Experimental Study on the Phlogopite Weathering Potential of Bacterial Communities Isolated from Different Soil Profiles

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Abstract

The silicate mineral weathering potential of different weathering pattern soil bacteria was characterized, and links between key habitat determinants and soil bacteria strategies were established in this work. Phlogopite weathering experiments were conducted under aerobic conditions and in closed systems with bacterial communities isolated from different soil profiles that are typical of a temperate climate. Our results suggest that the phlogopite weathering processes (i.e., acidolysis and complexolysis processes) of bacterial communities is determined by soil horizons as a function of (i) organic matter content, (ii) impoverishment in clays in leached horizons and (iii) the content of exchangeable cations. In our experiments, bacterial communities developed two strategies for iron release from phlogopite: the first was the K-strategy, which is used by bacterial communities extracted from soil rich in nutrient elements (especially in horizons with high contents of exchangeable Mg, Ca, etc.) and organic carbon. These communities produced strong chelating organic acids in low concentrations and used small amounts of carbon. The other was the r-strategy for bacterial communities extracted from soils poor in nutrient elements and organic carbon content. They produced weak chelating organic acids in large amounts and used a large proportion of the carbon source.

Keywords: weathering, organic acids, bacterial communities, soil horizons

1. Introduction

Mineral weathering is one of the most important geochemical phenomena occurring at the Earth’s surface because it controls the formation of soils.

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The weathering mechanism increases the availability of essential mineral elements in soils and influences the composition of natural waters. Two major processes of weathering, under various agents, can occur. One process corresponds to the physical disintegration of minerals associated with chemical leaching. The second is linked to biota (flora and microflora) activities in soil such as plants, fungi and bacteria. In symbiosis or not with plant, fungi and bacteria can accelerate elemental release from silicate through the release of protons or low molecular organic compounds (siderophores and organic acids) that influence pH, create complex cations, or change mineral saturation state (Watteau and Berthelin, 1994; Ullman et al., 1996; Liermann et al., 2000; Valsami-Jones and McEldowney, 2000; Hoffland et al., 2004). Heterotrophic bacteria play a specific role, as indicated by the many studies performed under laboratory or field conditions (Berthelin, 1983; Robert and Berthelin, 1986; Leyvalet et al., 1990; Drever and Stillings, 1997; Barker et al., 1998). Thus, via their diversity and their activities, bacteria mediate the health of soil ecosystems (fertility) (Uroz et al., 2009; Uroz et al., 2011a). In soils, bacterial diversity is due to habitat heterogeneity, which involves diversity in terms of resources, physicochemical conditions and biological interactions. Soils represent “oligotrophic” environments with several types of microhabitats (according to soil aggregates, decaying organic matter, porosity, soil water, etc.), in which physicochemical conditions can change rapidly over time. Bacteria must therefore adapt to these changes and develop strategies for their survival.

High bacterial activities permit increases in bacteria size and stimulate bacterial growth. These activities require energy and a variety of substrates from the environment needed for cellular material synthesis. In soil, the rhizosphere (volume of soil directly influenced by roots) is an adequate environment for bacteria growth. This area close to roots, in which elevated populations and activities of fast-growing bacteria are supported by the exudates of plants, is rich in carbon substrates. The rhizosphere is typically located in the upper horizons of soil, where roots are most abundant.

Moreover, soils are very different with regard to their parent rock materials, the climate in which they exist, their relief and the accompanying vegetation. Under temperate climates, biochemical weathering in soils is dependent on humus type, pH and biological activities.
Three main processes can be distinguished (adapted from Campy, 2003):

- **Acidolysis** in soils with mull-type humus and with medium cation exchange capacity that are only partly saturated with alkaline and alkaline earth cations (mainly Ca, K and Mg), such as cambisols.1. (WRB 2006);
- **Complexolysis** in soils with mor-type humus and with low cation exchange capacity that are saturated with $\text{Al}^{3+}$, such as podzols.1. (WRB 2006);
- **Neutral hydrolysis** in soils with mull-type humus and with high cation exchange capacity that are saturated with $\text{Ca}^{2+}$ and have high biological activity, such as calcaric soil.

The link between the biochemical processes listed above and the precise bacterial processes that enhance weathering of minerals has not been studied at the level of the soil profile. However, few studies have investigated the silicate weathering potential of bacterial strains isolated from the rhizosphere or mycorrhizosphere (i.e. the soil around roots and mychorriza) (Calvaruso et al., 2006; Calvaruso et al., 2007; Uroz et al., 2007; Koele et al., 2009; Leveau et al., 2010; Uroz et al., 2009). They have concluded that bacteria isolated from the rhizosphere and mycorrhizosphere feature high weathering ability in contrast to those isolated from bulk soil. Moreover, certain bacteria (efficient silicate weathering bacteria) are able to adapt to nutrient-poor conditions. However, these works focused on the first ten centimeters of the studied soils, which are often enriched in organic matter and exchangeable nutrients ($\text{Ca}^{2+}$, $\text{Mg}^{2+}$, etc.). To date, only one study (Wang et al., 2014) has investigated processes in the deep mineral horizons, and thus the whole soil profile, which is important considering that bacteria are ubiquitous in all environments. The results showed the depth-related changes in the weathering capacity and community structures of the cultivable mineral-weathering bacteria.

Therefore, several questions remain: What is the effect of the bacteria's environment of origin on their weathering potential? Does this potential differ among various soil horizons and soils? Is the mineral weathering potential of bacteria in upper horizons (close to conditions of rhizospheric environment) similar to that of bacteria growing in deep horizons?
The purpose of this study was to provide answers to these questions. To accomplish this, the objectives of the present work were (1) to characterize the phlogopite weathering potential of functional soil bacteria (i.e., acidolysis and complexolysis processes) isolated from contrasted soils (superficial and deep horizons) that are well characterized in terms of physico-chemical parameters and (2) to discuss the important environmental parameters that influence the strategies used by bacteria to weather silicate minerals in soil.

