Rhizosphere impact on the dissolution of test minerals in a forest ecosystem

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ABSTRACT

As soil minerals are the principal input of nutrients in non-fertilized forests, the parameters which influence their dissolution must be determined to predict ecosystem sustainability. Notably, biological activities within the rhizosphere, such as root and micro-organism exudation and respiration, considerably affect mineral dissolution rate. Numerous laboratory studies have even demonstrated that certain biological processes involved in mineral weathering can be stimulated in low-nutrient availability conditions, resulting in an improvement of plant nutrition. The objective of this work was to determine in the field if the mineral dissolution rate linked to root and root-associated micro-organism activity is increased in low-nutrient availability conditions. Here, the impact of the rhizosphere on the dissolution of test minerals containing Ca (fluorapatite and labradorite plagioclase) was assessed in an acid forest soil in two stands of mature beeches (*Fagus sylvatica*) presenting two levels of Ca availability: a control plot as well as a plot fertilized with Ca. Mineral-test bags were inserted at three different depths (-2.5, -10 and -20 cm) in the control and the Ca-fertilized plots into both a zone with roots as well as a zone where roots had been excluded, thus permitting to assess the effect of the rhizosphere on the mineral dissolution. After four years of incubation in the soil, the minerals were weighed and observed by scanning electron microscope. In the control stand, linear dissolution voids were only observed on the mineral surfaces incubated in the zone with roots, suggesting that local biological activities occurring in the rhizosphere affect mineral weathering. This positive effect of the rhizosphere in the control stand was confirmed by quantification of the mineral dissolution, which revealed an increase of fluorapatite and labradorite weathering, reaching factors 3 to 4 at 20-cm depth. In contrast, the beech rhizosphere did not increase mineral dissolution, hyphae colonisation or linear dissolution marks in the Ca-fertilized stand. These results suggest that the rhizospheric biological activities acting on mineral weathering could be regulated by the nutrient availability in the ecosystem. This plasticity of the rhizospheric biological activities may thus contribute to the maintenance of ecosystem sustainability.

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1. Introduction

The sustainability of ecosystems with low inputs depends notably on the flux of nutrients from soil mineral weathering (Likens et al., 1977; Ranger and Turpault, 1999). These fluxes are essential to plant nutrition and to the buffering of proton flux limiting acidification. During the last few decades, studies quantifying these fluxes and the parameters which influence them have been more numerous (for instance, White, 1995). The principal factors explaining mineral dissolution rates have been identified (i.e. specific surface areas of minerals, kinetic constants of minerals, hydrological parameters, weathering agents, temperature, ionic solution strength, etc.) and have been discussed in different reviews (White and Brantley, 1995; Drever, 2005). Some of these parameters are strongly affected by the biological activities of plants (Hinsinger et al., 2005) and micro-

organisms (Barker et al., 1997) such as physical disruption of rocks and minerals, absorption of nutrients, exudation of H^+ ions, organic acids and chelating molecules. These latter two have a double action on mineral weathering: (i) they attach and adhere to the mineral and literally extract nutrients from mineral particles by electron transfer, and (ii) they chelate ions present in the solution through their carboxyl and hydroxyl groups, indirectly accelerating the dissolution rate of the mineral by maintaining a constant concentration gradient.

As the rhizosphere constitutes a zone of intense biological activity and of privileged interactions between roots, micro-organisms, and minerals, numerous studies were focused on mineral weathering in this particular environment from the 80's. For example, by way of a pot experiment, Hinsinger and Jaillard (1993) have demonstrated that the process of mineral weathering can be accelerated in the rhizosphere. These authors observed thus the complete vermiculitization of phlogopite at a distance of 0.5 mm from the root mat within 32 days. Several studies equally revealed that the composition of root exudates (i.e. H^+ ions, organic acids and chelating molecules) varied according to the nutrient availability conditions in the system (Dakora and Phillips,

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2002). Thus, Römheld (1991) observed an increase of the production of phytosiderophores, low molecular weight organic ligands with a strong affinity for iron (Marschner and Römheld, 1994), in a condition of iron deficiency. In a column experiment in which Scots pine (*Pinus sylvestris*), mycorrhizospheric bacterial strain *Burkholderia glathei* PML1(12) and a K–Mg bearing mineral-test (biotite) were made to interact, Calvaruso et al. (2006) have demonstrated as well that the bacteria only promoted mineral dissolution when the nutrient solution did not contain K and Mg, thus contributing to plant nutrition when necessary. These results suggest that the biota can modify their activities in order to maintain a nutrient availability level sufficient to their growth. This capacity of plants and root-associated micro-organisms to respond to nutrient deficiency by implementing mechanisms favouring the mobilisation of nutrients from soil minerals is crucial for plant nutrition and therefore for maintaining ecosystem sustainability.

All of these studies, however, are based on the use of simplified ecosystems very different from natural ones. Indeed, not only are the kinetics of reactions very different from those in the ecosystems (White, 1995), but also it is impossible to integrate all parameters and their interactions and feedbacks as they occur *in situ*. Until now, the impact of the rhizosphere on mineral weathering in the field and at different levels of nutrient availability is still largely unknown. The aim of this study was therefore to quantify the effect of the rhizosphere biological activities on mineral weathering in a mature forest under two levels of Ca availability. To this goal we carried out both in control and Ca-fertilized plots, an experimental device combining two methods: (i) the test-mineral method which consists of determining the weathering rate of test minerals introduced for several years into the soil (Ranger and Nys, 1994; Augusto et al., 2000) and (ii) the soil trenching method (e.g. Hanson et al., 2000) which consists of creating a root exclusion zone. These combined methods allow thus to compare in the field the dissolution of minerals in the absence or presence of roots.

