Calcium carbonate biomineralization in ferrallitic, tropical soils through the oxalate-carbonate pathway

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Résumé

Biominéralisation de calcite dans des sols tropicaux ferralitiques par la voie oxalate-carbonate

Le temps de résidence du carbonate de calcium dans les sols est de l'ordre de 10^4 -10^6 ans. Par conséquent, le piégeage du CO_2 atmosphérique sous la forme de calcaire est presque irréversible, comparé à celui du carbone organique. L'oxalate de calcium, un métabolite souvent accumulé par les végétaux, est chimiquement très stable. En revanche, les bactéries oxalotrophes l'oxydent facilement, entraînant l'alcalinisation du milieu et la biominéralisation de carbonate de calcium, qui s'accumule finalement dans les sols. Dans les régions tropicales, sur des sols primitivement acides et non calcaires, ce phénomène représente un puits pour le CO_2 atmosphérique. Un arbre Iroko adulte peut, avec le taux actuel d'augmentation, stabiliser la concentration du CO_2 dans la colonne d'air au dessus d'une surface de 1000 m^2. Ce processus peut être appliqué dans des actions de reforestation, en agrosylviculture, de même que dans des mesures visant à la conservation et du développement durable du patrimoine forestier et pédologique tropical.

Abstract

Calcium carbonate has a residence time in soils in the order of 10^4 - 10^6 years. Therefore, sequestration of atmospheric carbon as $CaCO_3$ is almost irreversible, as compared to organic carbon. Calcium oxalate, a metabolite often accumulated in plants, is chemically highly stable. However, it is readily oxidized by oxalotrophic bacteria, with simultaneous alkalinization and biomineralization of calcium carbonate, which eventually accumulates in soil. In tropical countries, on mainly acidic, non calcareous soils, this represents a significant sink for atmospheric CO_2: at the present rate of increase, one adult Iroko tree may stabilize CO_2 concentration in the air column above a 1000 m^2 surface. This process may be applied in reforestation and agroforestry, as well as in conservation and sustainable development of tropical forests and soils.

Keywords: Oxalate, Calcium carbonate, Tropical soils, Biomineralization

1. Introduction

During the last century, the main gas responsible for the increase in the greenhouse effect and its consequent climatic changes is atmospheric carbon dioxide, whose concentration increased by 35%. Among the measures that allow this increase to be limited are, on one hand, a decrease in the use of fossil carbon utilization and, on the other hand, CO_2 capture by plants, through the light energy (photosynthesis). However, biomass represents only a transitory, short-term carbon sink (residence time between 1 and 1000 yrs), although it was the object of numerous studies during last twenty years.

Carbon transfer from atmospheric CO_2 to calcium carbonate (limestone) combining photosynthetic capture and biomineralization reactions would represent a much more efficient sink, since the residence time of limestone may be in the order of 10^4 - 10^6 years (RETALLACK, 1990). This would require a non-calcareous calcium source, together with the oxidation of organic material to CO_2 and alkalinization. Such a process was recently described in mainly acidic tropical soils under calcium-oxalate forming trees (BRAISSANT et al. 2002, 2004; CAILLEAU et al. 2004, 2005; VERRECCHIA et al. 2006).

2. Calcium oxalate in plants

A number of plants, including trees, accumulate large amounts of calcium oxalate, a low-soluble salt. It accumulates in the vacuoles of specialized cells, mainly as whewellite, the monohydrated salt. The reason for this accumulation is subject to controversy: either calcium ions are taken up by the plant to neutralize the otherwise strong oxalic acid, or oxalic acid is produced to maintain calcium concentration at a sub-toxic level (FRANCHESCHI and NAKATA, 2005). Otherwise, the presence of calcium oxalate crystals with its acute hedges could protect the plant webs against grazing. Depending on the plant species and growth conditions, the calcium oxalate content may be high (3-85% $w/w_{dry\ matter}$ FRANCESCHI and NAKATA 2005). Anyhow, this process results in a co-accumulation of both calcium and oxalate in a stable, crystalline form.

3. Calcium carbonate accumulation in tropical acidic soils

Most soils in the intertropical forest of the Old and New worlds are acidic and do not contain carbonates. However, CAILLEAU et al. (2005) observed large accumulations of limestone in African ferrallitic soils under certain tree species, particularly the Iroko tree, *Milicia excelsa (Moraceae)*, an overforested hardwood tree. In such soils, the pH under the tree was distinctly alkaline, reaching values between 8 and 9, whereas the soil at a distance from the tree was acidic with pH values < 6. This species accumulates important amounts of Ca-oxalate in its webs. Therefore, it was postulated that calcium carbonate originated from the oxidation of calcium oxalate, since no distinct accumulations of calcium oxalate was ever noticed in geological records. However, calcium oxalate is chemically and physically highly stable, with a very low solubility ($Kps = 4*10^{-9}\ M^2$). The question arose then of putative microbiological processes accompanying plant litter degradation, which would allow weathering of oxalate crystals and complete oxidation of the organic anion to CO_2 and OH^-.

