cell densities were measured in the most weatherable zone of Carlsbad  
216 Cavern than in the less weatherable zone [47]. Finally, using a grain-per-grain strategy on desert sand  
217 samples, Gommaux *et al.* [57] highlighted that the density of culturable bacteria was the same on  
218 different mineral types.

219 Qualitatively, Gleeson et al. [43-44] were among the first to apply molecular techniques on  
220 independent minerals extracted from granitic outcrops, determining the impact of the different mineral  
221 particles contained in granite (kaolinite, pegmatitic granite, unweathered granite) on the structure of the  
222 mineral-associated microbial communities. A differentiation of mineral-associated communities was  
223 confirmed here and in other studies performed on inclusions (muscovite, plagioclase, K-feldspar, and  
224 quartz) extracted from granite [10, 44], suggesting that different communities inhabited weatherable and  
225 poorly weatherable minerals. Similarly, several studies performed on marine basalt described the  
226 phylogenetic diversity of the basalt-associated bacterial communities in relation with basalt age [56-59],  
227 revealing a clustering of the bacterial communities according to basalt age (level of alteration).

228 However, none of these studies directly measured the level of weathering of the minerals analysed.  
229 Examples combining mineral weathering and molecular analyses are presented in **Box 2**. From all  
230 these observations, one may ask if these taxa are enriched on minerals due to their ability to weather  
231 minerals or because they are attracted due to the higher nutrient availability occurring near weathered  
232 mineral surfaces.

233

### 234 ***2.3 Artificial soils: a tool to decipher the impact of minerals on the bacterial communities***

235 In the last decade, several studies have tested how soil microbial communities are structured in  
236 artificial soils containing clay minerals, rock phosphate, silicate, or metal oxide [60-62]. A microcosm  
237 study by Carson *et al.* [60] revealed that bacterial communities from soil amended with mica did not  
238 cluster with those of other artificial soils. Additionally, the authors showed that the soil respiration was  
239 significantly higher in the soil amended with rock phosphate than in the soils amended with mica or  
240 basalt, suggesting that both the structure and function of the bacterial communities were modified in  
241 response to different amendments. Using a similar approach, Ding et al. [62] also reported significant  
242 differences in the relative abundance of Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes in  
243 relation to the mineral types present in the artificial soil. These taxa were significantly enriched in the

244 artificial soil containing a mix of quartz, montmorillonite and charcoal. These first studies clearly confirm  
245 that mineralogy drives the composition and functioning of soil bacterial communities.

246

### 247 **3. Mineral weathering ability and other functional abilities of the mineral-associated bacteria**

248 Aside from some specific bacterial taxa, which use insoluble terminal electron acceptors contained  
249 within secondary minerals [24], few studies have focused on the mineral-associated bacterial  
250 communities and their functional abilities [49, 63-66]. If minerals support the development of specific  
251 bacterial communities, it is logical then that for certain functionalities mineral selection should operate,  
252 permitting the development of adapted bacteria. In this context, we thus wonder whether mineral-  
253 associated bacteria possess specific metabolic and physiological abilities adapted to the specific  
254 mineral environments, and whether they are capable of weathering their mineral substratum. Due to the  
255 absence of known functional genes specifically associated with the mineral weathering activity, culture-  
256 based approaches remain an essential tool to address such questions.

257

#### 258 **3.1. Are all mineral-associated bacteria capable of weathering minerals?**

259 Such a question was addressed using microbial consortia and pure cultures isolated from various  
260 mineral environments. In aquifer systems, Bennett et al. [16] reported that the most intensively  
261 weathered minerals were the most colonized, suggesting a role of the mineral-associated  
262 microorganisms in the dissolution of minerals. Considering granite samples, Welch et al. [66] reported  
263 that granite-associated microbial consortia were able to weather both apatite and biotite in batch  
264 experiments through the production of organic acids during incubation. Other granite samples collected  
265 in front of the Damma glacier (Switzerland) were used to characterize the mineral weathering ability of a  
266 set of bacterial strains [63-64]. Mineral weathering assays revealed that around half of these Damma  
267 glacier bacterial strains were effective at weathering granite, with differences in the ability to release  
268 specific elements (Fe, Al, Ni). The highest mineral weathering potentials were obtained for bacterial

269 strains belonging to the *Arthrobacter*, *Janthinobacterium*, *Leifsonia* and *Polaromonas* genera. Effective  
270 mineral weathering bacteria belonging to the *Arthrobacter* and *Burkholderia* genera were also isolated  
271 from apatite particles incubated for several years in an acidic forest soil [49]. In the context of patrimony  
272 conservation, Qi-Wang et al. [59] revealed that bricks of the Nanjing Ming city walls (China) were  
273 inhabited by effective mineral weathering bacteria. Lastly, effective mineral weathering bacteria were  
274 found in the buccal cavity on the surface of human teeth, which are composed of hydroxyapatite  
275 minerals [13]. Altogether, these studies demonstrate that minerals, whatever their chemical nature and  
276 the environment considered, are colonized by specific taxa characterized by effective mineral  
277 weathering potentials.