2. Materials and methods

2.1. Ecosystem description

The study was carried out in Fougères forest (1660 ha of dominant beech trees) in the northeast of Ile-et-Vilaine (Brittany, France, 48°23′ N, 1°9′W, at a mean elevation of 150 m). The climate is oceanic with an average annual air temperature of 12.9 °C, a low range of inter-annual variations and only exceptional frost. Annual rainfall averages 868 mm, and is well distributed throughout the year. The soils are alocrisols–neoluvisols (AFES, 1995) developed in a 1.5 m thick, non-carbonated aeolian loam, containing approximately 15% clay and 75% silt (Lecointe et al., 2006), covering the late Cadomian Vire granite (600M years; Jonin, 1973). The humus ranges from an acid mull for the thicket stage, to a dysmoder in the old mature high forest stage which were the stands used in this study.

The choice of Fougères forest was motivated by the presence of i) a homogeneous, acid nutrient-poor soil, especially poor in Ca (Legout

et al., 2008), ii) an experimental design which had been established in 1973 (Le Tacon and Oswald, 1977) to determine the long term effects of the fertilization treatment principally CaCO$_3$ and iii) intensive measurements from 1997 of atmospheric deposition, litter-fall, biomass immobilisation, soil solution chemistry and soil properties in the beech chronosequence of six stands without fertilization and two fertilized stands. The spatial variability of the Fougères forest soil was very low and was due to heterogeneity inferior to decimetric scale (Wopereis et al., 1988; Lecointe et al., 2006; Legout et al., 2008).

2.2. Studied beech stands

Two 86 years old beech (*Fagus sylvatica*) stands (control and Ca-fertilized plots) were selected. They were part of a time sequence in the ORE F-ORE-T (Environmental Research Observatory) experimental site, and were well instrumented for biogeochemical cycle studies. The Ca-fertilized plot was one of the plots of a fertilization experiment set up in 1973 (Le Tacon and Oswald, 1977). In addition to 1500 kg ha^{-1} of CaO (CaCO$_3$, 80% of the particle size was less than 0.16 mm), 100 kg ha^{-1} of N (ammonium nitrate), 150 kg ha^{-1} of P$_2$O$_5$ (triple super phosphate), and 150 kg ha^{-1} of K$_2$O (potassium sulphate) were spread in Springs of 1973. The advantage of this ancient fertilization is a significant effect on the availability of Ca without strong effect on the other physico-chemical properties (Table 1).

The fertilization treatment had the largest impact on tree radial growth with an increase of 29% for the period after fertilizer application (1973–1988) as compared to the control; this effect having disappeared during the dry period of 1989–1991 (Elhani et al., 2005).

2.3. Test mineral

The choice of the test minerals was based on the following criteria:

1) They are present in a range of these soils;
2) They constitute the principal Ca sources in these types of acid soils;
3) Their weathering rate is fast enough to respond quickly to the treatments;
4) They are commercially available under chemically and miner-alogically pure form;
5) Their use in other experimental forest ecosystems for comparison purposes.

Two test minerals were chosen: i) a labradorite plagioclase from Norway and ii) a fluorapatite from Mexico (Durango). The initial plagioclase material was composed of 99.9% labradorite and 0.1% ilmenite (Augusto et al., 2000). Table 2 shows the chemical analyses of both minerals: apatite contained five times more CaO than plagio-clase. The structural formula of labradorite calculated from micro-probe analysis was Si$_{2.49}$Al$_{1.49}$K$_{0.02}$Ca$_{0.52}$Na$_{0.45}$O$_8$ (Augusto et al., 2000). The Durango apatite consisted of individual crystals which were hexagonal prisms with pyramidal extremities, 9–20 mm high and 12–45 mm in diameter. The chemical composition is given in

Table 1
Selected chemical characteristics of the soil (<2 mm) in the fertilized and control stands used in this study.

Depth (cm)	Treatment	pH$_{H_2O}$	Organic C (g kg^{-1})	Organic N	CEC[a] (cmol$_c$ kg^{-1})	Ca[b]	Mg[b]	Al[b]	P$_2$O$_5$ Du[c] (g kg^{-1})
0–5	Control	3.75 ± 0.06	108.2 ± 3.7	5.92 ± 0.27	8.89 ± 0.28	1.20 ± 0.21	0.84 ± 0.05	3.84 ± 0.21	0.23 ± 0.01
0–5	Fertilized	3.75 ± 0.04	88.2 ± 4.7	4.97 ± 0.28	8.98 ± 0.22	2.83 ± 0.21	0.59 ± 0.04	3.13 ± 0.18	0.36 ± 0.03
5–15	Control	3.96 ± 0.12	33.6 ± 4.4	1.63 ± 0.17	6.50 ± 0.31	0.12 ± 0.04	0.14 ± 0.02	4.84 ± 0.18	0.08 ± 0.01
5–15	Fertilized	4.05 ± 0.10	28.6 ± 3.7	1.49 ± 0.16	6.10 ± 0.47	0.37 ± 0.15	0.11 ± 0.02	4.31 ± 0.24	0.14 ± 0.04
15–30	Control	4.27 ± 0.14	20.4 ± 3.8	1.05 ± 0.19	4.74 ± 0.58	0.05 ± 0.02	0.06 ± 0.02	3.84 ± 0.42	0.08 ± 0.01
15–30	Fertilized	4.32 ± 0.13	18.1 ± 2.6	0.96 ± 0.14	4.62 ± 0.25	0.14 ± 0.03	0.05 ± 0.01	3.54 ± 0.17	0.09 ± 0.02

[a] CEC, cation exchange capacity.
[b] Exchangeable elements extracted by KCl 1M.
[c] P$_2$O$_5$ Du, P$_2$O$_5$ extracted by H$_2$SO$_4$ 0.004M and NaOH 0.1M (Duchaufour and Bonneau, 1959).

