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Increase of apatite dissolution rate by Scots pine roots associated or not with *Burkholderia glathei* PML1(12)Rp in open-system flow microcosms

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Abstract

The release of nutritive elements through apatite dissolution represents the main source of phosphorus, calcium, and several micronutrients (e.g., Zn, Cu) for organisms in non-fertilized forest ecosystems. The aim of this study was to quantify, for the first time, the dissolution rate of apatite grains by tree roots that were or were not associated with a mineral weathering bacterial strain, and by various acids known to be produced by tree roots and soil bacterial strains in open-system flow microcosms. In addition, we explored whether the mobilization of trace elements (including rare earth elements) upon apatite dissolution was affected by the presence of trees and associated microorganisms. The dissolution rate of apatite by Scots pine plants that were or were not inoculated with the strain *Burkholderia glathei* PML1(12)Rp, and by inorganic (nitric) and organic (citric, oxalic and gluconic) acids at pH 5.5, 4.8, 3.8, 3.5, 3.0, and 2.0 was monitored in two controlled experiments: "plant–bacteria interaction" and "inorganic and organic acids". Analyses of the outlet solutions in the "plant–bacteria interaction" experiment showed that Scots pine roots and *B. glathei* PML1(12)Rp produced protons and organic acids such as gluconate, oxalate, acetate, and lactate. The weathering budget calculation revealed that Scots pines (with or without PML1(12)Rp) significantly increased (factor > 10) the release of Ca, P, As, Sr, Zn, U, Y, and rare earth elements such as Ce, La, Nd from apatite, compared to control abiotic treatment. Scanning electron microscopy observation confirmed traces of apatite dissolution in contact of roots. Most dissolved elements were taken up by Scots pine roots, *i.e.*, approximately 50% of Ca, 70% of P, 30% of As, 70% of Sr, 90% of Zn, and 100% of U, Y, and rare earth elements. Interestingly, no significant additional effect due to the bacterial strain PML1(12)Rp on apatite dissolution and Scots pine nutrition and growth was observed. The "inorganic and organic acids" experiment demonstrated that the apatite dissolution efficacy of organic acids was higher than for the inorganic acid and varied in function of the acids: oxalic acid > citric acid > gluconic acid > nitric acid for pH $\leqslant 3.5$. In addition, apatite dissolution increased with increasing acidity for each acid. Only oxalic acid generated non-stoichiometric release of calcium and phosphorus from apatite in the solution at pH $\leqslant 3.5$, due to the precipitation of Ca-oxalate crystals at apatite surfaces. Comparison of the experiments revealed that the apatite dissolution rate by Scots pines supplied with nutritive solution at pH 5.5 reached 2.0×10^{-13} mol cm^{-2} s^{-1} and was equivalent to rates with nitric acid at pH 3.2, gluconic acid at pH 3.5, citric acid at pH 3.7, and oxalic acid at pH 3.8.

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Altogether our results highlight that, through the production of weathering agents, notably protons and organic acids, tree roots and root-associated microorganisms are able to significantly increase the release of macro- and micro-nutrients from apatite, thus maintaining high-nutrient conditions to support their growth.

1. INTRODUCTION

In non-fertilized acid forest ecosystems, trees acquire calcium and phosphorus from mineral phases, primarily apatite. Calcium and phosphorus present in the apatite mineral structure, however, are not directly available to organisms; apatite dissolution is thus crucial to satisfy plant nutritional needs. The structure of apatite also accommodates a relatively high abundance of many trace elements essential for biological functions (e.g., rare earth elements (REE), Cu, Zn), that are likely to be released upon apatite dissolution. Apatite ($Ca_{10}(PO_4)_6$ (F, Cl, $OH)_2$) is the most common phosphate mineral in nature and occurs as an accessory phase in igneous, metamorphic, and sedimentary rocks. It can be formed abiotically as well as biologically. The solubility and reactivity of apatite varies as a function of its composition (Jahnke, 1984; Anderson et al., 1985; Valsami-Jones et al., 1998; Harouiya et al., 2007; Feng et al., 2011; Dorozhkin, 2012).

Many experimental studies have demonstrated that plants, bacteria and ectomycorrhizal fungi, can accelerate the release of calcium, phosphorus, and trace elements from apatite by producing inorganic and organic acids (Lange-Ness and Vlek, 2000; Wallander, 2000; Welch et al., 2002; Blum et al., 2002; Rosling et al., 2007; Turpault et al., 2009; Leake et al., 2009; Goyne et al., 2010; Feng et al., 2011; Uroz et al., 2011; Smits et al., 2012). Hutchens et al. (2006) even demonstrated that the bacterial strain *Bacillus megaterium* can accelerate apatite dissolution rates without contact with the mineral phase. According to Sperber (1958), apatite-solubilizing microorganisms are common in the soil, especially in rhizosphere soil, and can thus significantly increase plant growth and nutrition (Anderson et al., 1985; Lapeyrie et al., 1991; Hinsinger and Gilkes, 1997). Moreover Uroz et al. (2012) revealed that apatite minerals dug into a forest soil over four years were strongly colonized by bacteria with homology to *Burkholderia* and *Collimonas* strains known to be efficient at mineral weathering.

A range of low-molecular-weight organic acids, including oxalic, formic, citric, malic, gluconic, and acetic acids, are produced in soils from root exudates and microbial metabolites (Stevenson, 1967; Graustein et al., 1977; Lynch, 1978; Cromack et al., 1979; Fox and Comerford, 1990; Jones and Darrah, 1994; Fox, 1995; Gadd, 1999). These aliphatic and aromatic compounds are commonly detected in soil solutions (Fox and Comerford, 1990; Baziramakenga et al., 1995; Krzyszowska et al., 1996; Van Hees et al., 2005) at concentrations ranging from micro- to millimolar (Stevenson, 1991; Jones, 1998; Jones et al., 2003). Although these organic acids are considered short-lived in soils, their continual production makes these acids and their conjugated anions chemically important (Mc Coll and Pohlman, 1986; Fox and Comerford, 1992; Jones et al., 2003). Indeed, organic acids may increase rock and soil mineral dissolution by increasing proton concentration (proton-promoted dissolution; Tan, 1986; Furrer and Stumm, 1986), forming surface complexes that weaken and break metal–oxygen lattice bonds (ligand-promoted dissolution; Furrer and Stumm, 1986; Stumm, 1997), and through the formation of aqueous metal–ligand complexes that reduce the extent of relative saturation of solution with respect to the dissolving phases (Drever and Stillings, 1997).

Though the importance of apatite as a source of bioavailable calcium and phosphorus in forest soils and the capacity of plants and soil microorganisms to dissolve apatite is well documented, there is little quantitative data to support this observation. In addition, the mobilization of trace elements during biological dissolution of apatite has rarely been studied.

The aim of the present study is to fill these gaps through two controlled experiments with open-system flow microcosms containing apatite, *i.e.*, a "plant–bacteria interaction" experiment and an "inorganic and organic acids" experiment. The objectives of these experiments were (i) to quantify the effect of Scots pine roots on apatite dissolution, *i.e.*, the mobilization of calcium, phosphorus and trace-elements, (ii) to determine the contribution of the rhizosphere bacterial strain *Burkholderia glathei* PML1(12)Rp on apatite dissolution and Scots pine nutrition and growth, and (iii) to compare the apatite dissolution potential of Scots pine roots with and without *B. glathei* PML1(12)Rp and those of different inorganic and organic acids commonly produced by plants and microorganisms. We hypothesized that Scots pine roots and *B. glathei* PML1(12)Rp produce weathering agents and accelerate apatite dissolution and that *B. glathei* PML1(12)Rp contributes to mineral weathering and improves Scots pine nutrition and growth. Our experimental device inside the growth chamber consisted of open-system flow microscosms containing apatite, either continuously supplied with a nutrient solution (devoid of all elements present in the apatite) with or without Scots pine seedlings and *B. glathei* PML1(12)Rp, or continuously supplied with solutions of nitric, gluconic, citric, and oxalic acids at pH 5.5, 4.8, 3.8, 3.5, 3.0, and 2.0. The inputs and outputs of Ca, P, and trace elements in solution, as well as the immobilization of these elements by pine seedlings, were measured to compare the apatite dissolution rate in the various treatments.

2. MATERIALS AND METHODS

2.1. Mineral

Fluoroapatite was chosen for this study because it is a phosphate mineral frequently present in acidic soils devel-

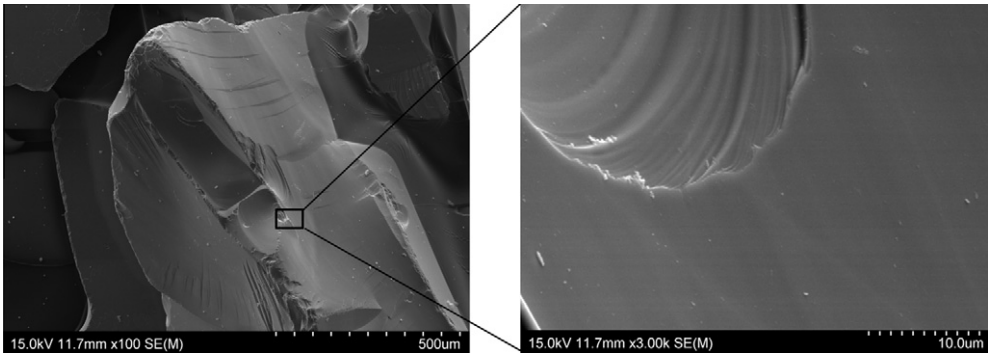

Fig. 1. SEM images of cleaned grains of apatite.

oped on granite that weathers relatively quickly, holds Ca and P, which are two elements essential for tree nutrition, and contains high amounts of trace elements notably REE. Natural fluoroapatite from Durango, Mexico, was used in this study. The main-section microprobe analysis of this sample showed that Durango apatite is well crystallized, pure (without inclusion), and homogeneous (absence of zonation). A total chemical analysis revealed that its composition was: 38.3 wt% Ca, 17.7 wt% P, 0.40 wt% Si, 0.31 wt% Na, 0.10 wt% Al, 0.06 wt% Fe, 0.05 wt% Mg, 4509 ppm Ce, 3485 ppm La, 1114 ppm Nd, 671 ppm As, 478 ppm Sr, 538 ppm Y, 362 ppm Pr, 211 ppm Th, 183 ppm Gd, 147 ppm Sm, 10 ppm U, 8 ppm Zn, and 5 ppm Cu. Its structural formula is consistent with $Ca_{9.77}La_{0.03}Ce_{0.04}Sr_{0.007}Fe_{0.003}Si_{0.06}$) ($P_{1.02}O_4$)$_6$ $F_{2.08}$ $Cl_{0.12}$ (Park et al., 2004).

Apatite crystals were ground, washed with distilled water, and ultrasonicated (three minutes at 100 volts) to remove fine particles. The apatite grains were then dry-sieved to obtain a size fraction between 0.5 and 1 mm. Scanning electron microscopy (SEM; Hitachi S4800 EDS connected to a Noran Vantage microanalysis system) observation of the starting mineral showed that the surfaces of apatite were relatively clean, without the fine particles that can be produced by the crushing procedure. A representative SEM image of these cleaned grains is provided in Fig. 1. As the specific surface area of apatite (0.5–1 mm) was too low to be measured using the BET method, it was calculated at 9.9 $cm^2\ g^{-1}$ using a spherical model. Pure quartz crystals, used as a physical culture substrate in our study, were prepared using the same method as for apatite and dry-sieved to obtain two size fractions: 0.5–1 and 1–2 mm. Apatite and quartz preparations were then sterilized by autoclaving (20 min at 120 °C). A previous experiment showed that the dissolution kinetics of both minerals was not modified by autoclaving.

2.2. Plant

Scots pine (*Pinus sylvestris* L.) was chosen for this study based on its hardiness in nutrient-poor soils and excellent growth under our laboratory conditions (Calvaruso et al., 2006). Scots pine seeds (provenance: Haguenau forest, France) were sown in a peat–vermiculite substrate (1/1). One hundred seedlings were grown in a greenhouse for 20 weeks under the following conditions: 60% humidity, a night temperature of 15 °C and a day temperature of 22 °C, a 16-h period of daylight, and watering of approximately 20 ml per seedling twice a day. After that, the seedling roots were washed very carefully four times successively with a brush in sterile ultra-pure water to remove remaining peat and vermiculite particles. Binocular microscope examination of each root was performed to check for the absence of peat and vermiculite particles and for fungal contamination. The excess water was removed using absorbent paper and the seedlings were weighed individually. Seedlings whose weight significantly differed from the average weight (155.1 ± 21.6 mg) were eliminated. Twenty six seedlings were randomly selected. Ten pines were used to quantify the initial root and shoot biomasses and initial Ca, P, and trace element contents of the seedlings, and sixteen pines were planted in the open-system flow microcosms of the "plant–bacteria interaction" experiment.

2.3. Bacterial strain

The bacterial strain *B. glathei* PML1(12) used in this study was isolated from the ectomycorrhizosphere of oak (*Quercus petreae*)–*Scleroderma citrinum* in the mineral soil horizon in the experimental forest site of Breuil-Chenue (Morvan, central France) (Calvaruso et al., 2007). This strain was selected because of its efficient mobilization of inorganic phosphorus and iron from tricalcium phosphate (TCP) and chrome azurol S (CAS) media, respectively (Calvaruso et al., 2007). Moreover, its ability to weather minerals has been demonstrated for biotite (Uroz et al., 2007) and apatite (Uroz et al., unpublished). The PML1(12) strain has also been demonstrated to increase the mobilization of K and Mg from biotite thus improving Scots pine nutrition and growth in open-system flow microcosms (Calvaruso et al., 2006).