278

### 279 **3.2. Are mineral-associated bacterial communities physiologically active or dormant?**

280 Using metabolic assays, Certini et al. [35] revealed that the metabolic potential of mineral-associated  
281 microorganisms was different from those of the surrounding soil, and that higher metabolic potentials  
282 were observed for the smaller rather than the larger mineral fractions. Epifluorescence microscopy,  
283 applied on mineral particles incubated several years in forest soil, also showed active bacterial cells  
284 using a vital stain [67]. Performing a BIOLOG analysis on effective mineral weathering bacterial strains  
285 isolated from these mineral particles, Lepleux et al. [49] highlighted that, in contrast to those of the  
286 surrounding bulk soil or the mycorrhizosphere, mineral-associated (mineralosphere) bacteria  
287 metabolized few substrates and with a very low intensity, suggesting an oligotrophic behaviour.  
288 Surprisingly, glucose appeared as the most intensively and unique substrate consumed by the  
289 mineralosphere bacteria. On the contrary, bacteria in the bulk soil preferentially metabolised amino and  
290 carboxylic acids with high intensity, with comparatively poor glucose metabolism. Finally, Frey *et al.* [63]  
291 showed that the most effective mineral weathering bacterial strains also produced high concentrations  
292 of oxalate. Similarly, tooth-associated bacteria are metabolically active and produce organic acids  
293 contributing to the formation of dental caries [13, 68]. These observations demonstrate that mineral-

294 associated bacterial isolates are physiologically active, metabolise organic substrates and produce  
295 metabolites, suggesting that they may participate in mineral weathering and nutrient cycling.

296

### 297 **3.3. Do minerals drive bacterial gene expression?**

298 While minerals appear to be colonized by bacterial communities harbouring particular functional  
299 abilities, our knowledge regarding the feedback effect of these minerals on the physiology of the  
300 mineral-associated bacteria remains limited. Many attempts have been undertaken using  
301 chemoheterotrophic bacteria capable of respiring metals contained within minerals. Differential gene  
302 expression or protein production were observed when the cells were forming biofilms on minerals [69].  
303 Comparatively, few analyses have been performed on heterotrophic bacteria. Olsson-Francis et al. [70]  
304 addressed the above question using a microarray approach to decipher the molecular mechanisms  
305 used by *Cupriavidus metallidurans* CH34 to weather basalt in a minimal medium lacking iron. Their  
306 microarray analyses revealed that siderophores were produced only in the absence of basalt, and that  
307 other functions (ca 4% of the genes) were up- or down-regulated. Notably, transport-related genes and  
308 multiple genes involved in motility were up-regulated only in the presence of basalt. On the contrary,  
309 genes encoding TonB-dependent outer membrane transporter and putative cytochromes were down-  
310 regulated in the presence of basalt. In another context (*Gaeumannomyces graminis/Pseudomonas*  
311 interaction), Almario et al. [71] while trying to decipher the impact of iron availability on the production of  
312 2,4-diacetylphloroglucinol by *Pseudomonas* CHA0, revealed a significantly higher induction of the  
313 metabolite production in the presence of iron-rich vermiculite than in presence of illite. Altogether, these  
314 results suggest that due to their physicochemical properties, minerals influence gene expression.