Table 2
Chemical composition of labradorite and apatite from microprobe and chemical analyses.

Mineral	Type of analysis		SiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	F	Cl	LOI	CeO_2	La_2O_3	Nd_2O_5
			(g kg^{-1})															
Labradorite	Microprobe[a] ($n=30$)		550.2	280.0	0.9	0.2	0.1	106.9	49.5	4.7	0.1	2.6	nd	nd	nd	nd	nd	nd
		SE	0.8	0.6	0.1	0.1	0.0	0.4	0.3	0.4	0.0	0.1						
Apatite	Chemical analysis 1		1.8	0.5	0.8	0.1	0.4	546.1	2.7	<0.5	<0.9	410.3	30.3	nd	5.5	5.2	8.2	2.0
	Micropro-be ($n=10$)		<8	<3	<1	<0.3	<4	547.9	2.7	<0.5	<0.9	400.6	25.9	4.3	nd	4.3	nd	nd
		SE						2.1	0.4			2.9	0.6	0.3		1.4		

LOI: loss on ignition; n: number of analyses from microprobe; SE: standard error; nd: non-determined.
  [a] From Augusto et al. (2000).

Table 1 and the analyses show a measurable variability, demonstrating heterogeneity in the trace element composition. The structural formula given by Park et al. (2004) was:

$(Ca_{9.77}La_{0.03}Ce_{0.04}Sr_{0.007}Fe_{0.003}Si_{0.06})(P_{1.02}O_4)_6F_{2.08}Cl_{0.12}$, which is not very different from the structural formula calculated from the data given in Table 2.

Initial mineral grains or crystals were ground in a jaw crusher. The labradorites and the apatites were sieved at 100–200 µm and 0.5–1 mm, respectively. The 100–200 µm fraction of labradorite was sorted magnetically in order to remove grains containing ilmenite. Apatite and labradorite grains were also treated ultrasonically and washed with distilled water in order to remove the fine particles. The size fractions used for labradorite (100–200 µm) and for fluorapatite (0.5–1 mm) were defined in function of the kinetic constants of these minerals and in order to make possible direct comparisons with previous studies (Augusto et al., 2000). The specific surface area of labradorite (100–200 µm) measured using the BET method was 0.11 ± 0.01 m^2 g^{-1} (Augusto et al., 2000). 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 0.0023 m^2 g^{-1} using a sphere model. Three grams ± 0.0005 g of labradorite (100–200 µm) and apatite (0.5–1 mm) were placed in bags (10×5 cm) of 20 µm and 300 µm mesh, respectively. The presence of a small mass of mineral in a fairly large bag results in a thin layer (a few mm), spread out in the bag implying that the whole of the test mineral is highly influenced by the roots growing in contact with the surface of the bag, despite the mesh of the bag.

2.4. Experimental design

The experimental design is presented in Fig. 1. Six labradorite bags of 20 µm mesh and four apatite bags of 300 µm mesh were introduced into each of three depths (-2.5, -10, and -20 cm) for the four soil treatments, i.e. control treatment (low Ca level) with and without roots and Ca-fertilized treatment (high Ca level) with and without roots. Bags of apatite and labradorite were inserted horizontally into the soil of each pit in April 2000 and the pits were then refilled horizon by horizon. It is important to note that the 300 µm mesh bags used for apatite allow root access and therefore direct contact between roots and apatite, whereas the 20 µm mesh used for labradorite only permits the penetration of fungal hyphae. In consequence we could not compare the dissolution rate of apatite and labradorite in our study. To obtain the root exclusion zone, 40-cm wide and 1-m deep trenches (15 m×3.5 m) were dug in a forest area without tree. A thick plastic sheet was introduced into the soil around this zone to prevent root penetration, and the trench was back-filled outside the plastic. In the root exclusion zones, the vegetation was pulled up regularly and treated once a year with a glycophosphate herbicide.

2.5. Analyses of the test minerals after incubation

After 4 years in the soil (April 2004), the mineral bags were collected and the soil sampled to determine the soil moisture. The minerals were washed and treated ultrasonically to remove soil particles. They were then dried and weighed (accuracy 0.0005 g).

These operations had to be carried out very carefully, by the same person, following a very strict method, so that none of the grains would be lost. The quality of the operator's work was tested on control bags which had not been inserted into the soil.

The percentage dissolution of each mineral was the weight lost during the four years in the soil. After washing, some samples were observed by scanning electron microscope (SEM; Hitachi S 2500 LB equipped with a Kevex Delta energy-dispersive system). To determine the number of hyphae and linear dissolution marks, 4 sub-samples of the apatite inserted at a depth of 2.5 cm in the soil with and without roots, were sampled from two bags in the control and Ca-fertilized treatments. Ten grains from each sub-sample oriented randomly, were systematically photographed at an enlargement of ×80 and the linear dissolution marks and the hyphae present were counted on the face observed for each grain (thus 20 per treatment).