For the inoculation experiments performed in this study, the rifampin-resistant mutant of strain PML1(12), strain PML1(12)Rp was used to accurately track the strain during the experiment. As described in Koele et al. (2009), 8 rifampin-resistant mutants were obtained by plating a cell suspension (ca. 10^{11} c.f.u. ml^{-1}) onto 10% TSA plates (3 g L^{-1} Tryptic Soy Broth from Difco and 15 g L^{-1} of agar) containing 100 mg rifampin per liter. After 5 days of incubation at 25 °C, the developing colonies were selected and transferred on same gelosed medium with 100 mg L^{-1} rifampin. The stability of the rifampin resistance was tested by subculturing the eight mutants on TSA agar plates 10 times. Each mutant was then plated

on TSA agar plates with or without 100 mg of rifampin per liter and bacterial counts were compared. The mineral weathering ability of each mutant was then tested using the microplate assay described in Uroz et al. (2007) and compared to the results obtained with the wild type strain. The mutant strain was stored at −80 °C.

The preparation of the bacterial inoculum consisted of the following three steps. Bacteria were grown on 10% TSA medium (3 g L^{-1} tryptic soy broth from Difco and 15 g L^{-1} agar) at 25 °C for 36 h. Bacteria were then suspended in sterile ultrapure water and washed twice. Finally, the suspension concentration was adjusted to 4×10^7 CFU ml^{-1} according to a A_{600} standard curve calibrated by plate enumeration. Two milliliters of this bacterial suspension were inoculated in each column for the treatments with strain PML1(12)Rp. Non-inoculated treatments received only two milliliters of water.

A preliminary 5-week study carried out in columns containing a mixture of apatite and quartz and continuously supplied with a carbon-free nutrient solution similar to that used in the biotic experiment (see below) revealed that strain PML1(12)Rp can survive under our experimental conditions (data not shown).

2.4. Solutions

Columns with biological material were supplied continuously with a sterile nutrient-poor solution. This solution contained all elements necessary for pine growth (except those present in apatite) in concentrations equivalent to those found in acidic soil (Typic Dystrochrept; Calvaruso et al. (2011)) from the Breuil-Chenue experimental forest site where the *B. glathei* PML1(12)Rp strain was isolated, *i.e.*, 10 mg L^{-1} of N (NO_3NH_4), 3 mg L^{-1} of K (KCl), and 1 mg L^{-1} of Mg and 1 mg L^{-1} of S ($MgSO_4$, $7H_2O$). The pH of the nutrient solution was approximately 5.5. The presence of the two forms of nitrogen in the nutrient solution was necessary to avoid the production of H^+ or OH^- by plants to balance ion charges within their cells. Indeed, if nitrogen was only available in the NH_4^+ form, plants would release H^+ to balance ion charges, thus acidifying the outlet solution (Haynes, 1990).

Columns without biological material were supplied continuously with a sterile nutrient-poor solution (see above) or 250 μM sterile solutions of different acids: nitric, citric, oxalic, and gluconic. Initial solution pH was adjusted to 5.5, 4.8, 3.8, 3.5, 3.0, and 2.0 ± 0.01 using dilute NaOH or HNO_3. Nitric acid was used as a model acidifying molecule. Citric, oxalic and gluconic acids were used as model chelating agents. These three organic acids were selected because they are produced by Scots pine roots (Leyval and Berthelin, 1991, 1993) and soil bacteria (Kim et al., 2005).

2.5. Experimental design and conditions (Fig. 2)

Our experimental device consisted of open-system flow microcosms containing apatite placed in a growth chamber with 60% humidity, a night temperature of 18 °C, a day temperature of 25 °C and a 17-h period of daylight. The microcosms consisted of columns made from sterile inert polypropylene Falcon tubes with corks, with the bottom cut and resealed with a 20 μm nylon membrane (Fisherbrand®). Each column was fitted with Tygon® tubes for inlet solutions. These tubes were fixed to the column through a hole drilled near the center of the cork. A preliminary test with fluorescein revealed that the central position of the Tygon® tubes allowed a distribution of the solution inlet over the whole reactor. In addition, the corks prevented contamination of the reactor. For the columns with a pine seedling, the cork was cut to allow the emergence of aerial parts. The inlet solutions were pumped at a constant rate with peristaltic pumps. To obtain the aerobic conditions indispensable for culturing tree seedlings, all columns were supplied with solution through the top. This prevented water saturation over the entire porosity (anaerobic conditions), part of the porosity remained filled with gas (the substrate moisture measured is described below). All outlet solutions were collected individually in 250 ml polyethylene flasks. For each column, the tube and flask were covered with aluminum foil to exclude light from the root and avoid algal development. Our study comprised two experiments, referred to as "plant–bacteria interaction" and "inorganic and organic acids" experiments.

For the "plant–bacteria interaction" experiment, the columns (15 cm depth, 4.1 cm inside diameter, size adapted to the culture of Scots pine seedlings) contained a sterile mixture of 4 g of fluoroapatite (0.5–1 mm diameter), 40 g of quartz (1–2 mm diameter), and 20 g of quartz (0.5–1 mm diameter). The pore volume represented approximately 46 ± 3% of the column. The quartz is used as chemically inert support for anchoring root system. Columns were or were not planted with Scots pines with or without inoculation with the bacterial strain *B. glathei* PML1(12)Rp and were continuously supplied (0.8 ml h^{-1}, turnover time of approximately 20 h) for 14 weeks with the nutrient-poor solution (Table 1). The abiotic control treatment, the treatment with PML1(12)Rp-only, and the treatments with pine seedlings non-inoculated or inoculated with PML1(12)Rp comprised 3, 6, 8, and 8 replicates, respectively. In addition, some columns without biological material were continuously supplied (0.8 ml h^{-1}) with solutions of nitric acid adjusted to pH 5.5, 3.8, 3.0, and 2.0 ± 0.01 over 14 weeks (Table 1). Each nitric acid treatment comprised 3 replicates. The "plant–bacteria interaction" experiment contained 34 columns.

For the "inorganic and organic acids" experiment, the columns (4.4 cm depth, 1.1 cm inside diameter) contained 2 g of sterile fluoroapatite (0.5 to 1 mm diameter). The pore volume represented approximately 45 ± 2% of the column. The columns were continuously supplied (0.5 ml h^{-1}, turnover time of approximately 1.5 h) with solutions of nitric, citric, oxalic, and gluconic acids adjusted to pH 5.5, 4.8, 3.8, 3.5, 3.0, and 2.0 ± 0.01 over 5 weeks (Table 1). Each treatment comprised 3 replicates; giving a total of 72 columns for the "inorganic and organic acids" experiment.

In preliminary studies, the size and composition of the open-system flow microcosms, as well as the inlet solution flow, were determined to obtain approximately the same apatite dissolution rate (r) in the "plant–bacteria interaction" and "inorganic and organic acids" experiments for pH ranging from 5.5 to 3.0 (Fig. 3).

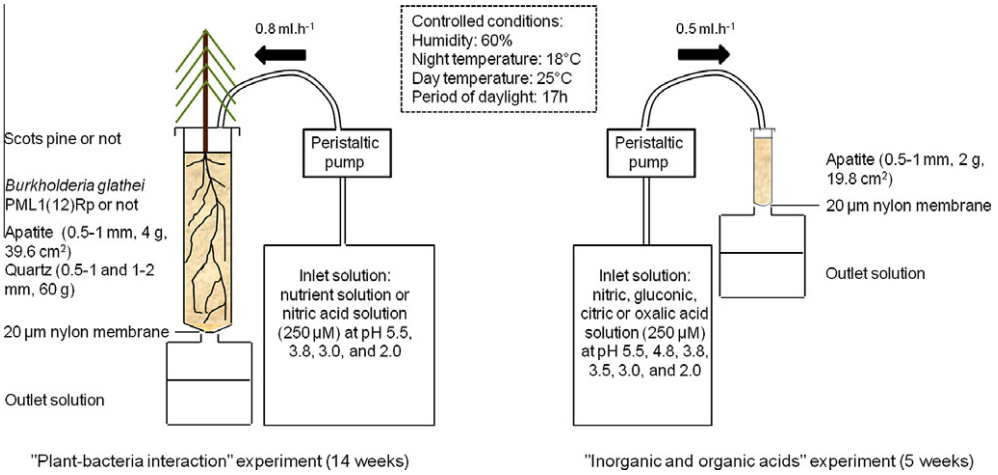

Fig. 2. Experimental set-up of the "plant–bacteria interaction" and "inorganic and organic acids" experiments. Each column, tube and flask was covered with aluminum foil to exclude light from the root and to avoid algal development. In the "plant–bacteria interaction" experiment, (i) columns with biological material were supplied with sterile nutrient solution containing all elements necessary to pine growth apart from those present in the apatite and (ii) columns without biological material were supplied with sterile nitric acid solution (250 µM) adjusted at pH 5.5, 3.8, 3.0, and 2.0.

Table 1
Experimental design and treatment setting.

Experiment	Treatment ID	Mineral		Solution[a]					Biological material	
		Apatite[b]	Quartz[c]	Nitric acid	Citric acid	Oxalic acid	Gluconic acid	Nutrient solution	*B. glathei* PML1(12)[d]	Scots pine
Plant–bacteria interaction	Scots pine	✓	✓					✓		✓
	Scots pine + PML1(12)	✓	✓					✓	✓	✓
	PML1(12)	✓	✓					✓	✓	
	Abiotic control	✓	✓[e]					✓		
	Nitric	✓	✓[f]	✓						
Inorganic and organic acids	Nitric	✓		✓[f]						
	Citric	✓				✓[f]				
	Oxalic	✓					✓[f]			
	Gluconic	✓						✓[f]		

[a] Solution was continuously supplied during the experiment at 0.8 ml h^{-1} and 0.5 ml h^{-1} for "plant–bacteria interaction" and "inorganic and organic acids" experiments, respectively.
[b] Four grams of apatite 0.5–1 mm in the "plant–bacteria interaction" experiment, 2 g of apatite 0.5–1 mm in the "inorganic and organic acids" experiment.
[c] Twenty grams of quartz 0.5–1 mm and 40 g of quartz 1–2 mm.
[d] The bacteria *B. glathei* PML1(12)Rp were isolated from the ectomycorrhizosphere of oak-*Scleroderma citrinum.*
[e] Solutions of pH 5.5, 3.8, 3.0, and 2.0.
[f] Solutions of pH 5.5, 4.8, 3.8, 3.5, 3.0, and 2.0.

Another preliminary study showed that the substrate moisture of our open-system flow microcosms (5 replicates) was 77.6 ± 4.7% and 80.5 ± 3.7% for the "plant–bacteria interaction" and "inorganic and organic acids" experiments, respectively.

2.6. Sample collection and analyses

2.6.1. Solution

Throughout both experiments, outlet solutions were collected weekly from each column. The volume and pH of each outlet solutions were measured, then a 10-ml aliquot was removed and the remainder was pooled each week to give a total outlet solution. The concentrations of Ca and P in the inlet and outlet solutions were measured weekly using an inductively coupled plasma atomic emission spectrometer (ICP-AES; Plasma torch JY180 ULTRACE). The evolution of the pH and Ca and P amounts in the outlet solutions was thus obtained for each column. The total amount of Ca and P in the outlet solutions was calculated for each column by adding the amounts of Ca and P measured each week. The trace-element concentration was measured only in the inlet and total outlet solutions for each column by ICP-MS spectrometer (ICP–MS, VG PlasmaQuad PQ2+).

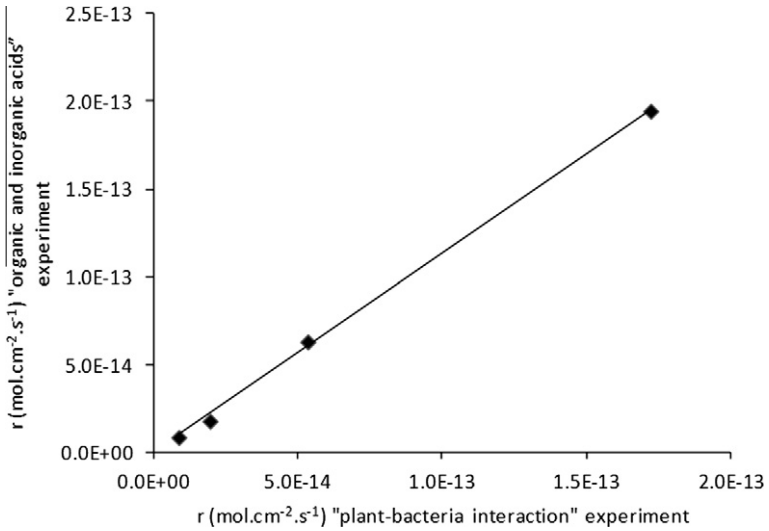

Fig. 3. Relation between the apatite dissolution rates for nitric acid at pH 5.5, 4.8, 3.8, and 3.0 in the "plant–bacteria interaction" experiment and in the "inorganic and organic acids" experiment. The equation is $y = 1.13x$ and $r^2 = 0.99$.

2.6.2. Mineral

For treatments without pine, 10 apatite particles were sampled from each column under sterile conditions. The particles were air-dried and glued to a glass slide with varnish, and the slides were coated with carbon (Goldstein et al., 2003). Images of the apatite surface and a semi-quantitative analysis of the constitutive elements of the sample were made simultaneously using a SEM (Hitachi S4800 EDS connected to a Noran Vantage microanalysis system).

For treatments with pine, the bulk mineral particles were separated from the root-adhering mineral particles with slight hand-shaking under sterile conditions. Root-adhering apatite grains were collected from the root system with the help of tweezers. Ten bulk and root-adhering apatite grains were sampled from each column. These apatite grains were treated and analyzed as described for the treatments without pine.