2. Materials and Methods

2.1. Site Description

The study area is located in the northeast of France. The climate of the region is temperate, with a mean annual precipitation of approximately 1000 mm and a mean annual temperature of approximately 10°C. Twenty-seven soil samples were taken from three different sites characterized by (1) different geomorphologies, (2) different parent rock materials, and (3) different vegetation covers. Soil sampling was performed in June 2008 and May 2009. The geological substrata included various sedimentary rocks (east of the Parisian Basin). Rocks from the western part of the study area are carbonated, with alternating marl and limestone layers forming cuesta successions (Cordier et al., 2006). Rocks from the eastern part of the study area (Vosges Mountains) are mainly sandstone (Figure 1). The first site that was investigated was situated in the plain near Nancy (200 m high) on marl material, with natural soils corresponding to gleyicluvisol (World reference base: WRB 2006). Based on their pedological characteristics (Jacquin and Florentin, 1988), typical soil profiles comprise four horizons: a surface horizon, $A_1$; a leached horizon, $E$; a mineral horizon, $B$; and a horizon of altered parental rock, $C$. In our study, the upper three horizons were sampled: $A_1$ (0-15 cm), $E$ (15-30 cm) and $B$ (30-60 cm). The soil was sampled under three different vegetation covers: forest (beech and oak), meadow, and cultivation (bare soil at the sampling moment). The second site that was sampled was situated on a plateau at the summit of the cuesta above Nancy (at 400 m high) and is composed of hard Bajocien limestone. The distance from the slope (erosion or accumulation processes) and the local presence of quaternary materials explained the high diversity among soils, whose levels of rock homogeneity varied. We sampled several typical soils under forest cover (mainly beeches and oaks):
- A calcic cambisol (WRB 2006) on the plateau directly developed from oolitic limestone; the 3 sampled soil horizons were A\textsubscript{1} (0-15 cm), B (15-30 cm) and C (>30 cm).

- Aluvisol (WRB 2006) on the plateau developed from thick loamy deposits mixed with coarse silicated alluvium covering limestone; three soil horizons were sampled: A\textsubscript{1} (0-15 cm), leached horizon E (15-30 cm) and B (>30 cm).

The third site was located on the Vosges Mountains on sandstones. Three soil profiles were investigated:

- Adystriccambisol (WRB 2006) under forest cover; three soil horizons were sampled: A\textsubscript{1} (0-30 cm), B (30-60 cm) and C (>60 cm).

- A podzol (WRB 2006) that had developed on steep slopes under a mature forest of European beech (Fagus sylvatica) and silver fir (Abies alba Mill); the 5 sampled soil horizons were A\textsubscript{1} (10-30 cm), E (30-40 cm), Bh (40-50 cm), Bs (50-70 cm) and C (>70 cm).

- A lepticpodzol (WRB 2006) on the plateau (top of the catchment) under young stands of Norway spruce (Picea Abies L.) with thick understory vegetation mainly composed of hair grass (Deschampsia flexuosa) and calluna (Calluna vulgaris L.); the uppermost three soil horizons, A\textsubscript{1}, E, and B, were sampled: A\textsubscript{1} (5-20 cm), E (20-30 cm), and B (30-70 cm).

In each profile, the soil samples from each horizon were stored in sterile plastic tubes at 4°C for analyses and extraction of bacteria.

2.2. Determination of Selected Chemical Characteristics of Soils

A number of soil parameters were measured to characterize the soils studied. Total carbon content (C) was determined in solid samples by combustion at 900°C in a CHNS-O (Flash EA 1112 ThermoFinnigan analyzer). Effective cation exchange capacity (CEC) was measured with the soil kept at the same pH at which it was sampled using a buffered 1 N NH\textsubscript{4}NO\textsubscript{3} salt solution (1:10 w/v) (Mathieu and Pieltain 2003).
The exchangeable cations (Ca, Mg, Na, K, Fe, Al) collected from the extracted solution were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES Varian). The apparent soil pH (pH$_{H_2O}$) was measured in a 1:2.5 (w/v) soil/water ratio suspension after overnight equilibration.

2.3. Extraction of Bacteria from Soil

Prior to the extraction of bacterial communities from soils, 5 g of each soil horizon was physically dispersed using 50 ml of a solution containing distilled water. For each sample, the soil-solution suspension was dispersed using a blender for 1 minute (3 times, 4 °C). Then 7 ml of this mixture was transferred to a centrifuge tube. Five milliliters of Nycodenz® (Nycomed Pharma, Norway) was carefully added to the bottom of the centrifuge tube containing the microbial-soil suspension using a syringe needle. The Nycodenz® medium is a non-ionic density gradient medium, as described by Bakken and Lindhal (1995). The efficiency of this substance was demonstrated by Bakken (1985). It has often been used to obtain purified bacterial suspensions and enumerate bacteria from soil (Bakken and Lindhal, 1995). Bacterial communities were extracted from the suspension using high-speed centrifugation in a swing-out rotor (Beckman) at 10,000 g for 1 hour (4°C) to establish the density barrier. A cell layer appeared in the middle of the tube and was then collected in another tube and centrifuged at 6,000 g for 15 min at 15°C. The supernatant was removed, and 1 ml of distilled water was added to wash the bacterial cells. This washing step was repeated three times to ensure the elimination of the extraction solution.