2.6. Measurements of root density

The root density in both control and Ca-fertilized plots was determined from a representative area of 20×28 m which was divided by a 4 m grid. All the intersections falling on a tree stump, (within a diameter of 1.5 m centred on the tree axis) were eliminated. Most of the fine roots (<2 mm) were sampled by hand from layers: 1 (0–8 cm), 2 (8–16 cm) and 3 (16–35 cm) of 19 pits, 70×35 cm. The roots were dried immediately in a steam-air dryer at 30 °C and after removing the soil for another experiment, they were weighed. A sample (1.5 kg) of the soil remaining after the first root extraction was taken from each horizon of each pit to determine the weight of roots remaining after wet sieving and drying (Huet, 2004). Thus the total weight of roots remaining after the first extraction was determined and the weight could be corrected for each horizon of each pit. The mean total was 43%±3% ($P=0.05$).
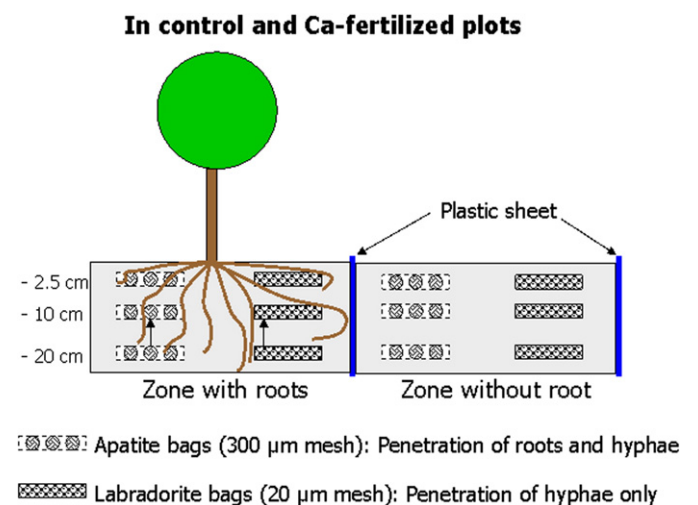

Fig. 1. Study experimental design.

2.7. Statistical analyses

Different statistical analyses were carried out using Unistat® software version 5.0 for excel. The initial statistical analyses of mineral dissolution data were carried out using three-way analyses of variance (ANOVA). Due to the non-significant interaction between the soil level factors and the root or fertilization treatment factors, the data were analysed by level using one- or two-way analyses of variance following a Student–Newman–Keuls test, to assess differences between treatments. Statistical tests were performed at the 0.05 significance level.

3. Results

3.1. SEM observations

Before their introduction into the soil, the mineral grains of apatite were ovoid (Fig. 2a) and the labradorite grains were more tabular (Fig. 2l). Apatite grains had clean-cut fracture faces which were frequently conchoidal and the surfaces were generally smooth (Fig. 2b and c).

After 4 years in the soil, weathering marks were clearly visible (Fig. 2d, e, f, g and j). In the same treatment, crystals could be
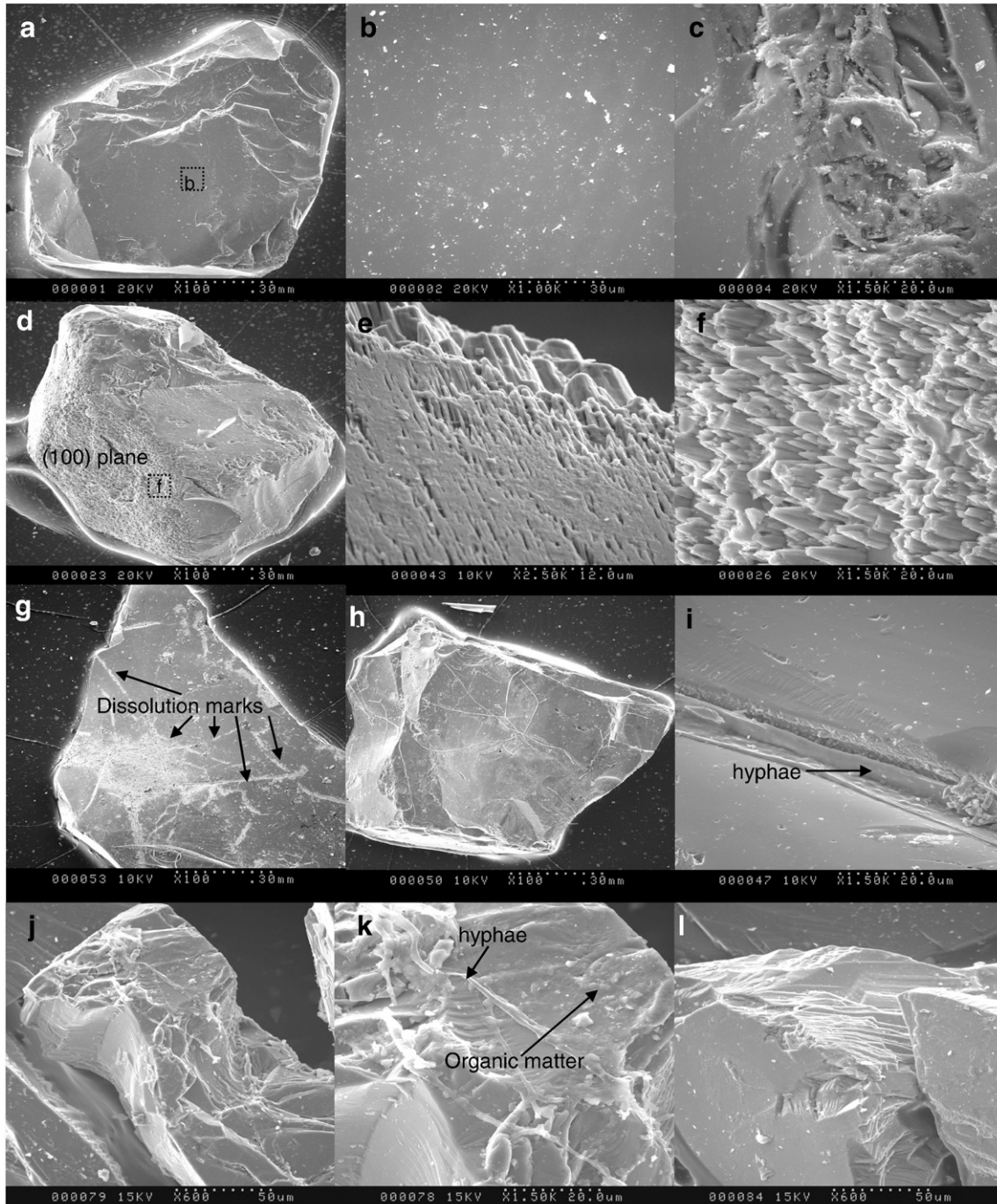