The root-adhering mineral particles were recovered by gentle washing of the roots in tubes containing 20 ml of sterile ultra-pure water. Approximately 100 mg of the bulk particles were also placed in tubes containing 20 ml of sterile ultra-pure water. Each tube was vortexed for one minute. An aliquot of the bulk and root-adhering suspensions obtained was used directly for PML1(12)Rp quantification by the method of dilution-spreading on selective medium. Another aliquot of these suspensions was incubated at 4 °C for one night and was used, the next day, for organic acid determination. Organic acids were measured using ion chromatography with conductivity detection (ICS 3000, Dionex Corp.) and an analytical column (IonPac® AS 11 HC, Dionex corp.) (Balland et al., 2010).

The mineral particle fractions collected were dried at 30 °C in a steam–air dryer for 7 days.

2.6.3. Plant

Ten pine seedlings pre-grown under the same conditions as those used in the column experiment, were sampled at the beginning of the column experiment to quantify the initial root and aerial part biomasses and initial Ca, P, and trace-element contents of the seedlings. After 14 weeks, the plants were removed from the columns and separated into aerial and root parts. The root systems were gently shaken by hand and washed very carefully in sterile ultra-pure water to remove mineral particles, which remained adhered to the roots. A binocular microscope examination was carried out to check the absence of mineral particles on the roots. The aerial and root parts were dried at 65 °C in a steam–air dryer (SR 1000 Thermosi) for five days to determine their dry weight. The aerial and root parts were cut with a chisel into 2–3 mm pieces. Approximately 150 and 100 mg (balance Mettler Toledo XS204) of aerial and root parts, respectively, were then placed in Teflon flasks containing 6 ml of HNO_3 (Merck Suprapur, 65%) overnight under a chemical hood. The next day, the aerial and root parts were digested with an ANTON PAAR Multiwave 3000 equipped with a 48MF50 rotor. Two international plant standards, *i.e.*, poplar leaves (LGC-Promochem NCS DC73350) and bush branches and leaves (LGC-Promochem NCS DC73349), and a nitric acid control were used in each series. The solutions obtained were filtered, and the resulting mineral composition was analyzed using ICP-AES (Plasma torch JY180 ULTRACE) for Ca and P and ICP-MS (ICP–MS, VG PlasmaQuad PQ2+) for trace-elements. The amounts of Ca, P, and trace-elements assimilated by pine plants during the experiment were thus calculated for the 8 replicates of each Scots pine treatment as the difference between final and initial Ca, P, and trace-element content in the pines.

2.7. Apatite weathering budget

A mineral weathering budget is a complex function of mineralogical, solution and physical variables. Because mineralogical and physical variables are constant for all experiments, it is possible to calculate the weathering budget for the different treatments.

For each column, the weathering budget of the apatite W was calculated for P as follows:

$$W = L + I$$

where W is the weathering budget based on P (mg); and L is the amount of P (mg) in the outlet solution, $L = Q * (C_o - C_i)$ with Q the total volume of the global outlet solution. C_o is the outlet concentration of P (mol L^{-1}) (outlet solutions); C_i, is the inlet concentration of P (nutrient solution, =0) (mol L^{-1}); and I is the amount of P (mg) immobilized by the pine plant during the experiment which corresponds to the difference between final and initial P content in the pine biomass (root and aerial part); $I = (M_{Pf} * C_{Pf} - M_{Pi} * C_{Pi})$ with M_{Pf} and M_{Pi} the final (after 14 weeks of experiment) and initial mass of Scots pine, respectively, and C_{Pf} and C_{Pi} the final (after 14 weeks of experiment) and initial concentration of P in Scots pine biomass, respectively.

2.8. Apatite dissolution rate

The apatite dissolution rate was calculated from the following formula:

$$r = \frac{w}{V_p * SA * M * \theta * t}$$

where r is the apatite dissolution rate (mol cm^{-2} s^{-1}); W is the weathering budget based on P (mol); V_P, is the P-stoichiometric coefficient of the apatite dissolution reaction calculated from the structural formula presented in the mineral section; SA is the initial apatite surface area (cm^2 g^{-1}); M is the initial apatite mass of the system (g); θ is the substrate moisture saturation; and t is the experiment duration (s). The r was calculated for the whole experiment because the part of P immobilized in the plant (I) can only be measured at the end of the experiment.

3. RESULTS

3.1. Plant–bacteria interaction experiment

3.1.1. *B. glathei PML1(12)Rp quantification*

The strain *B. glathei* PML1(12)Rp was inoculated in open-system flow microcosms at a concentration of approximately 10^6 CFU g^{-1} of dry substrate.

After 14 weeks, we confirmed that non-inoculated treatments were not contaminated with PML1(12)Rp and showed that PML1(12)Rp was still present in inoculated columns with or without pine, in concentrations higher than 10^5 CFU g^{-1} of dry bulk substrate (Table 2). In the rhizosphere of pine seedlings, the concentration of PML1(12)Rp reached 5×10^5 CFU g^{-1} of dry substrate, about five times that measured in the bulk soil, approximately 50% of the initial concentration.

3.1.2. *Acid production*

Only gluconate was detected in the substrate for the PML1(12)Rp alone treatment (Table 2). In return, gluconate, acetate, oxalate, and lactate were detected in the rhizosphere of Scots pines. Lactate was only detected in the rhizosphere of pine roots inoculated with PML1(12)Rp.

3.1.3. *Evolution of the pH of the outlet solution*

The pH of the outlet solutions collected under the columns inoculated or not with PML1(12)Rp and supplied with nutrient solution remained stable at approximately 6.5 all along the experiment (Fig. 4A).

In return, the pH of the outlet solutions collected under columns planted with pines with or without PML1(12)Rp decreased quickly to below 5 after only two weeks of experiment (Fig. 4A). After eight weeks of experiment, the pH of the outlet solutions collected under columns planted with pines with or without PML1(12)Rp reached 4.5 and 3.8, respectively, and remained stable until the end of the experiment. For nitric acid treatments, the pH of the outlet solutions reached approximately 6.5, 4.2, 3.3, and 2.3 for solution initially at pH 5.5, 3.8, 3.0, and 2.0, respectively.

3.1.4. *Evolution of Ca and P into the outlet solutions*

For the abiotic control treatment, the amounts of Ca and P released from apatite into the outlet solutions decreased slowly during the first five weeks of the experiment to reach values close to 0.05 mg of Ca and 0.02 mg of P per week until the end of the experiment (Fig. 4B and C). The same evolution of Ca and P into the outlet solutions was obtained for PML1(12)Rp, and nitric acid at pH 5.5 treatments (Fig. 4B and C). For nitric acid at pH 3.8, 3.0 and 2.0, the amounts of Ca and P released from apatite into the outlet solutions reached values close to 0.3, 1.2, 6.0 mg of Ca, respectively, and 0.1, 0.5, and 2.7 mg of P, respectively, by week from the sixth week until the end of the experiment.

The amounts of Ca and P in outlet solutions collected under columns planted with Scots pine seedlings (with or without PML1(12)Rp) were significantly higher than those of the abiotic control treatment throughout the experiment: 0.35–0.70 mg and 0.15–0.25 mg by week for Ca (Fig. 4B) and P (Fig. 4C), respectively.

Table 2
Concentrations of *B. glathei* PML1(12)Rp (in Log_{10} CFU g^{-1} DS (colony forming unit by gram of dry substrate)), and detection of organic acids in bulk and rhizosphere substrates at the end of the experiment.

	Compartments	Abiotic control	PML1(12)Rp alone	Pine	Pine + PML1(12)Rp
PML1(12)Rp (in Log_{10} CFU g^{-1} DS)	Bulk	0	5.9	0	5.1
	Rhizosphere	–	–	0	5.7
Organic acids	Bulk	–	Gluconate	Gluconate, acetate	Gluconate, acetate
	Rhizosphere	–	–	Gluconate, acetate, oxalate	Gluconate, acetate, lactate, oxalate

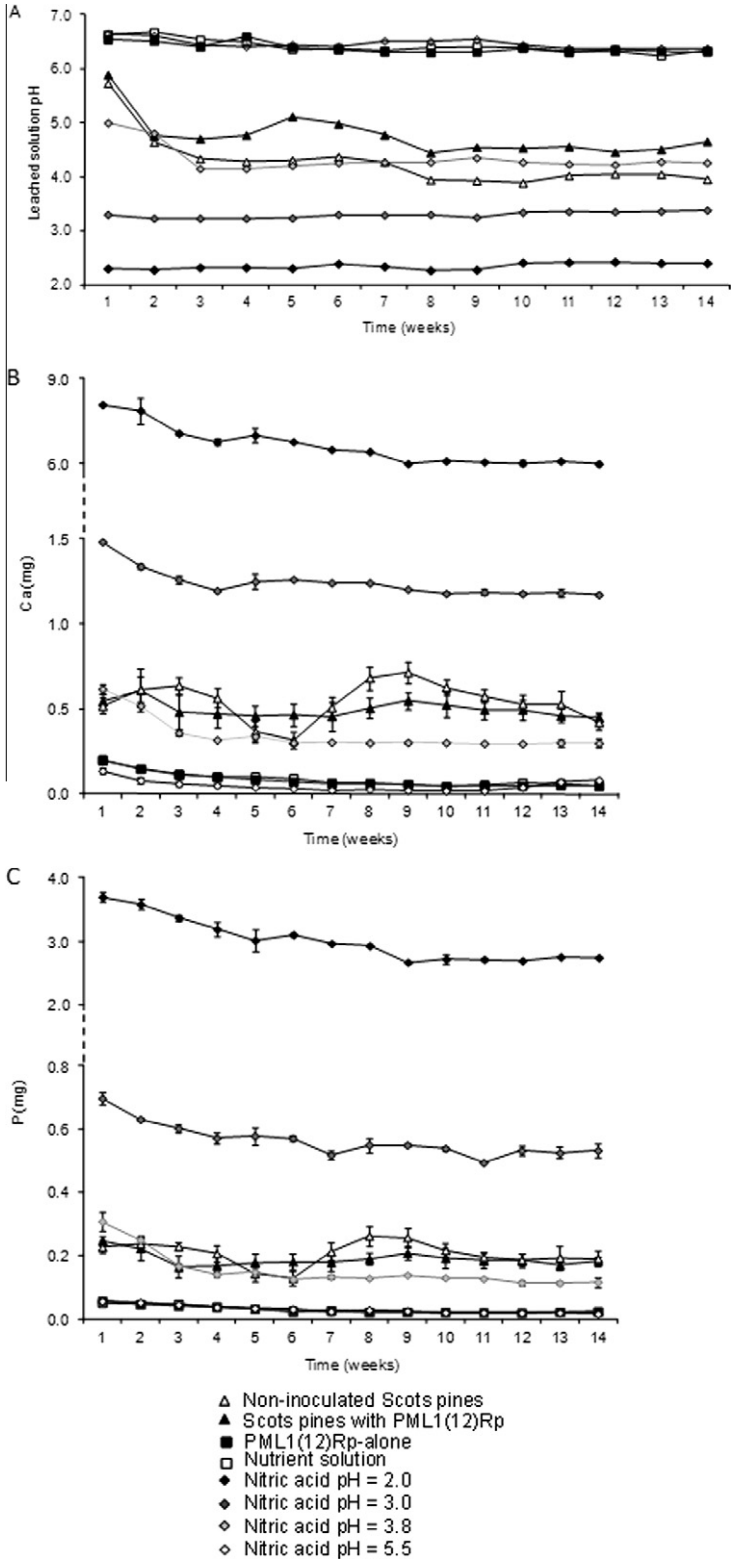

Fig. 4. Kinetic follow-up of the outlet solution pH (A) and of the amounts of Ca (B) and P (C) released into the outlet solutions during the 14 weeks of the biotic experiment. The white, the clear gray, the dark gray and the black rhombus represent the mean values of three replicates for nitric acid at pH 5.5, 3.8, 3.0, and 2.0, respectively. The white square represents the mean value of three replicates for the control treatment. The black square represents the mean value of six replicates for the bacteria treatment. The white and black triangles represent the mean values of eight replicates for the non-inoculated and *B. glathei* PML1(12)Rp Scots pine treatments, respectively.

Table 3
Apatite weathering budget for one element (*W*, in mg for Ca and P, and in μg for As, Sr, Zn, U, Y, and REE) and percentage of this element immobilized in the biomass of Scots pine (*I*, in %, corresponds to the amount of one element immobilized by pine/amount of this element released from apatite ratio) during the 14 weeks of the "plant–bacteria" experiment. Each value is the mean value of three (abiotic control, nitric acid pH 5.5, 3.8, 3.0, and 2.0), four (PML1(12)Rp), and eight replicates (Scots pine inoculated with PML1(12)Rp or not). In italics are presented standard deviations. For each element, values followed by the same letter are not significantly different according to a one-factor (treatment) ANOVA ($P = 0.05$), and the Bonferroni–Dunn test.