From each sample, the bacteria cells were quantified by acridine orange direct counts on a Thoma cell using microscopy (Bloem and Vos, 2004).

2.4. Phyllosilicate Weathering Potential of Bacteria: Experimental Set-Up

All of the experiments were performed under sterile conditions in the same miniature batch reactors used by Uroz et al. (2007). The experimental device featured a specific 96-well microplate (Multiscreen® microplates MAGVN22) with a 0.22-µm Durapore filter membrane at the bottom of the wells. This microplate allowed for a great number of replicates and the collection of solution without bacteria or particles. Each well received 7 mg of phlogopite (from Madagascar–Vohitrosy). The chemical composition of phlogopite is as follows (g/kg): SiO$_2$ 390; Al$_2$O$_3$ 150; Fe$_2$O$_3$ 52; MnO 0.9; CaO 2.5; MgO 294; K$_2$O 110; TiO$_2$ 11.3; Na$_2$O 1.3; P$_2$O$_5$ 1.1 (Leyval et al. 1990).
A dried 50- to 100-µm fraction was selected, and its specific surface area was 0.5 (±0.3) m²g⁻¹, as determined by N₂ adsorption via the Brunauer-Emmet-Teller method. Before starting the experiments, mineral powders were sterilized at 170°C for 2 hours under a dry atmosphere.

Before filling up the wells with culture media, microplates containing mineral samples were sterilized using UV irradiation for 2 hours. In all of the experiments, to simulate the chemical composition of a common soil solution (with low ionic strength) and to enhance mineral dissolution, an iron-magnesium free and diluted mineral medium (Bushnell-Hass) was used. Thus, the minerals were the only source of these elements. The culture medium (BHm) was made up of 20 mg.l⁻¹ KCl; 20 mg.l⁻¹ NaH₂PO₄, 2H₂O; 22.5 mg.l⁻¹ Na₃HPO₄, 2H₂O; 65 mg.l⁻¹ (NH₄)₂SO₄; 100 mg.l⁻¹ KNO₃; 20 mg.l⁻¹ CaCl₂ in distilled deionized water. The media were sterilized by autoclaving (30 min. at 110°C). The microbial dissolution of the phyllosilicates was studied in the presence of glucose (source of carbon and energy 2 g.l⁻¹) previously sterilized by filtration (0.2 µm).

Biotic experiments were conducted using a bacterial community directly extracted from soil horizons. Cell suspensions were incubated in mineral-filled microplates for 3 days with BHm media and glucose as iron-magnesium and energy and carbon sources, respectively. The operating conditions were as follows: 20 µl of cell suspension, 160 µl of BHm and 20 µl of glucose stock solution. The microplates were incubated at 28°C on a reciprocating shaker (New Brunswick, 120 rpm).

In each experiment, 12 replicates were created with the same bacterial community, for each analysis, 4 wells were used.

After incubation, the solutions were filtered to 0.2 µm. Phlogopite particles and bacteria were retained on filters for further analysis. Four replicates (wells) were used to determine the pH, iron concentration, glucose consumption and metabolites produced (organic acids). To compare our results with the different chemical pathways of mica dissolution in natural soil environments (i.e., proton-promoted and ligand-promoted dissolution), data regarding chemical experiments performed by Balland et al., (2010) under similar conditions were used.
2.5 Elemental Analyses of Solution

Organic Acids Released

Non-acidified samples were stored at -18 °C. The determination and quantification of 10 organic acids, those most typically released in solution by bacteria, were performed with an ionic chromatograph with conductivity detection (ICS 3000, Dionex Corp.) using an analytical column (AS 11 HC, Dionex corp.). The sample was eluted with KOH solution of varying concentrations (0.9 mM to 60 mM) over time (step gradient) with a flow of 1.3 ml/min. The synthetic reference materials used were sodium formate, D-gluconic acid, sodium butyrate, pyruvic acid sodium salt, sodium citrate tribasic, sodium oxalate, sodium propionate, sodium acetate, succinic acid disodium salt, DL-malic acid disodium salt (from Sigma-Aldrich) and sodium-L-lactate and malonic acid disodium salt (from Fluka). Uncertainties were better than 0.5% for all of the organic acids measured.

Colorimetric Determination of Iron, Protons and Glucose in Solution During Biotic Experiments

The amount of iron released (Fe$^{2+}$ and Fe$^{3+}$) in solution was determined by adding 20 µl of ferrospectral® (Merck, for iron determination) with a microplate reader (SAFAS Xenius FLX) at A$_{595}$ nm. The detection limit for Fe was 0.02 ppm. For pH determination, 10 µl of bromocresol green (1 g/l, Sigma) was added to 200 µl of leaching solutions; the reading was obtained using a microplate reader (SAFAS Xenius FLX) at 2 wavelengths, 440 and 620 nm. This colorimetric test allowed for the measurement of pH from 3 to 7.

The concentration of glucose in solution was determined by measuring the glucose remaining in a 3µl filtered solution after the addition of 300 µl of GOD-PAP (enzymatic kit, BioLabo). The solutions were left in the dark for 20 minutes; then measurements were performed using the microplate reader at A$_{520}$ nm.

According to the manufacturer’s instructions, calibration curves were built to determine the relationship between the absorbance and the amount of iron, protons and glucose for the ferrospectral, the bromocresol green and GOD-PAP (data not shown), respectively.
2.6. Statistical Analyses

For soil chemical characteristics (pH, Corg and exchangeable cations), each analysis was realized in triplicate. Significant differences between each treatment were determined by analyzing variance (ANOVA 1 factor) and by the Tukey HSD test (significance threshold of P <0.05 with n = 3) (XLSTAT version 2008 6.01).