Fig. 2. Scanning electron microscope photographs. (a): Apatite grain before incubation in the soil. (b) Detail of micrograph a) on the plane surface of an apatite grain. (c) Detail of micrograph a) on the edge of an apatite grain. (d) Apatite grain after four years in the soil at 2.5 cm (fertilized plot without roots). (e) Detail of micrograph d) at the intersection of two plane surfaces of an apatite grain. (f) Detail of micrograph d) on the surface showing residual weathering needles. (g) Apatite grain showing the dissolution marks as curved lines (control plot with roots at 10 cm). (h) Apatite grain surface covered by hyphae (control with roots at 10 cm). (i) Hyphae in a linear dissolution structure on the apatite surface (control with roots at 10 cm). (j) Labradorite grain surface covered by hyphae and organic matter (control with roots at 2.5 cm). (k) Detail of micrograph j). (l) Labradorite grain showing few dissolution marks (control without roots at 2.5 cm).
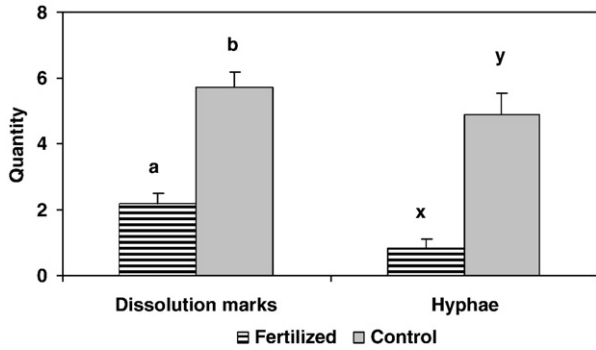
Fig. 3. Mean number of linear dissolution marks and hyphae observed on the apatite grain face after its introduction into the soil at a depth of 2.5 cm in fertilized and control plots.

weathered to greater or lesser degrees (Fig. 2g and h) and the marks were not distributed uniformly over the surface of the grains (Fig. 2d and g). In the treatments with roots, the roots had been separated from the grains, however the fungal hyphae were systematically observed on both types of mineral (Fig. 2h, i, j and k). Moreover, fairly wide (0.1 to 1.6 mm), generally curved linear features appeared frequently (Fig. 2g) in the control treatment with roots. Fig. 3 shows that there were three times as many of these marks on the apatite grains in the control relative to the fertilized treatment. These weathering marks were absent in the treatments without roots. There were thinner linear dissolution marks (10–100 μm) in which hyphae were sometimes present (Fig. 2i), but they were not systematically in these dissolution voids: they could either cross these weathered structures or travel around on the unweathered surfaces (Fig. 2k). The apatite grains from the control treatment were colonised by hyphae approximately six times more than the grains from the fertilized treatment (Fig. 3).

For apatite, the frequency and the geometry of these dissolution patterns were also dependant on their crystallographic orientation (Fig. 2e and f). The (100) planes were less corroded than the perpendicular faces which showed residual dissolution needles (Fig. 2e and f).

3.2. Mass balance

3.2.1. Apatite

For all treatments, the dissolution of apatite was between 0.4% and 1.8% of the initial weight (Fig. 4).
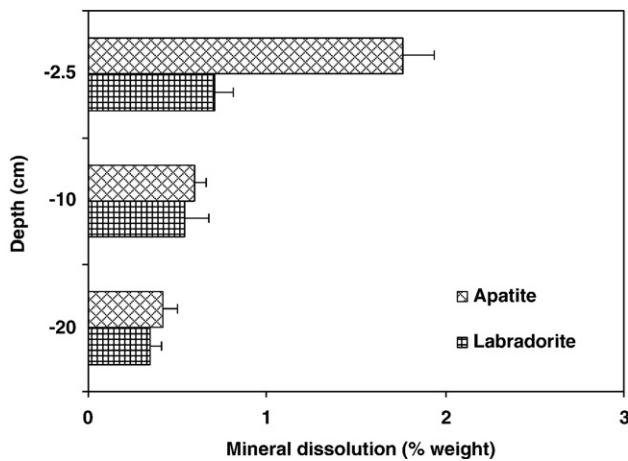

Fig. 4. Mean percentage of labradorite and apatite dissolution in each treatment at three depths.