Treatments	Ca		P		As		Sr		Zn		U		Y		Sum REE	
	W (mg)	*I* (%)	*W* (mg)	*I* (%)	*W* (mg)	*I* (%)	*W* (mg)	*I* (%)	*W* (mg)	*I* (%)	*W* (mg)	*I* (%)	*W* (mg)	*I* (%)	*W* (mg)	*I* (%)
Abiotic control	1.18^{d}	–	0.41^{e}	–	2.69^{e,f}	–	1.64^{e,f}	–	0.00^{d}	–	0.00^{d}	–	0.00^{e}	–	0.08^{e}	–
	0.09		*0.01*		*0.22*		*0.10*		*0.00*		*0.00*		*0.00*		*0.01*	
Nitric acid	0.70e	–	0.42^{e}	–	2.61^{f}	–	1.53^{f}	–	0.00^{d}	–	0.00^{d}	–	0.00^{e}	–	0.02^{f}	–
pH 5.5	*0.04*		*0.02*		*0.05*		*0.03*		*0.00*		*0.00*		*0.00*		*0.00*	
Nitric acid	4.25^{c}	–	1.91^{d}	–	9.04^{d}	–	4.06^{d}	–	0.04^{c}	–	0.02^{c}	–	3.89^{d}	–	61.6^{d}	–
pH 3.8	*0.02*		*0.03*		*0.29*		*0.13*		*0.00*		*0.00*		*0.13*		*2.0*	
Nitric acid	15.0b	–	6.83^{c}	–	36.2^{b}	–	16.1^{c}	–	0.28^{b}	–	0.31^{b}	–	17.7c	–	327.9^{c}	–
pH 3.0	*0.02*		*0.04*		*0.1*		*0.1*		*0.01*		*0.01*		*0.1*		*9.7*	
Nitricacid	92.9^{a}	–	42.14^{a}	–	168.1^{a}	–	72.9^{a}	–	1.52^{b}	–	1.79^{b}	–	83.3^{b}	–	1533^{b}	–
pH 2.0	*0.56*		*0.50*		*6.0*		*2.6*		*0.06*		*0.06*		*3.0*		*55*	
PML1(12)	1.09^{d}	–	0.41^{e}	–	3.00^{e}	–	1.77^{e}	–	0.00^{d}	–	0.00^{d}	–	0.00^{e}	–	0.09^{e}	–
	0.07		*0.01*		*0.14*		*0.07*		*0.00*		*0.00*		*0.00*		*0.01*	
Pine	16.9^{b}	54.1^{a}	7.81^{b,c}	65.7^{a}	32.7^{b,c}	37.9^{a}	42.4^{b}	77.2^{a}	27.5^{a}	93.1^{a}	3.21^{a}	99.5^{a}	143.0^{a}	99.9^{a}	2392^{a}	99.9^{a}
	2.4	5.5	*1.13*	*5.3*	*3.9*	*6.6*	*8.5*	*6.0*	*7.5*	*3.2*	*0.84*	*0.5*	*32.6*	*0.1*	*749*	*0.1*
Pine + PML1(12)	14.0^{b}	49.0^{a}	9.62^{b}	69.4^{a}	27.0^{c}	28.6^{a}	29.3^{b}	67.1^{a}	26.3^{a}	94.5^{a}	3.05^{a}	99.5^{a}	140.1^{a}	99.7^{a}	2560^{a}	99.9^{a}
	3.1	*10.1*	*1.30*	*8.5*	*5.6*	*11.3*	*8.6*	12.8	*10.2*	*2.9*	*1.00*	*0.4*	*44.9*	*0.2*	*626*	*0.1*

3.1.5. Release of Ca, P, As, Sr, Zn, U, Y, and REE from apatite

All pine seedlings were able to mobilize Ca and P from apatite (Table 3). The total amounts released during the 14 weeks of the experiment reached 14–17 mg for Ca and 8–10 mg for P. For both elements, there was no significant difference between Scots pine with and without PML1(12)Rp. Pines released significantly more Ca and P than abiotic control, nitric acid at pH 5.5 and 3.8 and PML1(12)Rp alone treatments. Only pines inoculated with PML1(12)Rp released significantly more P than nitric acid at pH 3.0.

All pine seedlings were able to mobilize As, Sr, Zn, U, Y, and REE from apatite (Table 3). The total amounts released during the 14 weeks of the experiment reached 33 μg for As, 42 μg for Sr, 27 μg for Zn, 3 μg for U, more than 100 μg for Y, and more than 2000 μg for REE. Pines released more As than the abiotic control, nitric acid at pH 5.5 and 3.8, and PML1(12)Rp alone treatments. Pines released significantly more Sr than all other treatments except nitric acid at pH 2.0. Pines released significantly more Zn, U, Y, and REE than all other treatments including nitric acid at pH 2.0.

For all elements, there was no significant difference between the non-inoculated Scots pines and those inoculated with PML1(12)Rp.

3.1.6. Uptake of elements issued from apatite weathering and Scots pine growth

According to the weathering budget for Ca and P, all pine seedlings were able to take up Ca and P released from apatite. During the 14 weeks of the experiment, 7.0 and 9.2 mg of Ca (issued from apatite weathering) was taken up by Scots pines inoculated with PML1(12)Rp and the non-inoculated Scots pines, respectively. These quantities correspond to approximately 50% of the Ca released from apatite (Table 3). More than 80% of the Ca taken up by the pines was translocated to above-ground biomass. During the 14 weeks, 6.7 and 5.2 mg of P (issued from apatite weathering) was taken up by Scots pines inoculated with PML1(12)Rp and the non-inoculated Scots pines, respectively, corresponding to approximately 70% of the P released from apatite (Table 3). More than 60% of the P taken up by the pines was translocated to above-ground biomass. During the 14 weeks, approximately 27–33 μg of As, 29–42 μg of Sr, 26–27 μg of Zn, 3 μg of U were taken up by Scots pine roots. These amounts correspond to about 30% of the As, 30–40% of the Sr, 90% of Zn, and 100% of the U released from apatite (Table 3). No significant difference between the non-inoculated Scots pines and those inoculated with PML1(12)Rp was observed for Ca, P, As, Sr, Zn, and U.

During the 14 weeks, almost 100% of the Y and REE released from apatite was taken up by Scots pines (Table 3). However, less than 1% of Y and REE taken up by Scots pines was translocated to the aerial parts (data not shown). The enrichment rate in Y and REE (the ratio of the amount of one element in the plant biomass at the end of the experiment: amount of one element in the plant biomass at the beginning of the experiment) of roots during the experiment was approximately 240 versus only 20 in the aerial parts.

The REE distribution pattern in Scots pines reveals that seedlings induce fractionation of REE and that this fractionation is different in the root and aerial parts (Fig. 5A and B). The REE distribution pattern confirms the absence of an effect of PML1(12)Rp on apatite dissolution under our experimental conditions.

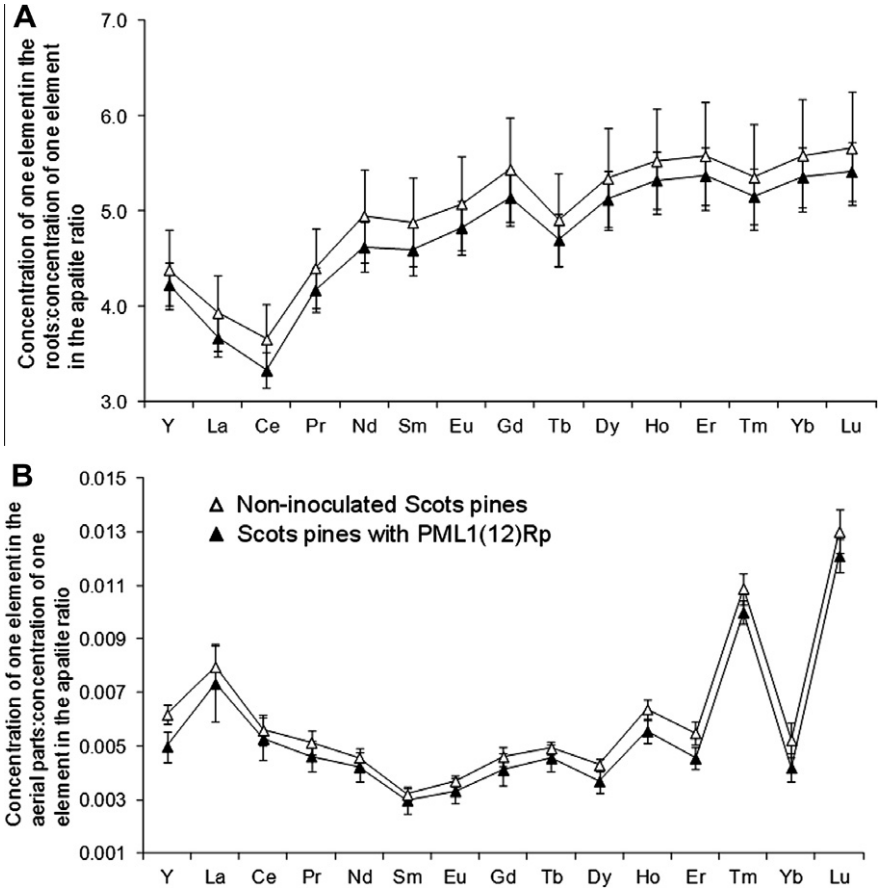

Fig. 5. REE distribution pattern in the Scots pine roots (A) and aerial parts (B). Dissolved REE are normalized to the concentration of REE in apatite. The white and black triangles represent the mean values of eight replicates for the non-inoculated and *B. glathei* PML1(12)Rp Scots pine treatments, respectively. Bars represent standard deviation.

3.1.7. Apatite weathering process based on Ca and P monitoring

Plotting the amount of Ca released from apatite against the amount of P released from apatite revealed that apatite was dissolved in a stoichiometric way for the following treatments: control, nitric acid at pH 5.5, 3.8, 3.0, and 2.0, non inoculated Scots pine, and PML1(12)Rp (Fig. 6). In contrast, apatite was dissolved in a non-stoichiometric way for the treatment with Scots pines inoculated with PML1(12)Rp (Fig. 6): there was a greater mobilization of P from apatite compared to that of Ca. The SEM observations of apatite particles sampled in the treatments with Scots pines clearly show traces of apatite dissolution in contact with roots, *i.e.*, formation of residual needles on the basal surface of apatite grains (Fig. 7).

3.1.8. Apatite weathering budget and apatite dissolution rate based on P monitoring

According to the total weathering budget based on P monitoring, between 44 and 54 mg of apatite were weathered by pine plants, representing between 1.1% and 1.4% of the apatite added to the column. There was no significant difference between the non-inoculated Scots pines and those inoculated with PML1(12)Rp. Significantly more apatite was weathered by Scots pines compared to all other treatments except nitric acid at pH 2.0.

Based on the formula presented in Section 2.8), the apatite dissolution rate based on P was $1.7–2.0 \times 10^{-13}$ mol cm^{-2} s^{-1} in the presence of Scots pines with or without *B. glathei* PML1(12)Rp, respectively.

3.1.9. Scots pine growth

During the 14 weeks, the biomass of pine seedlings with or without PML1(12)Rp increased by approximately 200%, *i.e.*, approximately 150 mg of dry biomass at the beginning of the experiment to 450–500 mg at the end of the experiment. All pine seedlings were healthy at the end of the experiment: no yellowing was observed, and the root system colonized the whole reactor. For the root, aerial part or total plant biomass, no difference was observed between the non-inoculated Scots pines and those inoculated with PML1(12)Rp (Fig. 8).

3.2. Inorganic and organic acids experiment

3.2.1. Apatite weathering process based on Ca and P monitoring

Plotting the amount of Ca released from apatite against the amount of P released from apatite revealed that apatite

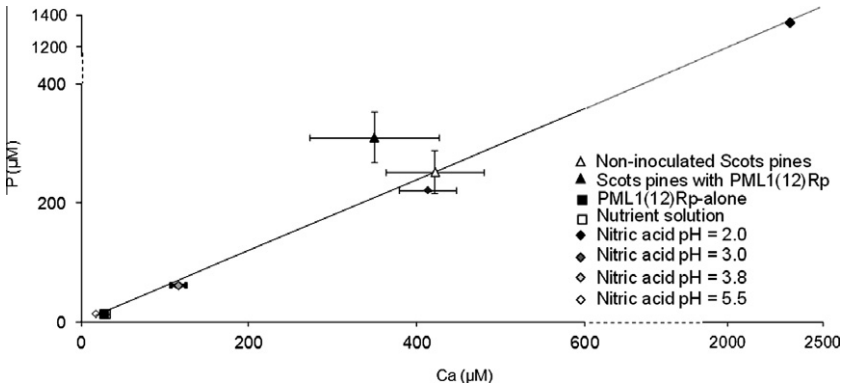

Fig. 6. Relation between Ca and P amounts in the outlet solutions and taken up by plants, *i.e.,* mobilized from the apatite. The white, the clear gray, the dark gray and the black rhombus represent the mean values of three replicates for nitric acid at pH 5.5, 3.8, 3.0, and 2.0, respectively. The white square represents the mean value of three replicates for the control treatment. The black square represents the mean value of six replicates for the *B. glathei* PML1(12)Rp treatment. The white and black triangles represent the mean values of eight replicates for the non-inoculated and *B. glathei* PML1(12)Rp Scots pine treatments, respectively. Bars represent standard deviation. The black curve represents the Ca/P stoichiometry in the Durango apatite which refers to a congruent dissolution process.

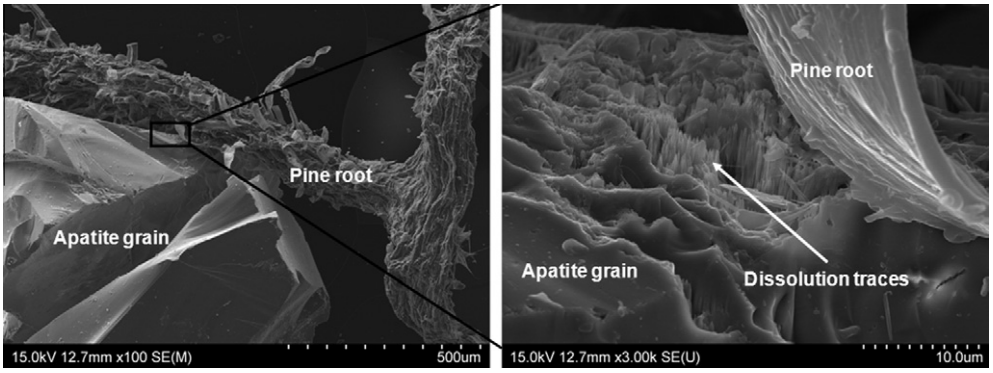

Fig. 7. SEM images of reacted apatite in the contact of Scots pine roots. Apatite surface is dissolved only under the Scots pine roots. The long spires form parallel to the *c* axis result from apatite dissolution.

was dissolved in a stoichiometric way for the nitric, citric and gluconic acid treatments at all pH and for the oxalic acid treatment at pH 5.5, 4.8 and 3.8 (Fig. 9). In contrast, apatite was dissolved in a non-stoichiometric way for the oxalic acid treatment at pH 3.5, 3.0 and 2.0 (Fig. 9). In these experiments, the Ca/P ratios in solution were much lower than expected. SEM examination of the apatite that reacted in oxalate solutions showed the formation of euhedral crystals precipitated on the apatite surfaces (Fig. 10A). These secondary phases were not observed in any other experiments and were likely to be calcium oxalates (Graustein et al., 1977; Cromack et al., 1979; Lapeyrie and Bruchet, 1986; Gharieb et al., 1998).