3. Results

3.1. Chemical Properties of Soil Horizons Sampled

Soil parameters such as pH, organic carbon content (Corg) and exchangeable element contents are presented in Table 1.

The pH values of the soils formed from sandstone, such as podzol, dystric cambisol and lepticpodzol, are in the acidic range (pH range from 3.7 to 4.8). Soils formed from loamy materials such as luvisol and gleyicluvisol have a pH range between 4.4 and 6.3. Soils formed from limestone, such as calcic cambisol, calcicaricleptosol and colluvialcalcicaricleptosol, have an alkaline pH range (from 5.9 to 8.5). In general, whatever the soil profile, the pH values in the upper horizon are slightly lower than the pH values in the deeper horizons.

The organic carbon content, as expected, decreases with depth, with a greater proportion of organic carbon in the A1 horizons of all profiles. A high organic carbon content was also observed in the Bh horizon in the podzol profile.

The cation exchange capacity (CEC) was very low (ranging from 0.14 to 3.2 meq. 100 g⁻¹ of soil) in soils developed on sandstone. This material parental is very poor in clays and in weatherable minerals that could provide nutrients such as Mg and Ca. The horizons with a higher organic content (A1 and Bh) have the greatest CEC (range from 1 to 3.2 meq. 100 g⁻¹). Whatever the depth, the most abundant cation in the CEC is Al³⁺ (toxic at low pH).
For soil developed on limestone (calcaricleptosol, colluvialcalcaricleptosol and B and C horizons of calcic cambisol), the CEC is very high, ranging from 17 to 46 meq. 100 g\(^{-1}\); Ca\(^{2+}\) represents more than 98% of the exchange cations in the soil exchangeable complex, indicating that these soils are saturated in calcium. The upper horizon of the calcic cambisol, which is decarbonated, has a CEC of approximately 1.3 meq. 100 g\(^{-1}\).

The cation exchange capacity of soils developed on loamy marl (luvisol and gleyicluvisol) ranged from 6 to 12 meq. 100 g\(^{-1}\). These soils are relatively rich in various nutrients such as Mg, Ca and K. The exchangeable iron content was below the detection limit.

3.2. Characterization of Phlogopite Weathering Processes Induced by Heterotrophic Bacteria

The two main biogeochemical mechanisms involved in bacteria-mineral interactions are referred to as proton-promoted and ligand-promoted interactions under aerobic conditions (Berthelin, 1983; Banfield et al., 1999). Most communities produced significant quantities of protons and metabolites during phlogopite weathering, and simultaneously, a large amount of iron was rapidly released in the media. The measurement of these parameters and the use of a model adapted from Balland et al. 2010 help define the processes involved in the weathering of phyllosilicate by bacterial communities. Balland et al. (2010) proposed three domains that are defined by the pH required for mica dissolution by bacteria: the first domain corresponds to the proton-promoted dissolution below pH 3 (or acidolysis), represented by a line called “acidolysis” on the model; the second domain corresponds to a combination of organic ligand (or complexolysis) and proton-promoted processes at weakly acidic pH, and finally the third domain corresponds to the iron immobilization at pH greater than pH 5 for phlogopite and corresponds to the domain below the line “acidolysis” (Figure 2). Based on the relative contribution of acidolysis, complexolysis and iron immobilization, soil bacteria will be associated with characteristic weathering pattern phenotypes.
3.2.1. Relationship between Bacterial Community Weathering Potential and Vegetation Covers in a Same Soil Type (Gleyic Luvisol)

After 72 hours of incubation, iron released from phlogopite and protons was measured in solution for each bacterial community isolated from the soil horizons of three gleyicluvisol profiles (WRB 2006) sampled under different vegetation covers. The relationship between iron release rates and measured pH (Figure 3) does not indicate any influence of vegetation covers on the weathering potential of bacterial communities extracted from leached horizon E. These communities are populated by chelating bacteria (domain II). These bacteria enhance the iron release rate from phlogopite ($r_{Fe} \approx 10^{-12} \text{mol m}^{-2} \text{s}^{-1}$) in a similar manner and at a similar pH (pH approximately 5.5). The communities extracted from the other horizons (A and B) were composed of acidifying bacteria under forest cover ($r_{Fe} \approx 6.3 \times 10^{-14}, 2 \times 10^{-12} \text{mol m}^{-2} \text{s}^{-1}$; and pH approximately 6.6, 3.2 for A and B, respectively) and chelating bacteria under meadow and cultivation ($r_{Fe} \approx 3.2 \times 10^{-12}, 7.9 \times 10^{-13} \text{mol m}^{-2} \text{s}^{-1}$; and pH approximately 5, 6 for A and B, respectively).

Our results suggest that the land use and vegetation conditions on the soil strongly affect the weathering potential of communities extracted from surface horizons. This effect seems to decrease with depth because communities isolated from E horizons release iron from phlogopite using the same process: complexolysis.

In addition, for gleyicluvisol, communities extracted from surface horizons (A) were more chelating than communities extracted from deep horizons (B). This suggests that communities extracted from surface horizons (A) produce a larger amount of chelating organic acids compared with communities extracted from deeper horizons (B).