Fertilization had a significant negative effect on the dissolution rate of apatite at −2.5 cm (Fig. 5). No difference on apatite dissolution rate was observed between the control and the Ca-fertilized plots in deeper soils.

In the control treatment, apatite dissolution rate significantly increased by a factor 3.1 in the presence of roots at −20 cm (Fig. 6a). In the fertilized treatment, apatite dissolution rate was not increased in the presence of roots, with the exception of the −20-cm depth (Fig. 6c). The dissolution rate of apatite was even significantly inferior in the presence of roots at −2.5 cm.

3.2.2. Labradorite

For all treatments, the dissolution of labradorite was between 0.3% and 0.7% of the initial weight (Fig. 4).

Fertilization had a significant negative effect on the dissolution rate of labradorite at −2.5 and −10 cm in the zone with roots (Fig. 5). This decrease in the dissolution rate of labradorite reached more than 50% in these two soil horizons.

In the control treatment, labradorite dissolution rate significantly increased in the presence of roots at all depths (Fig. 6b). Labradorite dissolution rate at −20 cm increased by a factor 2.8 in the presence of roots. In the fertilized treatment, labradorite dissolution rate was not increased in the presence of roots (Fig. 6d). The dissolution rate of labradorite was even significantly inferior in the presence of roots at −2.5 cm.

3.3. Root density

Fig. 7 shows the root density in the control and Ca-fertilized treatments outside the zone in proximity to the trees (1.5 m diameter). This density, which decreased with depth, was very different in both
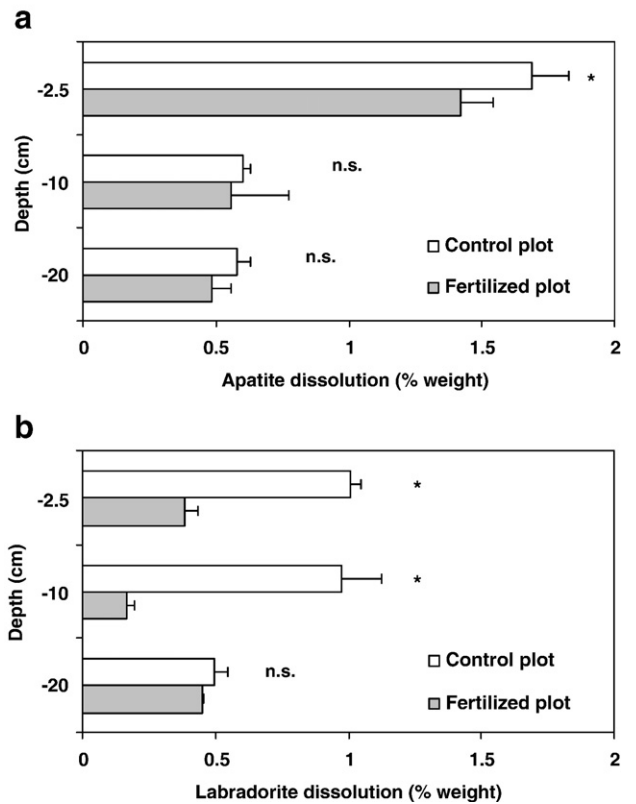