315

### 316 **4. Concluding remarks and future perspectives**

317 Based on the *in situ* observations and *in labo* demonstrations presented above, we propose to define  
318 the mineralosphere as the specific interface and habitat encompassing the rocks (or mineral surfaces)

319 and the surrounding soil, which are physically, chemically and biologically under the influence of  
320 minerals. Physically, the mineralosphere is characterized by several zones, including pores and cracks  
321 which modify water circulation and can be considered as microbial sanctuaries. Indeed, microorganisms  
322 can accumulate in these zones due to passive diffusion and develop with relative protection against  
323 external environmental pressures (e.g. pH, temperature, predation). Chemically, the mineralosphere  
324 can be considered as a nutrient reserve and a reactive interface. Surface charges and the exchange  
325 capacity of minerals have been shown to impact colonization of mineral surfaces. Indeed, positive  
326 charges (such as in the phyllosilicate interlayers) can attract negatively charged bacterial cells. Besides  
327 electrostatic processes, chemotactism can operate. The nutrients contained within minerals can attract  
328 or repel microorganisms due to their nutritive or toxic value. While some of the released nutrients can be  
329 directly available for microbes, other can form precipitates (oxides) requiring solubilisation by microbes  
330 to become available. These nutrients can be passively released from mineral surfaces due to abiotic  
331 processes, or actively due to biological activities. Biologically, the mineralosphere is enriched in specific  
332 microorganisms adapted to low carbon and mineral-rich environments, and potentially capable of  
333 contributing to mineral weathering. In this habitat, the mineral weathering ability of microorganisms may  
334 be regulated by their nutritional requirements, nutrient availability and/or the mineral type. Of course,  
335 this specific habitat is impacted by environmental conditions, which for soil include parameters such as  
336 pH and water availability, or the inputs of organic and inorganic nutrients.

337 Notably, the mineralosphere shares common properties with the well-known rhizosphere. As with the  
338 rhizosphere, minerals, due to their physico-chemical properties and the nutrients released during their  
339 dissolution, modify their environment chemically and physically. In this regard, we suggest that the  
340 mineralosphere may be considered as the inorganic twin of the rhizosphere (**Box 3**), where bacteria are  
341 not selected by organic nutrients coming from roots, but rather by the physico-chemical properties of  
342 minerals. This concept brings a new perspective to soil microbial ecology, as our current knowledge of  
343 the structure and diversity of microorganisms in soil environments is mainly based on composite

344 geologically heterogeneous samples. The selective effect of minerals on the soil bacterial communities  
345 may account at least partly for their heterogeneous distribution in the soil. Of course, more studies  
346 combining environmental geomicrobiology, geochemistry, mineralogy, microscopy and genomics (**Box**  
347 **4**) are required to fully comprehend the complex interaction of factors, both intrinsic and extrinsic,  
348 governing the bacterial colonisation of minerals as well as by fungi, and to determine their relative role  
349 in mineral weathering, nutrient cycling and ecosystem functioning.

350

351

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## **Glossary**

Corundum :  $\text{Al}_2\text{O}_3$

Critical-zone: interface of the lithosphere, atmosphere, and hydrosphere, which encompass the soil and terrestrial environments.

Quartz :  $\text{SiO}_2$

Intrinsic factors : Factors related to the mineral characteristics (chemical composition, porosity,...).

Eukaryotes: fungi, plants, lichens

Extrinsic factors : Factors related to environmental parameters (pH, nutrient availability, ...)

Geomicrobiology : The study of the interactions between minerals and microorganisms.

Mineral : Inorganic and solid compound characterized by a chemical formula, a crystal form and an atomic structure.

Mineralogy : The study of the chemistry and crystal structure of minerals.

Mineralosphere : Volume of soil under the influence of the nutritive and toxic elements contained into the minerals

Mycorrhizosphere : Volume of soil under influence of mycorrhizal-roots.

Nutritive content : The content of inorganic nutrients entrapped into the minerals.

Prokaryotes: archaea, bacteria

Rhizosphere : Volume of soil under root influence.

Rock : naturally occurring solid composed of one or more minerals.

Saprolite : Rock particles formed during weathering of bedrock and present in soil profiles.

Surface and subsurface minerals : Define the physical location of minerals submitted to atmospheric event (surface) or below ground (subsurface).