3.2.2. Relationship between Bacterial Community Weathering Potential and soil Types in Forested Ecosystems

Cambisols (sl): As shown in Figure 4A, communities extracted from deep mineral horizons (B of gleyicluvisol and luvisol, C of calcic cambisol and B, C of dystriccambisol) are populated by acidifying bacteria.
In these horizons, bacterial activity leads to iron release rates and pH values similar to those defined by the theoretical rate of chemical dissolution by protons, with strong acidification of the medium (pH range from 3 to 4.5). Communities extracted from surface horizons (A<sub>1</sub> of gleyicluvisol, calcic cambisol, luvisol and dystriccambisol) are chelating bacteria. They allowed a similar iron release rate ($r_{Fe}$ approximately $2 \times 10^{-13} \text{mol m}^{-2} \text{s}^{-1}$) to that allowed by bacteria extracted from deeper horizons, though at a higher pH (range from 5 to 7). Communities extracted from leached horizons, E, are chelating bacteria as well, leading to a high iron release rate ($r_{Fe}$ approximately $10^{-12} \text{mol m}^{-2} \text{s}^{-1}$) and low acidification of the medium (pH range from 5 to 6). Finally, communities extracted from surface horizons (A<sub>1</sub> and E) produce a large amount of chelating organic acids compared with communities extracted from deeper horizons (B, B and C of gleyicluvisol, luvisol, and dystriccambisol).

**Calcareaous soils (s.l):** As shown in Figure 4B, the community extracted from horizon C of colluvialcalciclephtosol is composed of acidifying bacteria, whereas communities extracted from surface horizons (A<sub>1</sub>) and C of calciclephtosol are chelating bacteria. The community extracted from horizon C of calciclephtosol is composed of chelating bacteria, which leads to a high iron release rate ($r_{Fe}$ approximately $1.2 \times 10^{-12} \text{mol m}^{-2} \text{s}^{-1}$) at a weakly acidic pH (approximately 6). This community seems to produce a large amount of chelating organic acids. For communities extracted from surface horizons (A<sub>1</sub>) of the two soils, the $r_{Fe}$ and pH values were very close ($r_{Fe}$ approximately $5.6 \times 10^{-13} \text{mol m}^{-2} \text{s}^{-1}$, and pH approximately 4.8), indicating a low production of chelating organic acids by bacteria.

**Podzols (s.l):** As shown in Figure 4C, communities extracted from lepticpodzol exhibit different behavior compared with communities extracted from podzol. Communities extracted from horizons E, Bs and C of the podzol are acidifying bacteria, whereas communities extracted from horizons A<sub>1</sub>, E, Bs and C of lepticpodzol and A<sub>1</sub>, Bh of podzol are chelating bacteria. For podzol, communities extracted from organic horizons (A<sub>1</sub> and Bh) were very efficient at weathering phlogopite, with a high iron release rate ($r_{Fe}$ approximately $3.2 \times 10^{-12} \text{mol m}^{-2} \text{s}^{-1}$) and high acidification of medium. Differences in vegetation cover could explain this difference: a mature forest of beeches and firs is developed on podzol, whereas a young plantation of spruces with thick herbaceous strata is developed on lepticpodzol.
Our results suggest that the potential phlogopite weathering processes adopted by bacterial communities extracted from soils under forest cover depend on soil horizons as a function of (i) organic matter content (A1 surface horizons) and (ii) the leaching of horizons (with a low clay content, which decreases the cation exchangeable capacity and depletes the exchangeable base cations (Ca, Mg, etc.)).

4. Discussion

Silicate mineral weathering is an important mechanism that controls soil evolution and the availability of nutrient elements that are essential for soil organisms and vegetation. In our study, we used the phlogopite weathering potential of soil functional bacteria (i.e., acidolysis and complexolysis processes) to

- Establish links between key habitat determinants (soils and horizons scale) and soil bacteria strategies;
- Study various soil bacteria communities or the functional diversity of pure strains.

No tendencies related to the genus of bacterial strain, soil type, horizons, organic matter content or CEC depletion in exchangeable base cations (Ca, Mg, etc.) are observed in contrast to the studies of Leveau et al, 2009; Uroz et al, 2007 and Uroz et al, 2010. They demonstrate that the genus Burkholderia sp and Sphingomonas sp are the most efficient to weather silicate minerals in the rhizosphere. These results suggest that the interaction between the different strains in the bacterial community modify weathering processes relative to those adopted by an isolated bacterial strain. To summarize, the silicate minerals weathering by bacteria isolated from “bulk” soil would be controlled by the bacterial phenotypes, the mineral, the environmental conditions and a “community” effect and no by the genus of bacterial strains.

4.1. Link between Global Soil Profiles, Key Habitat Determinants at the Horizon Scale and Weathering Potential of Bacterial Communities

In this study, the soil profiles investigated have variable physical and chemical characteristics linked to their very different parent rock materials, geomorphologic localization and local climate.
The specific biochemical processes known to control the formation and the vertical differentiation of soil profiles are acidolysis for cambisols, complexolysis for podzols, and neutral hydrolysis for calcareous soil (Campy, 2003). However, these processes were not found to be the weathering processes that were used by bacterial communities isolated from the different soil horizons. For communities isolated from podzol, the main process used to release iron from phlogopite was acidolysis, whereas for communities isolated from cambisol, the main process used was complexolysis. These differences in the biochemical processes in the soil and in experimental conditions can be explained by the fact that communities extracted from these soils are the only bacteria that are able to survive under our experimental conditions and that less than 1% of total soil bacteria are cultivable. These results are quite reproducible and agree with the preliminary work performed using other types of soils and bacterial strains studied (Balland et al., 2010; Balland-Bolou-Bi and Poszwa, 2012). In addition, the work of Vandevivere et al. (1994) highlighted the existence of a “highly reactive zone” in the bacteria micro-environment, where extreme physico-chemical conditions (pH and metabolites gradients) could induce more mineral dissolution than would be expected from bulk solution. Under natural soil conditions, bacteria influence the weathering of minerals at the local scale, and the effects may be diluted or bulked across the profile. For example, the process of complexolysis is the predominant process leading to the differentiation of podzolic soil profiles. However, in our experiments, the process used by bacteria is acidolysis, with a drastic acidification of the medium by bacteria. However, the amount of carbon metabolized by these bacteria is very large, in the range between 5 and 15%, indicating a very high production of organic ligands. However, in our experiments, at pH 3, these molecules were protonated and acted as strong acids.