Fig. 5. Percentage of apatite (a) and labradorite (b) dissolution after four years in fertilized (with roots) and control (with roots) treatments.
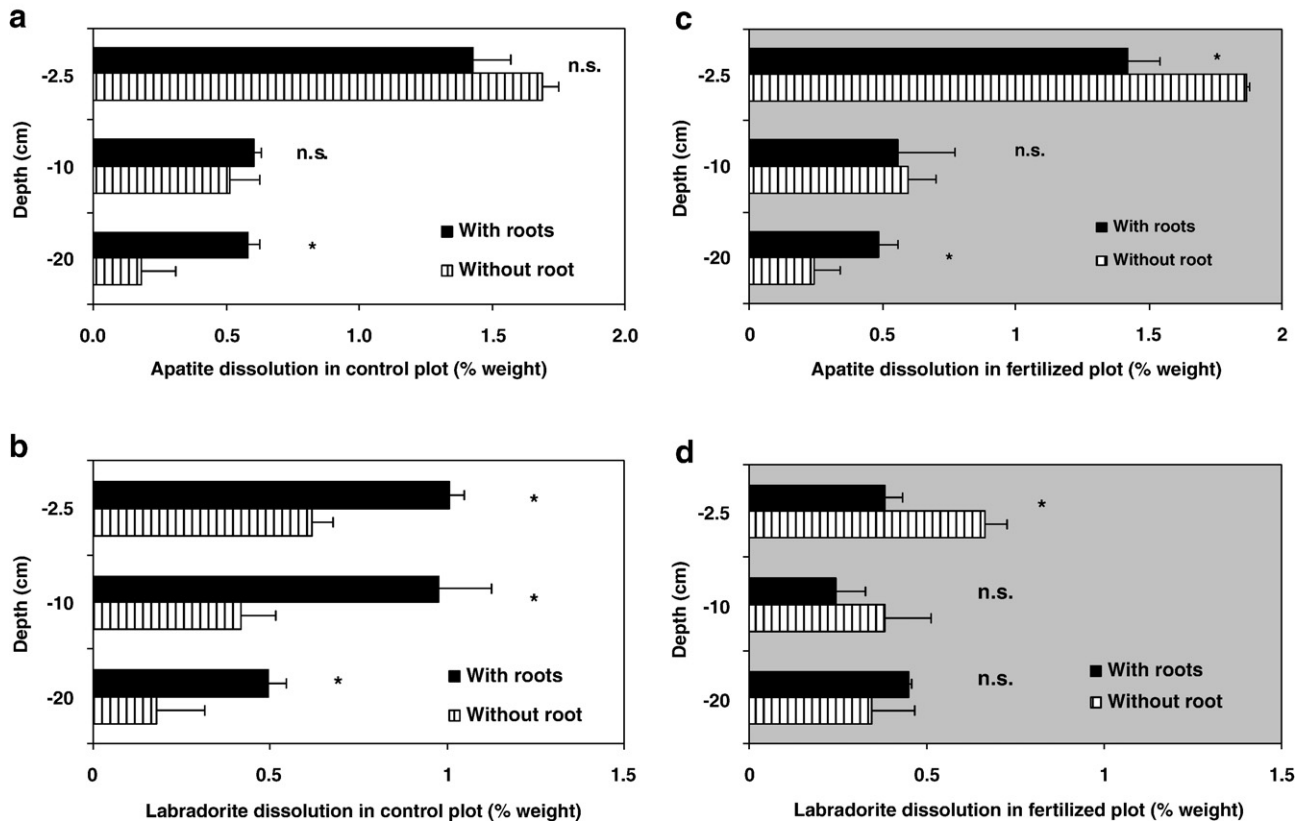
Fig. 6. Percentage of apatite and labradorite dissolution in control (a and b) and fertilized treatments (c and d), with and without roots, after four years of incubation in the soil.

treatments. At the top of the soil profile, root density doubled in the control plot as compared to that of the fertilized treatment even though this was inversed in the deep horizons.

4. Discussion

In order to quantify the impact of the rhizosphere on mineral weathering process, we used an approach combining mineral-test and root exclusion zone methods for the first time in this study.

4.1. Site comparison

The test-mineral method has already been used by Augusto et al. (2000), especially with labradorite, in different non-fertilized acid ecosystems in the north of France. The site and soil physico-

Fig. 7. Root distribution (density) in the control and Ca-fertilized plots at three depths.

chemical characteristics of these experimental sites are presented in Table 3.

In our study, the labradorite dissolution rate was of the same order of magnitude as those measured by Augusto et al. (2000) in the forest sites but, within this range, showed some of the highest values (Fig. 8; Augusto et al., 2000). Thus, the comparison of the dissolution kinetics of labradorite introduced in the presence of tree roots in non-fertilized plots revealed that the forest site of Fougères was one of the most "weathering" ecosystems out of those tested. At the surface, its potential weathering was equivalent to the ecosystems under Norway spruce at Monthermé and Eu. At depth (-15 to -25 cm), this potential was greater than all ecosystems studied by Augusto et al. (2000) and also greater than that of a comparable site developed on loess silts which were very poor in weatherable minerals. This difference may result from the important activity of beech roots and micro-organisms in the soil at the Fougères site, but also from the higher acidity at Fougères (pH_{H_2O} 5–15 cm = 3.96) as compared to Eu (pH_{H_2O} 5–15 cm = 4.20) (Table 3).

4.2. Rhizosphere effect

The geometric form of the dissolution graphs for apatite seems to be controlled by two factors:

(i) a crystallographic factor which applied to all treatments: apatite dissolution was characterized by the formation of residual needles on the basal surface as has already been observed by Thirioux et al. (1990) on the same fluorapatite. This seems to indicate a large contribution by this mineral face to mineral dissolution as already shown for other types of minerals like the inosilicates (Xie and Walther, 1994) or the phyllosilicates (Turpault and Trotignon, 1994).
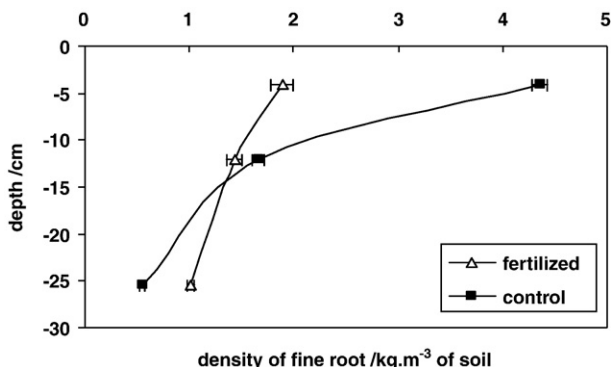
Table 3
Principle site and soil (layer 5–15 cm) characteristics from Augusto et al. (2000).

Site	Bedrock	Soil (F.A.O)	Rainfall (mm)	Temperature (°C)	Tree species	pH_{H_2O}	Organic C (g kg^{-1})	CEC[a] (cmol$_c$ kg^{-1})	BS[b] (%)
Fougères (this study)	Aeolian silt	Dystric Cambisol	868	12.9	Beech	3.96	34	6.5	
Monthermé[c] (Mon)	Sandy silt	Dystric Cambisol	1100	8	Spruce	4.10	64	9.3	4
					Oak	4.20	52	10.5	7
Eu[c] (Eu)	Aeolian silt	Dystric Cambisol	780	10	Spruce	4.20	20	5.3	11
					Oak	4.20	11	6.3	7
Argonne[c] (Ar)	Gaize	Haplic Podzol	790	7	Spruce	3.30	51	3.7	23
					Beech	3.75	21	2.9	19

[a] CEC, cation exchange capacity.
[b] BS, base saturation.
[c] From Augusto et al. (2000).