Weatherability : weathering capacity of a mineral in specific conditions

*Mineralosphere : a microbial habitat ?*

## Box and Figure legend

### Figure 1: Mosaic of environments and microbial colonization.

**A.** Thin layer section of soil surfaces imaged with a stereomicroscope. This panel presents the soil heterogeneity, with tree roots (r), pores (p), mineral particles (mp), clays and organic matter (com). **B.** Focus on a granite particle (q, quartz; wm, white mica; bm, black micas; f, feldspaths). The particle was imaged using a polarised microscope. **C.** Granitic saprolite showing the different mineral particles present (quartz, micas, feldspaths) and the variability of size and colour, imaged with a stereomicroscope. **D.** Focus on an apatite particle imaged with a scanning electron microscope. This figure presents the different cracks and fissures, which can be used, as habitat by microorganisms. **E.** Apatite particles imaged after several years of incubation in soil conditions (mesh bag approach). The apatite particles appeared covered by a complex organo-mineral biofilm containing fungal hyphae and bacteria. **F.** Bacterial cells on biotite surfaces imaged by epifluorescence microscopy. Bacteria colonize mineral surfaces where they can establish complex biofilms. Such biofilms have been observed *in situ* on the surface of various stone monuments and aquifer minerals [40], *in labo* using pure-culture based experiments [41], but rarely *in situ* on soil minerals. However, panel E of this figure clearly shows a complex organo-mineral structure on the surface of the apatite particles. Interestingly, Certini et al [35] reported the presence of complex mats on mineral surfaces considering sandstone rocks.

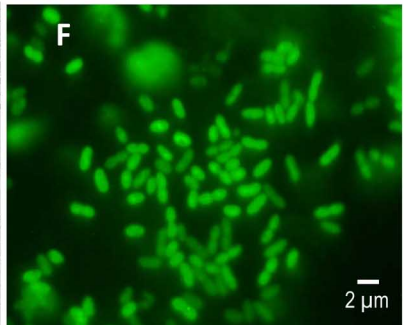
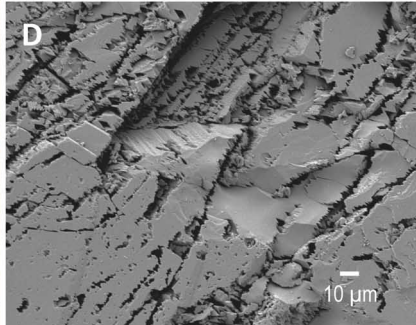
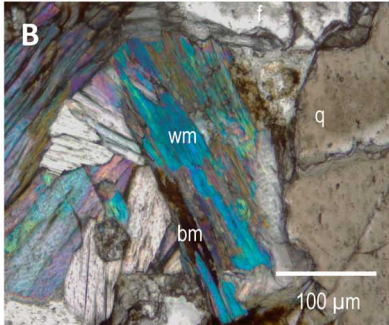
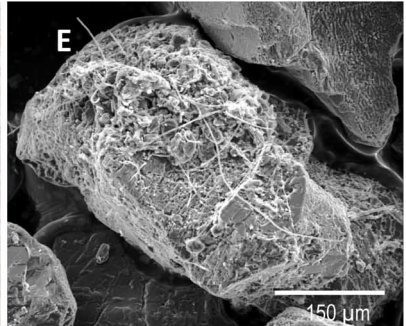
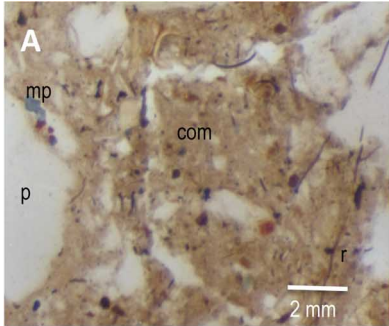
### Box 1: a simple method to analyse mineral-associated microbial communities: the mesh bags.