To better explain our results, we must consider the weathering phenotype of the communities in addition to discussing the process involved.

Nevertheless, our results show a clear correlation between processes adopted by the communities and the physico-chemical characteristics of the horizons. Under forest cover, bacterial communities extracted from organo-mineral surface horizons and intermediate leached horizons are chelating bacteria, whereas bacterial communities extracted from deeper horizons are acidifying. In other words, bacterial communities extracted from surface and leached horizons produce a large amount of chelating organics acids compared with communities extracted from deeper horizons.
Our results demonstrate a strong correlation between the carbon organic content and mineral weathering potential of bacteria. This trend is logical because organic matter provides an energy source for the soil heterotrophic bacterial community and is also the main supply of nutrients such as N, P and S. Moreover, soil pH represents a major determinant of soil microbial diversity and activity. However, in our work, the distribution and activity of soil bacteria as a function of soil pH was not simply determined by physiological pH preference (Van Elsas et al., 2007). The results do not show a simple correlation between soil pH and the phlogopite weathering potential of bacterial communities. Thus, the phlogopite weathering processes adopted by bacterial communities seem to depend on soil horizons as a function of (i) organic matter content and/or rhizospheric effect (A1 horizons), (ii) leaching of horizons and decreases in clay content, which modify the exchangeable cation capacity, and (iii) the chemical characteristics of soil, which are affected by the exchangeable base cation (available nutritive elements) content and stores. These observations are in accordance with those of Uroz et al. 2009, who demonstrated that the mineral weathering mechanisms of Collimonas bacteria are adjusted based on the available carbon sources as well as the biogeochemical conditions of the bacteria's environment. In this way, recent works (Balland and Poszwa 2012, Uroz et al., 2011) showed that bacterial communities isolated from an acidic soil limed with carbonates and salts weathered phyllosilicates more efficiently than bacterial communities isolated from the same type of soil without inputs. The nutrient availability of limed soil was improved as a result of Mg and Ca release from carbonated and easily weathered salted rocks.

4.2. Influence of Organic Acids Produced by Bacteria on Phlogopite Weathering: Is There an Adaptation Strategy Used by Bacterial Communities Regarding Phlogopite Weathering?

Acidification has generally been linked to the production of bacterial organic acids and protons, and complexolysis has been linked to the production of bacterial chelating molecules such as siderophores (Berthelin, 1983; Liermann et al., 2000) or organic acids. In this study, organic acids were measured by ionic chromatography in each experiment performed with bacterial communities (Table 2), but the presence of siderophores was not tested. There was a great variability and diversity of organic acids produced. Bacterial communities produced organic acids that were more or less chelating.
Communities isolated from GL-B, L-B, CC-C, DC-C, P-C, P-Bs and LP-Bs leached iron from phlogopite by releasing large amounts of non-chelating organic acids (in the range of 100 to 500 μmol.l⁻¹), such as gluconic, lactic, acetic, butyric, pyruvic and formic acid, which acidified the medium. On the other hand, communities that weather phlogopite by complexolysis, such as those extracted from surface horizons (GL-A₁, L-A₁, P-A₁, CC-A₁) and from leached horizons (GL-E and L-E), produced few μmol.l⁻¹ of chelating organic acids, particularly oxalic and citric acids. The presence of these polyfunctional acids could increase the silicate dissolution rate by 3–10 times compared to the silicate dissolution rate in experiments performed with monofunctional acids such as acetic acid (Robert and Berthelin, 1986; Welch and Ullman, 1993). Thus, a positive correlation was found between the amounts of citric and gluconic acid produced and the iron release rate and pH, which is consistent with the chemical model we developed (Balland et al., 2010) (data not shown) and the results from previous studies (Van de Viver et al., 1994; Uroz et al., 2007; Wu et al., 2008). Chelating organic acids such as citric or oxalic acid are produced via the Krebs cycle. In general, bacteria that use the Krebs cycle are chelating and possess an anabolism that requires high energy and thus an efficient catabolism that requires a large initial carbon source. In contrast, formic lactic and acetic acids are acids slightly chelating and result from fermentation. Anaerobe or facultative anaerobe bacteria, which produce these organic acids, are acidifying. They have an anabolism that requires low energy and thus a catabolism that is more efficient and requires a small initial carbon source (Gottschalk, 1986).

Soil bacteria are traditionally divided into autochthonous and zymogenous types. In general, autochthonous bacteria are oligotrophic; they are able to metabolize and can grow using a low concentration of nutrients (K-strategists). Zymogenous bacteria are able to metabolize and grow extremely quickly when the nutrient supply is large (r-strategists) (Van Elsas et al., 2007). Our results demonstrate (precedent section) that bacterial communities extracted from A₁ horizons rich in organic matter content are chelating and that communities extracted from deeper horizons that are poor in organic matter content are acidifying. As shown in Figure 5, a positive and significant correlation between the amounts of carbon metabolized by bacteria (or organic acids produced, normalized to consumed glucose) and iron release rates was observed. Moreover, two iron release strategies could be highlighted based on key habitat determinants (linked to the soil horizon origin).
In acidic soils (developed on sandstone), a large amount of carbon was metabolized by the bacteria extracted from the deeper horizons, which resulted in a maximum iron release rate of approximately $2 \times 10^{-12}\text{mol m}^{-2}\text{s}^{-1}$; meanwhile, a small amount of carbon was metabolized by the bacteria extracted from the surface horizons, which resulted in a maximum iron release rate of approximately $3.2 \times 10^{-12}\text{mol m}^{-2}\text{s}^{-1}$. The community extracted from the deeper horizons produced weak chelating organic acids such as lactic and acetic acids in large amounts; thus, the bacteria required small amounts of carbon ($r$-strategy). In contrast, the community extracted from surface horizons produced strong chelating organic acids such as citric acid in low concentrations; thus, the bacteria needed a large amount of carbon ($K$-strategy). This tendency is observed for the bacterial communities extracted from podzol, lepticpodzol, and dystriccambisol.