3.2.2. Apatite weathering budget and apatite dissolution rate based on P monitoring

Because Ca precipitates were observed on the apatite surfaces while P was not precipitated, dissolved P was used as an indicator of apatite weathering budget and apatite dissolution in our open-flow microcosms. The effect of pH and acids on apatite dissolution was ascertained by comparing the amount of P released into solution for experiments under similar conditions.

Whatever the acid considered, the apatite weathering budget increased with decreasing pH (Table 4). The apatite weathering budget based on P indicated that between pH 5.5 and 2.0, the amount of apatite dissolved during the five weeks ranged from 0.2 mg for all acids to approximately 47 mg, 140 mg, 232 mg, and 107 mg for nitric, citric, oxalic, and gluconic acids, respectively (Table 4). This represented 0.1% to 1.2%, 3.5%, 5.8%, and 2.7% of the apatite added to the column for nitric, citric, oxalic, and gluconic acids, respectively.

At pH 5.5, the apatite weathering budget based on P does not significantly differ for the different acids. At pH 4.8, apatite dissolution increases as follows: citric acid > nitric acid = oxalic acid = gluconic acid. At pH 3.8, the apatite weathering budget based on P increases as follows: citric acid > oxalic acid > gluconic acid = nitric acid. At pH 3.5, 3.0, and 2.0, the apatite weathering budget based on P increases as follows: oxalic acid > citric acid > gluconic acid > nitric acid.

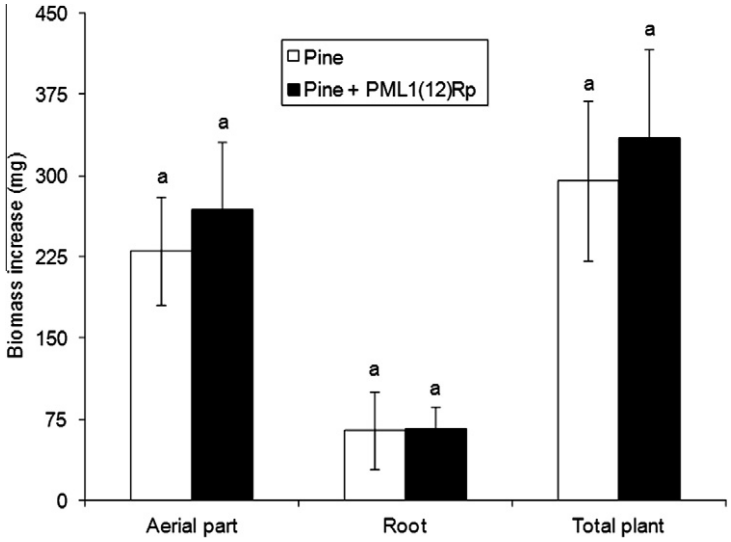

Fig. 8. Increase of root, aerial part, and total plant biomass during the 14 weeks of the column experiment for the non-inoculated Scots pine (white) and *B. glathei* PML1(12)Rp inoculated Scots pine (black) treatments. Each plot is a mean value of eight replicates. Bars represent standard deviation. For each variable (root, aerial part, total plant biomass), treatments associated with the same letter are not significantly different according to a one-factor (fungal treatment) ANOVA ($P = 0.05$), and the Bonferroni-Dunn test.

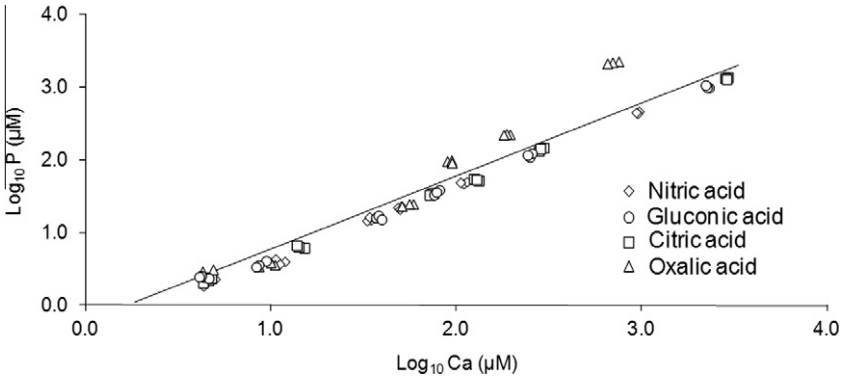

Fig. 9. Relation between Ca and P amounts released from apatite by the different acids and at different pH. The rhombus, the triangles, the squares, the circles represent the nitric acid, the oxalic acid, the citric acid, and the gluconic acid, respectively. The black curve represents the Ca/P stoichiometry in the Durango apatite which refers to a congruent dissolution process.

Apatite dissolution rates based on P release in nitric, citric, oxalic and gluconic acid solutions from pH 5.5 to 2.0 ranged from approximately 1.0×10^{-14} to 2.1×10^{-12} mol cm^{-2} s^{-1}, 1.0×10^{-14} to 6.1×10^{-12} mol cm^{-2} s^{-1}, 1.3×10^{-14} to 1.0×10^{-11} mol cm^{-2} s^{-1}, and 1.1×10^{-14} mol cm^{-2} s^{-1} to 4.7×10^{-12} mol cm^{-2} s^{-1}, respectively (Table 5).

4. DISCUSSION

While numerous studies clearly demonstrated the importance of apatite as a source of bioavailable calcium and phosphorus, notably in nutrient-poor forest ecosystems, and the capacity of tree roots and soil microorganisms to dissolve apatite and to release nutrients, the literature still lacks quantitative data. Thanks to open-system flow microcosms not saturated in water, our study allowed us for the first time to quantify (i) the impact of tree roots associated or not with a mineral weathering bacterial strain on apatite dissolution rates, as well as the contribution of this strain to tree nutrition, and (ii) the impact of various inorganic and organic acids on apatite dissolution rates. In fine, these experiments allowed us to compare the apatite weathering potential of Scots pines with these different acids.

4.1. Apatite dissolution rates

The dissolution of fluoroapatite in soils occurs according to the following ideal reaction (Khasawneh and Doll, 1978): $Ca_{10}(PO_4)_6F_2 + 12\ H^+ \leftrightarrow 10\ Ca^{2+} + 6\ H_2PO_4 + F^-$ (1). For the forward reaction to continue, an adequate supply of protons (H^+) and removal of the dissolution products, e.g., Ca^{2+}, H_2PO_4, and F^-, from the reaction site are required. The open-system flow microcosms used in this

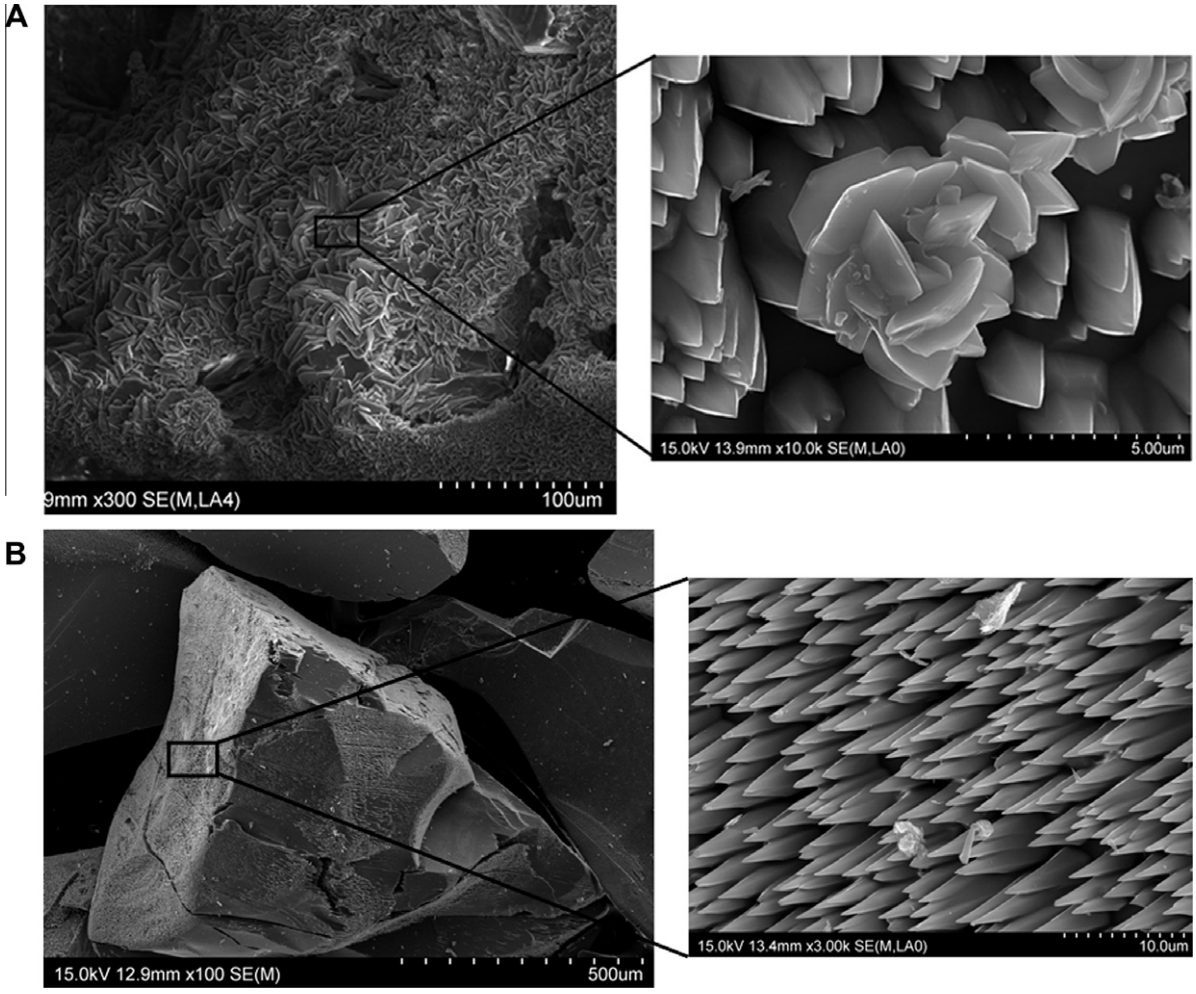

Fig. 10. SEM images of (A) Ca oxalate crystals on apatite surface during dissolution by oxalic acid at pH 2.0 and (B) formation of residual needles on the basal surface of apatite during dissolution by nitric acid at pH 2.0.

study met these criteria because Scots pines and bacteria in the "plant–bacteria interaction" experiment and the inorganic and organic acids in the "inorganic and organic acids" experiment supplied protons, and because the dissolution products left the column through the outlet solutions.

The evolution of Ca and P in the outlet solutions revealed steady-state conditions in the "plant–bacteria interaction" experiment for the values in the abiotic control treatment. This result indicates that abiotic factors, notably mineral factors, were stable throughout the experiment, allowing us to assess the effect of biotic factors on the apatite weathering process. In addition, we observed at the end of the experiment, that PML1(12)Rp was only present in microcosms initially inoculated with this strain (no contamination of other treatments) in concentrations of approximately 10^5 CFU g^{-1} of dry substrate. These observations show the ability of this strain to maintain in our experimental conditions, thus allowing us to assess the effect of PML1(12)Rp on apatite dissolution and Scots pine nutrition and growth.

The weathering budgets based on P monitoring demonstrated that *B. glathei* PML1(12)Rp alone did not significantly increase apatite dissolution compared to the abiotic control treatment. This most likely results from the absence of a carbon source in the nutrient solution to support microbial metabolism. The strain *B. glathei* PML1(12)Rp may have been in a state of metabolic dormancy, as suggesting by the lack of acidification of the outlet solution.

In return, the weathering budgets demonstrated that whatever the bacterial status (with or without *B. glathei* PML1(12)Rp), pine roots significantly increased apatite weathering compared to the abiotic control. Scots pines dissolved more than 1% of the initial apatite during the 14 weeks of the experiment. This result demonstrates the capacity of Scots pines to significantly increase the bioavailability of nutrients trapped in soil minerals. This dissolution rate corresponds to that measured by Turpault et al. (2009) for the same Durango apatite (0.5–1 mm) incubated for four years in a beech stand, *i.e.*, 0.6–1.8%. This result clearly demonstrates that the mineral weathering process is intensified 15-fold in our open-system flow microcosms compared to field studies. This difference is mainly due to experimental conditions that are conducive to mineral dissolution, *i.e.*, high temperature, water flow, soil moisture,

Table 4
Apatite weathering budget for one element (W, in mg for Ca and P) during the 5 weeks of the "inorganic and organic acids" experiment. Each value is the mean value of three replicates for Ca and P. In italics are presented standard deviations. For each element and each pH, values followed by the same letter are not significantly different according to a one-factor (acid type) ANOVA ($P = 0.05$), and the Bonferroni–Dunn test.