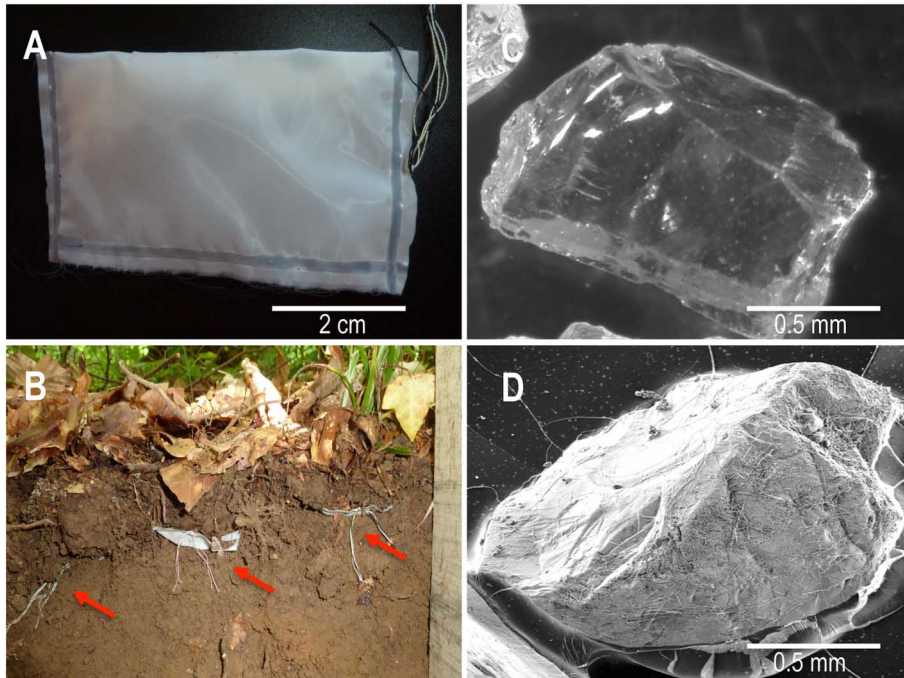
Although the technics based on microscopy have strongly evolved, the bottleneck still remains the rocks or minerals themselves. Indeed, if it is possible to analyse the functional and taxonomic diversity of microbial communities colonizing minerals collected in the field, these minerals are rarely pure, not of the same size and porosity, giving conclusion difficult to establish. To limit such problems, several studies have developed a pure mineral strategy. Pure and calibrated minerals can be introduced directly *in situ* or conditioned in mesh bags in terrestrial and aquatic environments. Mesh bags containing minerals are used since decades [73], and this method was initially used to determine mineral weathering in environmental conditions (Figure I). More recently, this approach was used to determine the impact of plants [74-75] on minerals, and which fungi [76] or bacteria [22,50] are able to colonize minerals. Interestingly, this approach can be modulated to test single type or mixed minerals, different size of minerals and permits or not the penetration of plant roots inside the mesh bags. Notably, Augusto et al. [74] developed a method permitting to measure mineral weathering based on mass loss. Similar systems have been developed in aquatic environment. Figure I: Mesh bags approach. **A.** Pure and calibrated apatite particles (3 grs; particle size 0.5-1 mm) have been conditioned in mesh bags (mesh size 50  $\mu\text{m}$ ; 4  $\times$  10 cm). **B.** Installation of mesh bags in the organic horizon of forest soil. Red arrows indicate the location of the mesh bags. Minerals have been incubated several years in soil before mineralogical and microbial analyses. **C.** Apatite particle imaged before incubation in soil conditions. **D.** Apatite particle imaged after several years incubation in soil conditions. This image illustrates the complex biofilm formed on apatite surface.

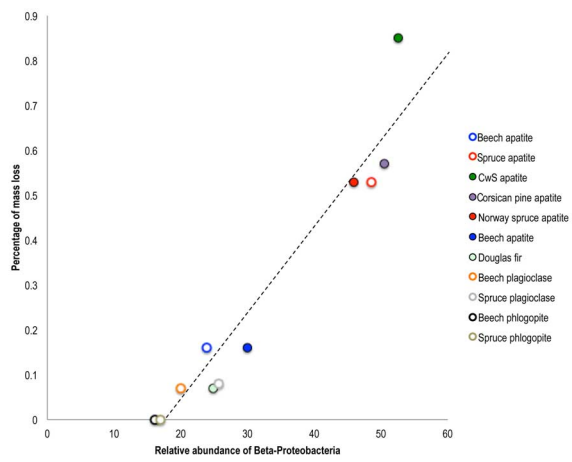
**Box 2: Relationship between mineral weatherability and distribution of the bacterial communities** In order to get inside the potential relation between mineral weatherability and bacterial

diversity, pure and calibrated minerals (apatite, plagioclase, phlogopite) were incubated in acidic forest soil conditions during four years using the mesh bag approach. Such approach has permitted to determine both mineral weathering level and bacterial diversity. Using a small-scale 16S rRNA clone library (70 sequences per condition), Uroz et al. [22] revealed that the mineral-associated bacterial communities were distinct from those of the surrounding soil. This analysis also highlighted that the most weathered minerals (apatite) were characterized by a decrease of diversity (shannon index) and an enrichment of specific taxa such as Beta-Proteobacteria in comparison to the less weathered minerals (phlogopite), suggesting that a correlation may exist between the bacterial diversity and the level of weathering of the minerals. Notably, this finding was confirmed using a more resolving approach (16S rRNA pyrosequencing; 30,000 sequences per condition) on apatite samples incubated in different soil conditions [50]. Interestingly, a significant correlation (Figure 1) between the level of weathering and the abundance of 16S rRNA sequences was obtained for the Beta-Proteobacteria, the Burkholderiales as well as the Burkholderia, which have been described as effective mineral weathering taxa on this experimental site. These observations suggest that these effective mineral weathering taxa colonize minerals and may play an important role in mineral weathering and nutrient cycling. Figure 1: Relation between mineral weathering of apatite and relative abundance of 16S rRNA gene sequences affiliated to Beta-Proteobacteria (based on 16S rRNA sequences). Minerals have been incubated in soil conditions (4 years) in the experimental site of Breuil-Chenue below different tree species (beech, douglas fir, spruce, Corsican pine, coppice with standards). Open circles correspond to analyses performed by cloning-sequencing and solid circles to analyses performed by 16S rRNA pyrosequencing.