These communities produced larger amounts of organic acids than the communities extracted from the other soils. Organic carbon content represents a major determinant of soil microbial diversity and activity. However, the distribution and activity of soil bacteria is not determined by organic carbon content alone. For soils developed on limestone (calcaricleptosol, colluvialcalcaricleptosol and calcic cambisol) and on loamy materials (luvisol and gleycluvisol), both strategies were observed but were not correlated to organic matter content. These soils have high nutrient contents and stores compared to acidic soils developed from sandstone. Communities extracted from calcareous soil produced weak chelating organic acids such as lactic and acetic acids in large amounts; thus, these bacteria require small amount of nutrients. In contrast, the communities extracted from loamy soils produced strong chelating organic acids such as citric acid in low concentrations; thus, the bacteria require a large amount of nutrients. The $K$-strategy is adopted by communities extracted from loamy soils (except communities extracted from B horizon of luvisol), and the $r$-strategy is adopted by communities extracted from calcareous soils.

The sum of exchangeable elements (without taking into account exchangeable Ca) is significantly lower in calcareous soil (range from 0.3 to 1) than in loamy soils (range from 1.5 to 3.5). This observation regarding exchangeable elements also applied to soils developed on minerals or a deep horizon of sandstone.
To summarize, bacterial communities extracted from soil horizons rich in nutrients and organic carbon (A1 and Bh horizons of lepticpodzol, dystriccambisol, podzol, luvisol and gleyicluvisol) developed strategy K to weather phlogopite; bacterial communities extracted from soils poor in nutrients and organic carbon (mineral or horizons of lepticpodzol, dystriccambisol, podzol, calcaricleptosol and colluvialcalcicluvisol) developed strategy r to weather phlogopite.

Thus, the origins of bacterial communities are crucial to their phlogopite weathering potential. Several studies (Hameeda et al., 2006; Calvaruso et al., 2007; Uroz et al., 2007; Haichar et al., 2008; Patel et al., 2008; Leveau et al., 2010) have described weathering potentials that vary according to the nature of the carbon source, and they have shown that the carbon source present in root exudates or in mycorrhizosphere (sugars of type aldose) has a significant influence on the phylogenetic structure and functions of soil bacterial communities.

Our work and the experimental approach that we adopted contribute to the development of new indicators of soil biological quality. Further research will be needed to (i) better characterize the two strategies (r and K) used by bacterial communities, as well as their structure and (ii) precisely define the representativeness and the impact of these communities in different soil profile.

5. Conclusion

Depending on the origins of bacterial communities in soils, the efficiency with which bacteria weather phlogopite is variable. Thus, the phlogopite weathering processes adopted by bacterial communities seem to depend on soil horizons as a function of (i) organic matter content, (ii) the leaching of horizons and (iii) the depletion of base cations with respect to the exchange capacity.

Bacterial communities use two different strategies to release iron from phlogopite: the first is the K-strategy, which is used by bacterial communities extracted from soils rich in nutrient elements and organic carbon. These bacteria produce large amounts of weak chelating organic acids from small amounts of carbon. The other is the r-strategy, which is used by bacterial communities extracted from soils poor in nutrients and organic carbon. These bacteria produce small amounts of strong chelating organic acids using a large amount of carbon.
The focus of this study was to gain a better understanding of soil function with respect to mineral weathering and the potential availability of nutrients. The study deepens our knowledge about the factors that control the functional biodiversity of soil. The biotest used to determine the weathering potential of bacteria and to study the nature and quantities of organic acids produced by bacteria serves as a new way of determining the biochemical quality and potential fertility of soil.

Acknowledgements

We are grateful to Géraldine Kintsinger and David Billet for their assistance during the organic acids analysis, to Hervé Marmier for cations analysis, to Patrick Billard for his advice on the isolation of bacteria and to Jean-Pierre Boudot for his help during the sampling campaigns.

Table 1: Selected Chemical and Physical Characteristics for Both Soils “below Detection Limit”