	Treatments	Ca W (mg)	P W (mg)
pH 5.5	Nitric acid	0.19[a]	0.09[a]
		0.01	*0.01*
	Citric acid	0.18[a]	0.09[a]
		0.01	*0.01*
	Oxalic acid	0.18[a]	0.10[a]
		0.01	*0.01*
	Gluconicacid	0.17[a]	0.10[a]
		0.01	*0.01*
pH 4.8	Nitric acid	0.45[b]	0.16[b]
		0.03	*0.01*
	Citric acid	0.58[a]	0.26[a]
		0.03	*0.01*
	Oxalic acid	0.39[b,c]	0.15[b]
		0.04	*0.01*
	Gluconicacid	0.35[c]	0.15[b]
		0.02	*0.01*
pH 3.8	Nitric acid	1.36[d]	0.62[c]
		0.03	*0.04*
	Citric acid	2.96[a]	1.38[a]
		0.07	*0.04*
	Oxalic acid	2.21[b]	0.98[b]
		0.16	0.05
	Gluconicacid	1.53[c]	0.65[c]
		0.05	*0.04*
pH 3.5	Nitric acid	1.93[d]	0.89[d]
		0.04	0.05
	Citric acid	5.16[a]	2.16[b]
		0.75	*0.06*
	Oxalic acid	3.72[b]	3.85[a]
		0.12	*0.15*
	Gluconicacid	3.15[c]	1.45[c]
		0.10	*0.11*
pH 3.0	Nitric acid	4.40[b]	1.97[d]
		0.78	*0.06*
	Citric acid	11.50[a]	5.70[b]
		0.36	*0.30*
	Oxalic acid	7.51[c]	9.08[a]
		0.30	*0.13*
	Gluconicacid	10.00[b]	4.71[c]
		0.27	*0.29*
pH 2.0	Nitric acid	38.15[c]	18.14[d]
		0.65	*0.62*
	Citric acid	115.2[a]	53.7[b]
		1.92	*1.63*
	Oxalic acid	28.10[b]	88.74[a]
		2.03	*2.85*
	Gluconicacid	90.59[b]	41.09°
		193	*182*

surface of contact between apatite grains and roots, tree age, etc.

The weathering budgets also demonstrated that with or without *B. glathei* PML1(12)Rp, pine roots significantly increase the release of As, Sr, Zn, U, Y, and REE from apatite. According to Hinsinger (2000), the bioavailability of trace elements is related to root-induced chemical changes in the rhizosphere, *i.e.*, accumulation and depletion of ionic species, acidification and alkalinization, oxidation and reduction, and complexation and chelation. Mailloux et al. (2009) observed in laboratory experiments that phosphate-limited cells of *Burkholderia fungorum* mobilize ancillary As from apatite. According to these authors, As mobilization is a by-product of apatite lattice weathering for nutrient acquisition. In column flow experiments, Luo and Gu (2011) also demonstrated that complexing organic ligands such as EDTA (Ethylenediaminetetraacetic acid) and citrate can mobilize U by dissolving U-bearing minerals, even under in a strict anaerobic environment. Regarding REE, Feng et al. (2011) demonstrated that two soil bacteria, *i.e.*, *Pantoea agglomerans* and *Bacillus megaterium*, increase the release of REE from fluoroapatite significantly more than the abiotic treatment and scavenge REE during their growth. In addition, Cao et al. (2001) showed that La, Ce, Gd and Y release increased gradually with a decrease in pH from 7.5 to 3.5, highlighting the strong impact of the production of protons and organic acids by plants and microorganisms on REE mobilization from mineral phase.

Table 5
Apatite dissolution rate r (mol cm^{-2} s^{-1}) for the different acids and for the different pH in the "inorganic and organic acids" experiment. Each value is the mean value of three replicates. For each variable, values followed by the same letter are not significantly different according to a one-factor (acid type) ANOVA ($P = 0.05$), and the Bonferroni–Dunn test.

pH	r (mol cm^{-2} s^{-1})			
	Nitric acid	Gluconic acid	Citric acid	Oxalic acid
5.5	9.5×10^{-15a}	1.1×10^{-14a}	1.0×10^{-14a}	1.3×10^{-14a}
4.8	1.8×10^{-14b}	1.7×10^{-14b}	2.9×10^{-14a}	1.7×10^{-14b}
3.8	6.7×10^{-14c}	7.4×10^{-14c}	1.6×10^{-13a}	1.1×10^{-13b}
3.5	1.0×10^{-13d}	1.7×10^{-13c}	2.5×10^{-13b}	4.4×10^{-13a}
3.0	2.3×10^{-13d}	5.4×10^{-13c}	6.5×10^{-13b}	1.0×10^{-12a}
2.0	2.1×10^{-12d}	4.7×10^{-12c}	6.1×10^{-12b}	1.0×10^{-12a}

The geometric form of the dissolution traces on apatite grains seems to be controlled by two factors in our study. First, a crystallographic factor was applied to all treatments: apatite dissolution was characterized by the formation of residual needles on the basal surface (Figs. 7 and 10B), as already observed by Thirioux et al. (1990), Welch et al. (2002), Harouiya et al. (2007), and Turpault et al., 2009 on fluoroapatite. This seems to indicate a large contribution of this mineral face to mineral dissolution as already shown for other types of minerals such as inosilicates (Xie and Walther, 1994) or the phyllosilicates (Turpault and Trotignon, 1994). Second, there was a "biological contact" factor: in the treatment with Scots pines, dissolution traces were only observed on mineral faces in contact with roots, as observed by Turpault et al. (2009), (Fig. 7).

Apatite dissolution rates based on P release reached 2.0×10^{-13} mol cm^{-2} s^{-1} in the presence of Scots pines. Compared to the "inorganic and organic acids" experiment,

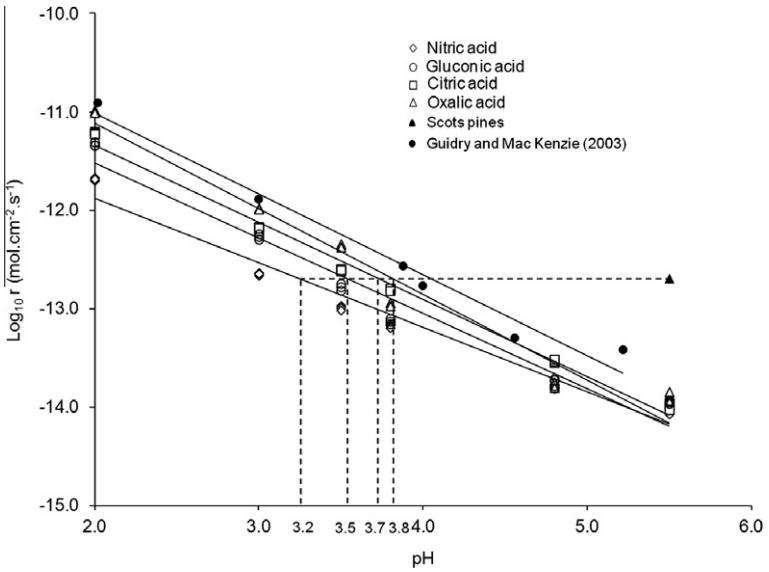

Fig. 11. Comparison between the $\log_{10}$ of apatite dissolution rate by Scots pines and those by inorganic and organic acids in the "geochemical" experiment. Note that the pH is the pH of the inlet solutions. The equations are $y = -0.65x - 10.58$ ($r^2 = 0.97$), $y = -0.77x - 10.0$ ($r^2 = 0.96$), $y = -0.78x - 9.8$ ($r^2 = 0.99$), and $y = -0.87x - 9.4$ ($r^2 = 0.96$) for nitric (rhombus), gluconic (circle), citric (square), and oxalic (triangle) acids, respectively. The black points correspond to the apatite dissolution rate measured by Guidry and Mackenzie (2003). The apatite dissolution rate by Scots pine is equivalent to those by nitric acid at pH 3.2, gluconic acid at pH 3.5, citric acid at pH 3.7, and oxalic acid at pH 3.8.

this value was equivalent to those of nitric acid at pH 3.2, gluconic acid at pH 3.5, citric acid at pH 3.7, and oxalic acid at pH 3.8 (Fig. 11). The stimulation of mineral weathering by plant roots associated with rhizosphere bacteria was already observed by Leyval and Berthelin (1991), Puente et al. (2004), and Calvaruso et al. (2006). The latter showed, in similar open-system flow microcosms, that Scots pine seedlings with or without *B. glathei* PML1(12) increased biotite weathering by factors of 1.7 and 2.5, respectively. The effect of plant roots and root-associated microorganisms on mineral weathering may result from physical and/or biochemical processes linked to root and microbial activities. These processes have ever been widely described, e.g., fragmentation of soil minerals, improvement of soil structure, nutrient uptake, exudation of a wide range of compounds such as CO_2, protons, anions, cations, inorganic acids, organic acids, organic ligands, and discussed in different reviews (Robert and Berthelin, 1986; Jongmans et al., 1997; Kelly et al., 1998; Barker et al., 1997; Landeweert et al., 2001; Berner et al., 2005; Gadd, 2007; Uroz et al., 2009; Andrews et al., 2011). In our study, we observed a drastic decrease in the outlet solution pH in the presence of Scots pines with or without *B. glathei* PML1(12)Rp: from pH 6.5 for the abiotic control treatment to pH 4.0–4.5 for Scots pine treatments. This acidification results from the production of CO_2 during respiration, from the release of organic compounds in the root and microbial exudates, and from the balance of ion charges within the roots, which depends on the excretion of one H^+ ion for every cation absorbed (Barker et al., 1997; Hinsinger et al., 2005).

We have also demonstrated that Scots pines and *B. glathei* PML1(12)Rp produce organic acids such as gluconate, oxalate, acetate, lactate, confirming the previous observations of Leyval and Berthelin (1991, 1993) for Scots pines and Kim et al. (2005) for *B. glathei*. According to Moghimi et al. (1978), organic acids produced in the rhizosphere can enhance the dissolution of phosphate compounds both by supplying protons and by complexing Ca^{2+} ions. Because of the short life of organic acids, it is likely that our method for organic acid sampling allows us to access only a fraction of the diversity of the organic acids produced in our experimental conditions. As an example, citric acid, generally produced by Scots pines and *B. glathei*, was not detected in our substrate, most likely because of its quick degradation in the rhizosphere (Braissant et al., 2002).

In our geochemical experiment, we clearly demonstrated that all organic acids tested (oxalate, citrate, and gluconate) more efficiently weather apatite and release Ca and P than inorganic acids for pH $\leqslant$3.5. This is in agreement with the observation of Welch et al. (2002) who demonstrated that oxalate increases apatite dissolution compared to inorganic solution at pH 3.0 by a factor of approximately 2.5. The effect of organic acids on mineral weathering has been demonstrated on other minerals, including plagioclase (Welch and Ullman, 1993; Welch et al., 1999), hornblende (Liermann et al., 1999; Van schöll et al., 2006; Hausrath et al., 2009), feldspars (Manley and Evans, 1986; Hutchens et al., 2003), phlogopite (Paris et al., 1996), augite (Hausrath et al., 2009), biotite (Wallander and Wickman, 1999), sphene (Hausrath et al., 2009), microcline (Wallander and Wickman, 1999), and muscovite (Van schöll et al., 2006).

In addition, we have demonstrated an increase in apatite dissolution rate with increasing acidity of inlet solutions from pH 5.5 to pH 2.0 for each acid. These results confirm the observations of Guidry and Mackenzie (2003) for pH comprised between 2.0 and 6.0 and Chaïrat et al. (2007)

for pH comprised between 3.0 and 7.0. This increase in mineral dissolution with increasing acidity of solutions is a common feature for many mineral dissolution reactions (*i.e.*, Blum and Lasaga, 1988; Drever and Vance, 1994; Welch et al., 2002). Dissolution of fluoroapatite under acidic conditions can be described by the reaction (1), presented above. It is clear from this equation that elevated levels of protons should accelerate dissolution reaction. Apatite dissolution rates based on P release in inorganic solutions from pH 5.5–2.0 ranged from approximately 1.0×10^{-14} to 2.1×10^{-12} mol cm^{-2} s^{-1}. These values are consistent with those of Valsami-Jones et al. (1998), Guidry and Mackenzie (2003), and Chaïrat et al. (2007) in open-system microcosms that measured fluoroapatite dissolution rates based on Ca release by HCl, *i.e.*, 10^{-14} to 10^{-11} mol cm^{-2} s^{-1} for inlet solution pH ranging from 6.0 and 2.0. The discrepancy between rates can be partially explained by differences in procedures, *i.e.*, microcosm volumes, inlet solution flows, etc. The composition of the apatite, which influences its solubility and reactivity (Jahnke, 1984; Anderson et al., 1985; Valsami-Jones et al., 1998; Harouiya et al., 2007), could also explain the difference of apatite dissolution rates between our study and those of Valsami-Jones et al. (1998), Guidry and Mackenzie (2003), and Chaïrat et al. (2007). Notably, the proportion of *F* is higher in the Durango apatite used in our study than in the Paraïba apatite used by Chaïrat et al. (2007). The increase in *F* in the apatite structure decreases its solubility. Harouiya et al. (2007) also demonstrated that the dissolution rate of apatite significantly increases with increasing temperature. The studies of Valsami-Jones et al. (1998), Guidry and Mackenzie (2003), and Chaïrat et al. (2007) were carried out at 25 °C while our study was performed at 25 °C during the day (17 h) and 18 °C at night (7 h).