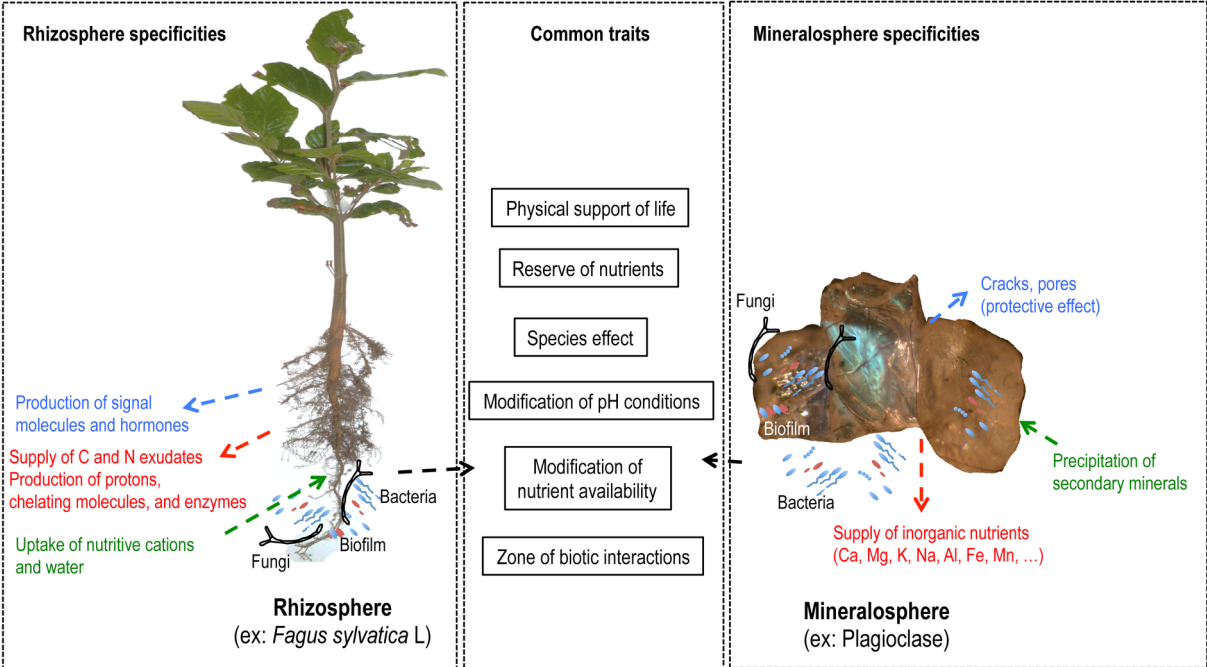
**Box 3: The mineralosphere: the inorganic twin of the rhizosphere ?** The structuring effect of the microbial communities by the tree root system was reported in various environments and for various plants (77). Here, we are presenting the common ecological traits and the specificities of the rhizosphere and mineralosphere habitats.











#### **Box 4 : Outstanding questions**

- 1) *Given the environmental and mineral heterogeneity, can we develop tools applicable to any mineral/rock environments, especially in soil to test mineral chemistry effect on microbial communities (i.e. mesh bag systems, artificial labelled minerals)?*
- 2) *How can we best assess mineral surfaces, and especially image mineral-associated microorganisms, given both methodological challenges and the complexity of mineral/rock samples?*
- 3) *Can we demonstrate if mineral-associated microbes are selected by the physico-chemical properties of a mineral or by the higher nutrient availability occurring in its vicinity?*
- 4) *Do mineral-associated microorganisms characterized by functional and physiological specificities? Do mineral-associated microorganisms regulate their physiology according to the mineral types? Is there a functional complementation between the mineral-associated microbial communities?*
- 5) *More studies are needed to analyse in situ geochemistry of minerals, release of nutrients from their surface and transfer to plants ? How to determine in situ the relative contribution of soil microorganisms to this transfer of nutrients?*