(ii) a "biological contact" factor which only affected the grains introduced into the treatments with roots: when roots are present with the grains, they form linear curves which seem to correspond to the root imprints. Similar forms due to root activity have been observed in limestone (Hinsinger et al., 2003). In our study, mineral-root contact was not preserved as the minerals had been treated ultrasonically before observation by SEM. Given the shape and size of the marks and the fact that they were only found in treatments containing roots, these marks could clearly be attributed to the functioning of roots and their associated micro-organisms. Also, there were many more imprints in the control treatment as compared to the Ca-fertilized treatment, which could be the result of local rhizospheric activity dependent on the treatment. The finer marks were furthermore observed in all treatments with roots. Some marks were colonised by hyphae or underneath hyphae, thus strongly suggesting the role of fungi in the formation of these dissolution marks. The role of ectomycorrhizal fungi, especially through their hyphae, in the dissolution of minerals has been demonstrated frequently (Paris et al., 1995; Wallander and Wickman, 1999), notably via the tunnelling formation on some soil minerals in boreal ecosystems (Jongmans et al., 1997).

In the control stand, mineral weathering in the zone with roots was more intense than in the zone without roots. This positive impact of the rhizosphere on the weathering increased with depth, reaching factors 3 and 4 at −20 cm. These factors were probably under-estimated because of the absence of root uptake in the zone of root exclusion generated an increase in soil moisture which stimulated mineral weathering. This increase in soil moisture (on average 11% ± 5%, i.e. one third more moisture, Epron, personal communication) is particularly important at the surface, explaining thus the weak difference between the mineral dissolution in the zone with roots and in the zone of root exclusion observed in this soil horizon. Therefore, in this study we showed that the rhizosphere of the control

stand developed on an acid soil poor in nutrients, played a positive role in mineral weathering in this temperate ecosystem. These results confirm those obtained by Gobran et al. (2005) in a boreal spruce forest using another root exclusion method. These authors showed that fluorapatite dissolution was increased in bags with 540 μm mesh permitting root access in comparison to that measured in bags with 50 μm mesh preventing root access. These data on the increase of mineral weathering in the rhizosphere also confirm the results obtained many times in laboratory experiments (Hinsinger et al., 1992) as well as in the field (Bormann et al., 1968; Courchesne and Gobran, 1997; Calvaruso et al., 2009).

4.3. Possible regulation of the rhizospheric biological activity by nutrient availability

The positive effect of the rhizosphere on the weathering of Ca-bearing minerals in the control plot of the Fougères site was not observed in the Ca-fertilized plot which even showed a converse tendency to that measured at the surface. Thus, it seems that rhizospheric biological activities contributing to Ca-bearing weathering are regulated by Ca availability in the ecosystem. In other words, certain biological functions intervening in the mineral weathering process, such as weathering agent production, could be stimulated when the nutrient availability in the system is low. Furthermore, the rhizosphere activity in the control plot was most likely underestimated due to the fact that the root density was significantly inferior in the control plot as compared to that in the fertilized plot at −20 cm.

The same regulation effect was observed at a site in southern Sweden (Gobran et al., 2005). Numerous other examples can be cited indicating this type of biological regulation, and notably root exudation (Dakora and Phillips, 2002). For example, Tadano and Sakai (1991) showed that trees can produce acid phosphatases under phosphorus-deficient conditions. Recent study of Wallander and Thelin (2008) showed that the addition of bags containing apatite had a positive influence on the growth of EM mycelia but this effect diminished when the forests were fertilized with PK. According to these authors, the extent of the EM growth response to apatite was strongly correlated to the needle P status which supports the view that the forest trees regulate growth of EM fungi in response to P source in the soil.

4.4. Conclusion

Through the use of an approach combining mineral-test and root exclusion zone methods in a temperate forest ecosystem, our study clearly demonstrated that the biological activities within the rhizosphere can significantly increase the dissolution of Ca-bearing minerals such as labradorite and apatite but only in low-Ca availability conditions. These observations suggest a high degree of plasticity of rhizospheric biological activities to produce weathering
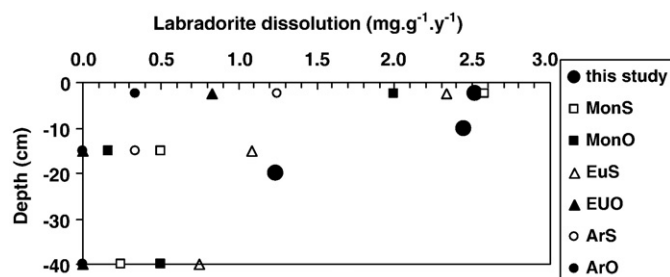

Fig. 8. Percentage of labradorite dissolution in the Fougères site under beech (this study) in comparison with other sites (Mon: Monthermé; Eu; and Ar: Argone) and different species (S: spruce; O: oak) from Augusto et al. (2000).

agents like protons or organic acids and consequently to contribute to the maintenance of nutrient availability in the ecosystem.

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