We demonstrated that the apatite weathering efficacy of different acids differs according to the acid. Notably, at pH 3.5, 3.0, and 2.0, we observed that oxalic acid was the most efficient to weather apatite and solubilize P. Solubilization of P compounds by organic acids is achieved by complex formation between organic acids/anions and metal ions such as Fe, A1, and Ca. Complex formation depends on the number and position of carboxyl (–COOH) and phenolic (–OH) functional groups in the organic acids. The presence of two carboxyl groups in oxalic acid confers a strong ability to complex metal ions and particularly Ca, to this organic acid. That is in agreement with our observations, *i.e.*, dissolution of apatite in a non-stoichiometric way, e.g., the Ca/P (mol/mol) ratio in outlet solution reached 0.8 at pH 2 while the stoichiometric ratio for apatite is of 1.6, and the presence of Ca oxalate crystals on apatite surfaces for pH $\leqslant$3.5 (Fig. 10A). Apatite dissolution rates based on P release in gluconic, citric, and oxalic acid solutions from pH 5.5 to 2.0 ranged from approximately 1.1×10^{-14} to 4.7×10^{-12} mol cm^{-2} s^{-1}, 1.0×10^{-14} to 6.1×10^{-12} mol cm^{-2} s^{-1}, and 1.3×10^{-14} to 1.0×10^{-11} mol cm^{-2} s^{-1}, respectively.

4.2. Scots pine nutrition

Our study revealed that Scots pines with or without *B. glathei* PML1(12)Rp were able to significantly take up Ca and P released from apatite. Approximately 50% of the Ca and 70% of the P issued from apatite weathering were immobilized in Scots pine biomass: more than 80% of Ca and more than 60% of P were translocated to above-ground biomass. The weight of the seedlings increased on average by more than three during the 14 weeks of the experiment. This confirms the importance of apatite as a source of bioavailable nutrients for plants as shown by Blum et al. (2002). The strain *B. glathei* PML1(12) did not significantly increase root uptake and plant nutrition. This result agrees with the previous study of Calvaruso et al. (2006) carried out under the same conditions (*i.e.,* open-system flow microcosms, climate conditions, biological material, etc.) but with biotite as source of mineral nutrients (K and Mg). Indeed, these authors demonstrated that the inoculation of Scots pine roots by *B. glathei* PML1(12)Rp improves Scots pine nutrition and growth only in nutrient-deficient conditions. In our study, the amounts of bioavailable Ca and P remained high in the outlet solutions during 14 weeks in the presence of Scots pines alone (approximately 4.0 mg L^{-1} for Ca and 0.9 mg L^{-1} mg for P). The presence of such concentrations of Ca and P in the solution suggests that Scots pines released enough nutrients from apatite to support their nutritional needs. In these rich-nutrient conditions, pines did not invest more energy to release nutrients, *i.e.*, production of weathering agents and/or production of carbon compounds stimulating the activity of the rhizosphere microflora. Through microcosm experimentations, Smits et al. (2012) have already demonstrated that trees invest proportionally more photosynthate energy into the ectomycorrhizal fungal partner under low-P conditions. Our study also revealed that Scots pines with and without *B. glathei* PML1(12)Rp were able to take up significant amounts of trace elements released from apatite. Approximately 95% of the Zn and 100% of the REE issued from apatite weathering were immobilized in the Scots pine biomass. Approximately 70% of Zn was translocated to above-ground biomass revealing the importance of the apatite dissolution in Scots pine nutrition. Interestingly, only a very small fraction of Y, REE, and U was translocated to the aerial parts of Scots pines (less than 1%). The sum of REE (sum of elemental concentrations from La to Lu) was approximately 650-fold higher for roots compared to aerial parts. Moreover, the enrichment factors between the initial plants and the organs after experimentation (elemental ratio between concentration of final and initial organ) was higher for roots (2300 on average for SREE) than for aerial parts (18 on average for SREE), showing a preferential concentration in/on the roots. Stille et al. (2006) observed in the Strengbach catchments that beeches were strongly enriched in REE originating from apatite dissolution. Plants took up REE from the circulating catchment waters and the enrichment of the very small roots (1–2 mm in diameter) reached levels up to 100,000-fold higher than the stream water. In addition, the root signature of Scots pines was closer to the apatite signature than was the aerial part signature (Fig. 12): the aerial parts showed a higher enrichment in light REE (La) compared to middle REE (Sm) and higher enrichment in heavy REE (Yb + Lu) compared to middle REE (Pr + Nd) than

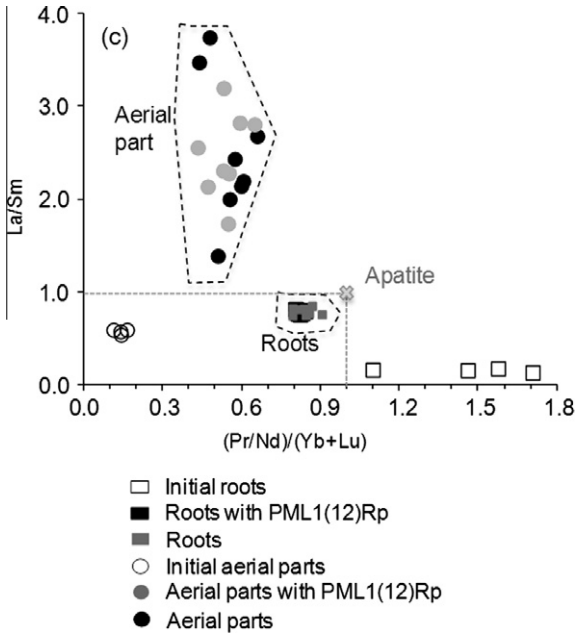

Fig. 12. Fractionation among the REE elements of initial, inoculated with PML1(12)Rp and non inoculated roots and aerial parts. Ten pine seedlings pre-grown under the same conditions as those used in the column experiment, were sampled at the beginning of the experiment to quantify the initial REE contents in roots and aerial parts.

the enrichment in the roots. These observations are in agreement with the work of Ding et al. (2007), which showed that REE fractionations are dominated by a fixation mechanism in soybean roots caused by cell wall absorption and precipitation, and by the combined effects of fixation and transport mechanisms in above-ground parts caused by solution complexation by intrinsic organic ligands. According to Köhler et al. (2005), who studied the REE release rates during apatite dissolution from pH 2.8 to 9.2 in open systems, REE can precipitate in the solid phase as rhabdophane ($REE(PO_4)\ nH_2O$) when outlet solutions are supersaturated with respect to Nd-rhabdophane ($Ksp \approx 10^{-24.5}$). In our experimental conditions, outlet solutions were not supersaturated with respect to Nd-rhabdophane ($Q < 10^{-24.5}$) suggesting that precipitation of REE onto roots is not at the origin of the enrichment of roots in REE.

5. CONCLUSION

Thanks to open-system flow microcosms under controlled conditions, we confirmed the significant impact of Scots pine roots with or without *B. glathei* PML1(12)Rp on mineral weathering. The weathering budgets revealed that Scots pine roots increase Ca, P, As, Sr, Zn, U, Y, and REE release from apatite by a factor > 10 compared to abiotic control treatment. Under our experimental conditions, which corresponded to relatively favorable nutrient conditions, we observed no contribution of the bacterial strain *B. glathei* PML1(12)Rp to mineral weathering and no improvement in pine nutrition and growth. Although no quantitative difference was determined when Scots pines were inoculated with *B. glathei* PML1(12)Rp, a modification of the apatite dissolution process was observed. Indeed, inoculation with *B. glathei* PML1(12)Rp associated with pines led to faster release of P than Ca from apatite leading to a non-stoichiometric dissolution of apatite. This effect of the *B. glathei* PML1(12)Rp is most likely linked to activities specific to this strain. The dissolution rate of apatite by Scots pines supplied with a nutrient solution of pH 5.5 was equivalent to that for nitric acid at pH 3.2, gluconic acid at pH 3.5, citric acid at pH 3.7, or oxalic acid at pH 3.8. This notably results from the production of protons and organic acids (gluconate, acetate, oxalate, and lactate) by roots and microbes. This study reveals that tree roots and root-associated microorganisms can release nutrients from apatite, thus maintaining high-nutrient conditions to support their nutrition. A great distance remains in our journey to transfer these microcosm results to predictions for the effect of tree roots and soil bacteria on apatite weathering in the field, as well as their relative impact on Ca, P and trace element cycles in forest ecosystems.

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REFERENCES

Anderson D., Krussow W. R. and Corey R. (1985) Phosphate rock dissolution in soil: implications from plant growth studies. *Soil Sci. Soc. Am. J.* **49**, 918–925.

Andrews M. Y., Leake J. R., Palmer B. G., Banwart S. A. and Beerling D. J. (2011) Plant and mycorrhizal driven silicate weathering: Quantifying carbon flux and mineral weathering processes at the laboratory mesocosm scale. *Appl. Geochem.* **26**, 314–316.

Balland C., Poszwa A., Leyval C. and Mustin C. (2010) Dissolution rates of phyllosilicates as a function of bacterial metabolic diversity. *Geochim. Cosmochim. Acta* **74**, 5478–5493.

Barker W. W., Welch S. A. and Banfield J. F. (1997) Biogeochemical weathering of silicate minerals. In *Geomicrobiology: Interactions Between Microbes and Minerals* (eds. J. F. Banfield and K. H. Nealson). Miner. Soc. Am., Washington, DC, pp. 391–428.

Baziramakenga R., Simard R. R. and Leroux G. D. (1995) Determination of organic acids in soil extracts by ion chromatography. *Soil Biol. Biochem.* **27**, 349–356.

Berner E. K., Berner R. A. and Moulton K. L. (2005) Plants and mineral weathering: present and past. In *Surface and Ground Water, Weathering and Soils* (ed. J. I. Drever), pp. 169–188. Treatise on Geochemistry Series. Elsevier, Amsterdam.

Blum A. and Lasaga A. C. (1988) Role of surface speciation in the low temperature dissolution of minerals. *Nature* **331**, 431–433.

Blum J. D., Klaue A., Nezat C. A., Driscoll C. T., Johnson C. E., Siccama T. G., Eagar C., Fahey T. J. and Likens G. E. (2002) Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature* **417**, 729–731.

Braissant O., Verrecchia E. P. and Aragno M. (2002) Is the contribution of bacteria to terrestrial carbon budget greatly underestimated? *Naturwissenschaften* **89**, 366–370.

Calvaruso C., Turpault M.-P. and Frey-Klett P. (2006) Root-associated bacteria contribute to mineral weathering and to mineral nutrition in trees: a budgeting analysis. *Appl. Environ. Microbiol.* **72**, 1258–1266.

Calvaruso C., Turpault M.-P., Leclerc E. and Frey-Klett P. (2007) Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. *Microb. Ecol.* **54**, 567–577.

Calvaruso C., N'Dira V. and Turpault M.-P. (2011) Impact of common European tree species and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) on the physicochemical properties of the rhizosphere. *Plant Soil* **342**, 469–480.

Cao X., Chena Y., Wanga X. and Deng X. (2001) Effects of redox potential and pH value on the release of rare earth elements from soil. *Chemosphere* **44**, 655–661.

Chaïrat C., Schott J., Oelkers E. H., Lartigue J. E. and Harouiya N. (2007) Kinetics and mechanism of natural fluorapatite dissolution at 25 °C and pH from 3 to 12. *Geochim. Cosmochim. Acta* **71**, 5901–5912.

Cromack, Jr., K., Sollins P., Graustein W. C., Speidel K., Todd A. W., Spycher G., Li C. Y. and Todd R. L. (1979) Calcium oxalate accumulation and soil weathering in mats of the hypogenous fungus *Hysterangium erassum*. *Soil Biol. Biochem.* **14**, 463–468.

Ding S., Liang T., Yan J., Zhang Z., Huang Z. and Xie Y. (2007) Fractionations of rare earth elements in plants and their conceptive model. 50, 47–55.Dorozhkin S. V. (2012) Dissolution mechanism of calcium apatites in acids: A review of literature. *Sci. China C Life Sci* **50**, 47–55.

Dorozhkin S. V. (2012) Dissolution mechanism of calcium apatites in acids: a review of literature. *World J. Methodol.* **2**, 1–17.

Drever J. I. and Stillings L. L. (1997) The role of organic acids in mineral weathering. *Colloids Surf. A Physicochem. Eng. Asp.* **120**, 167–181.

Drever J. I. and Vance G. F. (1994) Role of soil organic acids in mineral weathering processes. In *Organic acids in geological processes* (eds. E. D. Pittman and M. D. Lewan). Springer-Verlag, New York, pp. 138–161.

Feng M.-H., Ngwenya B. T., Wang L., Li W., Olive V. and Ellam R. M. (2011) Bacterial dissolution of fluorapatite as a possible source of elevated dissolved phosphate in the environment. *Geochim. Cosmochim. Acta* **75**, 5785–5796.

Fox T. R. (1995) The influence of low-molecular-weight organic acids on properties and processes in forest soils. In *Carbon Forms and Functions in Forest Soils*, vol. 99 (eds. W. W. McFee and J. M. Kelly). Soil Science Society of America, Madison, WI, pp. 43–62.

Fox T. R. and Comerford N. B. (1990) Low-molecular-weight organic acids in selected forest soils of the south eastern USA. *Soil Sci. Soc. Am. J.* **54**, 1139–1144.

Fox T. R. and Comerford N. B. (1992) Influence of oxalate loading on phosphorus and aluminium solubility in spodosols. *Soil Sci. Soc. Am. J.* **56**, 290–294.

Furrer G. and Stumm W. (1986) The coordination chemistry of weathering: I. Dissolution kinetics of δ-Al_2O_3 and BeO. *Geochim. Cosmochim. Acta* **50**, 1847–1860.

Gadd G. M. (1999) Fungal production of citric and oxalic acid: Importance in metal speciation, physiology and biogeochemical processes. *Adv. in Microbial Physiol.* **41**, 47–92.

Gadd G. M. (2007) Geomycology: biogeochemical transformation of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycol. Res.* **111**, 3–49.