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<tr>
<th>Soil</th>
<th>Soil profile</th>
<th>Depth (cm)</th>
<th>C org (%)</th>
<th>pH</th>
<th>Exchangeable elements (meq/100g)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CEC</td>
</tr>
<tr>
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<td>5-20</td>
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<td></td>
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<td>4.33b</td>
<td>0.90b</td>
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<td>3.74a</td>
<td>1.75a</td>
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<td>0.3b</td>
<td>4.54b</td>
<td>0.75c</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>&gt; 60</td>
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<td>3.96ab</td>
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<td>4.30ab</td>
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<tr>
<td></td>
<td>C</td>
<td>30-60</td>
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</tr>
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<td>Soil Type</td>
<td>Horizon</td>
<td>pH</td>
<td>C (mg/kg)</td>
<td>CEC (cmol/kg)</td>
<td>Interpretation</td>
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<td>Colluvial Calcicleplosol</td>
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<td>7.2c</td>
<td>7.80a</td>
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<td>C 30-60</td>
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<td>Calcic cambisol</td>
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<td>5.90a</td>
<td>1.31a</td>
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<tr>
<td></td>
<td>B 15-30</td>
<td>0.2a</td>
<td>6.60ab</td>
<td>17.62b</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>C &gt; 30</td>
<td>0.6ab</td>
<td>7.90b</td>
<td>19.49b</td>
<td>0.49</td>
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<td>5.40a</td>
<td>4.17b</td>
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<tr>
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<td>E 15-30</td>
<td>1.0ab</td>
<td>4.40ab</td>
<td>1.74a</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>B &gt; 30</td>
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<td>4.70b</td>
<td>6.78c</td>
<td>0.89</td>
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<tr>
<td>Gleyicluvisol (under mor)</td>
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<td>3.4b*</td>
<td>5.00a</td>
<td>10.39a</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>E 15-30</td>
<td>1.3a</td>
<td>5.90b</td>
<td>13.94b</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>B 30-60</td>
<td>-</td>
<td>6.30b</td>
<td>18.21c</td>
<td>1.35</td>
</tr>
<tr>
<td>Gleyicluvisol (under forest)</td>
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<td>2.1b*</td>
<td>4.80a</td>
<td>6.64a</td>
<td>0.77</td>
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<tr>
<td></td>
<td>E 15-30</td>
<td>1.1a</td>
<td>5.70b</td>
<td>6.61a</td>
<td>1.04</td>
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<tr>
<td></td>
<td>B 30-60</td>
<td>0.8a</td>
<td>6.00b</td>
<td>9.61b</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Each value is a mean of three replicates. For each soil horizons of one type of soil, different letters (a,b,c) indicate that the pH or the amount of C and exchangeable cations capacity (CEC) are significantly different according to a one-factor ANOVA (p < 0.05). For each type of soil (Lepticpodzol, Lystriccambisol, Podzol, Calcricleplosol, ColluvialCalcicleplosol, Calcic cambisol, Luvisol and Gleyicluvisol) different letters (*,$) indicate that the pH or the amount of C and CEC of the upper horizons A1 are significantly different according to a one-factor (compartment) ANOVA (p < 0.05).
Table 2: Organic acids released in µmol l⁻¹ during bioweathering of phlogopite by bacterial communities isolated from different horizons of gleyicluvisol (GL-A1; GL-E; GL-B), dystriccambisol (DC-A1; DC-B; DC-C); calcic cambisol (CC-A1; CC-B, CC-C) and luvisols (L-A1; L-E; L-B), colluvialcalcaricleptosol (CCL-A1; CCL-C) and calcaricleptosol (CL-A1; CL-C), podzol (P-A1; P-E; P-Bh; P-Bs; P-C), lepticpodzol (LP-A1; LP-E; LP-Bs; LP-C). The vegetation covers are the forest.

<table>
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<td>GL-A1</td>
<td>324.9</td>
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<td>108.6</td>
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<td>0.0</td>
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<td>11.1</td>
<td>44.4</td>
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<td>3.7</td>
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<td>Location</td>
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<td>Granitic, volcanic...</td>
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**Figure 1:** Simplified Geologic Map of the Studied Sites
Figure 2: Model of limits and domains for process determination adapted from Balland et al., 2009. The measured iron release rates are represented on a log scale (log rFe) as a function of average pH during (bio) weathering of phlogopite.

Domain 1 represents by black line corresponds to the proton-promoted dissolution or acidolysis process (rFe = KH). At weakly acidic pH, both complexolysis and acidolysis processes were involved represented by a grey area in the figure (rFe = KH + KL). When pH is greater than 5 (dashed line n°2), the iron is immobilized (Domain 3).
Figure 3: Log-log plots of iron release rate in solution ($r_{Fe}$ moles m$^{-2}$ s$^{-1}$) versus pH for experiments performed with bacterial communities extracted from different horizons of gleicluvisol (GL-A$_1$; GL-E; GL-B) under different land use: forest (GLF), cultivation (GLC) and meadow (GLP). Each symbol represents the mean and standard deviation calculated from 4 replicates.
Figure 4: Log-log plots of iron release rate ($r_{Fe}$ moles m$^{-2}$ s$^{-1}$) versus pH for experiments performed with bacterial communities extracted from (A) different horizons of gleyicluvisol (GL-A1; GL-E; GL-B); dystric cambisol (DC-A1; DC-B; DC-C); calcic cambisol (CC-(B)/C) and luvisols (L-A1; L-E; L-B); (B) colluvial calcicleptosol (CCL-A1; CCL-C) and calcicleptosol (CL-A1; CL-C) and; (C) podzol (P-A1; P-E; P-Bh; P-Bs; P-C); and leptic podzol (LP-A1; LP-E; LP-Bs; LP-C) under forest covers. Each symbol represents the mean and standard deviation calculated from 8 replicates.
Figure 5: Percentage of metabolized carbon (amount of organic acids produced normalized at consumed glucose) versus iron release rates \( (r_{Fe} \text{ moles m}^{-2}\text{s}^{-1}) \) for all experiments. Black diamonds correspond to experiments performed with bacterial communities extracted from A1 and Bh horizons podzol, lepticpodzol, and dystric cambisol. White diamonds correspond to experiments performed with bacterial communities extracted from Bs and C horizons podzol, lepticpodzol, and dystric cambisol. Black circles correspond to experiments performed with bacterial communities extracted from loamy soil (luvisol and gleyicluvisol). White circles correspond to experiments performed with bacterial communities extracted from soil developed on limestone (calcaricleptosol, colluvialcalcaricleptosol and calcic cambisol). Each symbols represents the one measure of a mean sample.
References