Gharieb M. M., Sayer J. A. and Gadd G. M. (1998) Solubilization of natural gypsum ($CaSO_4{}^*2H_2O$) and the formation of calcium oxalate by *Aspergillus niger* and *Serpula himantioides*. *Mycol. Res.* **102**, 825–830.

Goldstein J., Newbury D. E., Joy D. C., Lyman C. E., Echlin P., Lifshin E., Sawyer L. C. and Michael J. R. (2003) *Scanning Electron Microscopy and X-ray Microanalysis*. Plenum Press, Third Edition.

Goyne K. W., Brantley S. L. and Chorover J. (2010) Rare earth element release from phosphate minerals in the presence of organic acids. *Chem. Geol.* **278**, 1–14.

Graustein W. C., Cromack, Jr., K. and Sollins P. (1977) Calcium oxalate: Occurrence in soils and effects on nutrient and geochemical cycles. *Science* **198**, 1252–1254.

Guidry M. W. and Mackenzie F. T. (2003) Experimental study of igneous and sedimentary apatite dissolution: control of pH, distance from Equilibrium, and temperature on dissolution rates. *Geochim. Cosmochim. Acta* **67**, 2949–2963.

Harouiya N., Chaïrat C., Köhler S. J., Gout R. and Oelkers E. H. (2007) The dissolution kinetics and apparent solubility of natural apatite in closed microcosms at temperatures from 5 to 50 °C and pH from 1 to 6. *Chem. Geol.* **244**, 554–568.

Hausrath E. M., Neaman A. and Brantley S. L. (2009) Elemental release rates from dissolving basalt and granite with and without organic ligands. *Am. J. Sci.* **309**, 633–660.

Haynes R. J. (1990) Active ion uptake and maintenance of cation–anion balance. A critical examination of their role in regulating rhizosphere pH. *Plant Soil* **126**, 247–264.

Hinsinger P. (2000) Bioavailability of trace elements as related to root-induced changes in the rhizosphere. In *Trace Elements in the Rhizosphere* (eds. G. R. Gobran, W. W. Wenzel and E. Lombi). CRC Press LCC, Boca Raton, USA, pp. 25–41.

Hinsinger P. and Gilkes R. J. (1997) Dissolution of phosphate rock in the rhizosphere of five plant species grown in an acid, P fixing mineral substrate. *Geoderma* **75**, 231–249.

Hinsinger P., Gobran G. R., Gregory P. J. and Wenzel W. W. (2005) Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phytol.* **168**, 293–303.

Hutchens E., Valsami-Jones E. and Mc Eldowney S. (2003) The influence of heterotrophic bacteria and fungi on K-feldspar dissolution. *Geochim. Cosmochim. Acta* **67**, 163.

Hutchens E., Valsami-Jones E., Harouiya N., Chaïrat C., Oelkers E. and McEldowney S. (2006) How do microorganisms enhance mineral dissolution rates? An experimental investigation of the effect of *Bacillus megaterium* on apatite dissolution rates. *Geomicrobiol. J.* **23**, 177–182.

Jahnke R. A. (1984) The synthesis and solubility of carbonate fluoroapatite. *Am. J. Sci.* **284**, 58–78.

Jones D. L. and Darrah P. R. (1994) Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* **166**, 247–257.

Jones L. D. (1998) Organics acids in the rhizosphere – a critical review. *Plant Soil* **205**, 1849–1859.

Jones D. L., Dennis P. G., Owen A. G. and Van Hees P. A. W. (2003) Organic acid behavior in soils - misconceptions and knowledge gaps. *Plant Soil* **248**, 31–41.

Jongmans A. G., Van Breemen N., Lundstrom U., Van Hees P. A. W., Finlay R. D., Srinivasan M., Unestam T., Giesle R.,

Melkerud and Olsson M. () Rock-eating fungi. *Nature* **389**, 682–683.

Kelly E., Chadwick O. A. and Hilinski T. E. (1998) The effect of plants on mineral weathering. *Biogeochemistry* **42**, 21–53.

Khasawneh F. E. and Doll E. C. (1978) The use of phosphate rock for direct application to soils. *Adv. Agron.* **30**, 159–206.

Kim Y. H., Bae B. and Choung Y. K. (2005) Optimization of biological phosphorus removal from contaminated sediments with phosphate-solubilizing microorganisms. *J. Biosci. Bioeng.* **99**, 23–29.

Koele N., Turpault M.-P., Hildebrand E. E., Uroz S. and Frey-Klett P. (2009) Interactions between mycorrhizal fungi and mycorrhizosphere bacteria during mineral weathering: budget analysis and bacterial quantification. *Soil Biol. Biochem.* **41**, 1935–1942.

Köhler S. J., Harouiya N., Chaïrat C. and Oelkers E. H. (2005) Experimental studies of REE fractionation during water–mineral interactions: REE release rates during apatite dissolution from pH 2.8 to 9.2. *Chem. Geol.* **222**, 168–182.

Krzyszowska A. J., Blaylock M. J., Vance G. F. and David M. B. (1996) Ion-chromatographic analysis of low molecular weight organic acids in spodosol forest floor solutions. *Soil Sci. Soc. Am. J.* **60**, 1565–1571.

Landeweert R., Hoffland E., Finlay R. D., Kuyper T. M. and Van Breemen N. (2001) Linking plants to rock: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends Ecol. Evol.* **16**, 248–253.

Lange-Ness R. and Vlek P. L. G. (2000) Mechanism of calcium and phosphate release from hydroxy-apatite by mycorrhizal hyphae. *Soil Sci. Soc. Am. J.* **64**, 949–955.

Lapeyrie F. F. and Bruchet G. (1986) Calcium accumulation by two strains, calcicole and calcifuge, of the mycorrhizal fungus *Paxillus involutus*. *New Phytol.* **103**, 133–141.

Lapeyrie F., Ranger J. and Vairelles D. (1991) Phosphate-solubilizing activity of ectomycorrhizal fungi in vitro. *Can. J. Bot.* **69**, 342–346.

Leake J. R., Duran A. L., Johnson I., Bonneville S. and Smits M. M. (2009) Hydroxyapatite weathering by pine mycorrhizas- the role of oxalic acid. *Geochim. Cosmochim. Acta* **73**, A732.

Leyval C. and Berthelin J. (1991) Weathering of a mica by roots and rhizospheric micro-organisms of pine. *Soil Sci. Soc. Am. J.* **55**, 1009–1016.

Leyval C. and Berthelin J. (1993) Rhizodeposition and net release of soluble organic compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi. *Biol. Fertil. Soils* **15**, 259–267.

Liermann L. J., Kalinowski B. E., Brantley S. L. and Ferry J. G. (1999) Role of bacterial siderophores in dissolution of hornblende. *Geochim. Cosmochim. Acta* **64**, 587–602.

Luo W. and Gu B. (2011) Dissolution of uranium-bearing minerals and mobilization of uranium by organic ligands in a biologically reduced sediment. *Environ. Sci. Technol.* **45**, 2994–2999.

Lynch J. M. (1978) Production and phytotoxieity of acetic acid in anaerobic soils containing plant residues. *Soil Biol. Biochem.* **10**, 131–135.

Mc Coll J. G. and Pohlman A. A. (1986) Soluble organic acids and their chelating influence on Al and other metal dissolution from forest soils. *Water Air Soil Pollut.* **31**, 917–927.

Mailloux B. J., Alexandrova E., Keimowitz A. R., Wovkulich K., Freyer G. A., Herron M., Stolz J. F., Kenna T. C., Pichler T., Polizzotto M. L., Dong H., Bishop M. and Knappett P. S. K. (2009) Microbial mineral weathering for nutrient acquisition releases arsenic. *Appl. Environ. Microbiol.* **75**, 2558–2665.

Manley E. P. and Evans L. J. (1986) Dissolution of feldspars by low-molecular-weight aliphatic and aromatic acids. *Soil Sci.* **141**, 106–112.

Moghimi A., Lewis D. G. and Oades J. M. (1978) Release of phosphate from calcium phosphates by rhizosphere products. *Soil Biol. Biochem.* **10**, 277–281.

Paris F., Botton B. and Lapeyrie F. (1996) In vitro weathering of phlogopite by ectomycorrhizal fungi. 2. Effect of K^+ and Mg^{2+} deficiency and N sources on accumulation of oxalate and H^+. *Plant Soil* **179**, 141–150.

Park C. Y., Fenter P., Zhang Z., Cheng L. W. and Sturchio N. C. (2004) Structure of fluorapatite (100)-water interface by high-resolution X-ray reflectivity. *Am. Min.* **89**, 1647–1654.

Puente M. E., Bashan Y. and Lebsky V. K. (2004) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. I. Root colonization and weathering of igneous rocks. *Plant Biol.* **6**, 629–642.

Robert M. and Berthelin J. (1986) Role of biological and biochemical factors in soil mineral weathering. In *Interactions of Soil Minerals with Natural Organics and Microbes* (ed. P. M. Huang). Soil Sci. Soc. Am., Madison, WI, pp. 453–495.

Rosling A., Suttle K. B., Johansson E., Van Hees P. A. W. and Banfield J. F. (2007) Phosphorous availability influences the dissolution of apatite by soil fungi. *Geobiology* **5**, 265–280.

Smits M. M., Bonneville S., Benning L. G., Banwart S. A. and Leake J. R. (2012) Plant-driven weathering of apatite - the role of an ectomycorrhizal fungus. *Geobiol.* **10**, 445–456.

Sperber J. I. (1958) The incidence of apatite solubilizing organisms in the rhizosphere and soil. *Aust. J. Agric. Res.* **9**, 777–781.

Stevenson F. J. (1967) Organic acids in soil. *Soil Biochem.* **1**, 119–146.

Stevenson F. J. (1991) Organic matter–micronutrient reactions in soil. In *Micronutrients in Agriculture* (eds. J. J. Mortvedt, F. R. Cox, L. M. Shuman and M. Welch). Soil Sci. Soc. Am., Madison, WI, pp. 145–186.

Stille P., Steinmann S., Pierret M.-C., Gauthier-Lafaye F., Aubert D., Probst A., Viville D. and Chabaux F. (2006) The impact of vegetation on fractionation of rare earth elements (REE) during water–rock interaction. *J. Geochem. Explor.* **88**, 341–344.

Stumm W. (1997) Reactivity at the mineral–water interface. dissolution and inhibition. Colloids Surf. *A Physicochem. Eng. Asp.* **120**, 143–166.

Tan K. H. (1986) Degradation of soil minerals by organic acids. In *Interactions of Soil Minerals with Natural Organics and Microbes* (eds. P. M. Huang and M. Schnitzer). Soil Sci. Soc. Am., Madison, WI, pp. 1–27.

Thirioux L., Baillif P., Touray J. C. and Ildefonse J. P. (1990) Surface reactions during fluorapatite dissolution–recrystallisation in acid media (hydrochloric and citric acids). *Geochim. Cosmochim. Acta* **54**, 1969–1977.

Turpault M.-P. and Trotignon L. (1994) The dissolution of biotite single crystals in dilute HNO_3 at 24 °C: evidence of an anisotropic corrosion process of micas in acidic solutions. *Geochim. Cosmochim. Acta* **58**, 2761–2775.

Turpault M.-P., Nys C. and Calvaruso C. (2009) Rhizosphere impact on the dissolution of test-minerals in a forest ecosystem. *Geoderma* **153**, 147–154.

Uroz S., Calvaruso C., Turpault M.-P., Pierrat J. C., Mustin C. and Frey-Klett P. (2007) Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the soil bacterial communities involved in mineral weathering in a forest soil. *Appl. Environ. Microbiol.* **73**, 3019–3027.

Uroz S., Calvaruso C., Turpault M.-P. and Frey-Klett P. (2009) Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends Microbiol.* **17**, 378–387.

Uroz S., Oger P., Lepleux C., Collignon C., Frey-Klett P. and Turpault M.-P. (2011) Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems. *Res. Microbiol.* **162**, 820–831.

Uroz S., Turpault M.-P., Delaruelle C., Mareschal L., Pierrat J.-C. and Frey-Klett P. (2012) Minerals affect the specific diversity of forest soil bacterial communities. *Geomicrobiol. J.* **29**, 88–98.

Valsami-Jones E., Ragnarsdottir K. V., Putnis A., Bosbach D., Kemp A. J. and Cressey G. (1998) The dissolution of apatite in the presence of aqueous metal cations at pH 2–7. *Chem. Geol.* **151**, 215–233.

Van Hees P. A. W., Jones D. L., Jentschke G. and Godbold D. L. (2005) Organic acid concentrations in soil solution: effects of young coniferous trees and ectomycorrhizal fungi. *Soil Biol. Biochem.* **37**, 771–776.

Van Schöll L., Smits M. M. and Hoffland E. (2006) Ectomycorrhizal weathering of the soil minerals muscovite and hornblende. *New Phytol.* **171**, 805–814.

Wallander H. (2000) Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi. *Plant Soil* **218**, 249–256.

Wallander H. and Wickman T. (1999) Biotite and microcline as potassium sources in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings. *Mycorrhiza* **9**, 25–32.

Welch S. A., Barker W. W. and Banfield J. F. (1999) Microbial extracellular polysaccharides and plagioclase dissolution. *Geochim. Cosmochim. Acta* **63**, 1405–1419.

Welch S. A. and Ullman W. J. (1993) The effect of organic acids on plagioclase dissolution rates and stoichiometry. *Geochim. Cosmochim. Acta* **57**, 2725–2736.

Welch S. A., Taunton A. E. and Banfield J. F. (2002) Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiol. J.* **19**, 343–367.

Xie Z. and Walther J. V. (1994) Dissolution stoichiometry and adsorption of alkali and alkaline earth elements to the acid-reacted wollastonite surface at 25 °C. *Geochim. Cosmochim. Acta* **58**, 2587–2598.

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