4. Oxalate metabolism in bacteria

Although only second to CO_2 as the most oxidized carbon compound, oxalate may be utilized as energy and carbon source by several bacteria, both anaerobic and aerobic (SAHIN, 2003), i.e. the oxalotrophs. They include the anaerobic *Oxalobacter formigenes*, as well as a number of aerobes belonging to different taxonomic groups, both Gram positive and Gram-negative. Briefly, oxalate anion is taken up and decarboxylated to formate. This latter is secreted in anaerobes (ALLISON et al, 1986), whereas it is utilized as a low potential respiratory substrate in aerobes, being then oxidized to CO_2. In parallel, oxalate is assimilated through either one of two pathways, the serine pathway (BLACKMORE and QUAYLE, 1970) or the glycerate pathway (QUAYLE et al., 1961). The overall equation of oxalate oxidation by aerobes is therefore:

$$(COO^-)_2 + \frac{1}{2} O_2 + H_2O \rightarrow 2\ CO_2 + 2\ OH^-$$

combining carbon oxidation to CO_2 and alkalinisation.

In an aqueous suspension of Ca-oxalate crystals, free oxalate concentration is 63 µM. Although low, this concentration is saturating for bacterial needs, since the half rate uptake affinity is in the order of 1 µM. So, Ca-oxalate is readily dissolved in the presence of oxalotrophic, aerobic bacteria (Fig.1).

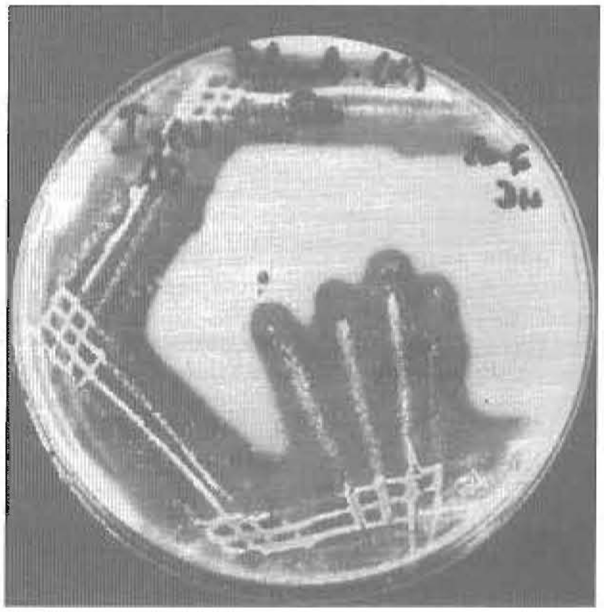

Fig. 1 : Dissolution of Ca-oxalate precipitate by a strain of *Cupriavidus necator*, on mineral agar + Ca-oxalate as sole C-source. From Tamer and Aragno (1980)

5. The oxalate-carbonate pathway

Calcium carbonate biomineralization is therefore possible in acidic, tropical soils, combining:

- weathering of non carbonateous calcium containing minerals, such as plagioclases or apatite;
- transfer of Ca^{++} ions to the trees;
- CO_2 fixation by photosynthesis, and biosynthesis of oxalate;
- formation of Ca-oxalate crystals in vacuoles of specialized cells;
- degradation of oxalate-containing plant webs during the littering process, through saprophytic fungi, and liberation of Ca-oxalate crystals;

- solubilisation of Ca-oxalate crystals through oxalate uptake by oxalotrophic, aerobic bacteria, and consequent liberation of Ca^{++} ions;
- catabolic oxidation of oxalate to CO_2, with concomitant release of hydroxide ions;
- precipitation of calcium carbonate at pH > 8.5, accompanied by the liberation of one CO_2 per oxalate molecule.

The overall equations of Ca-carbonate formation from Ca-oxalate is therefore:

$Ca(COO)_2 \leftrightarrow Ca^{2+} + (COO)_2^{2-}$
(Ca-oxalate dissolution - weathering)
$(COO)_2^{2-} + \frac{1}{2} O_2 + H_2O \rightarrow 2\ CO_2 + 2\ OH^-$
(Oxalate catabolism through aerobic respiration)
$Ca^{2+} + CO_2 + 2\ OH^- \rightarrow \underline{CaCO_3} + H_2O$
($CaCO_3$ precipitation)

$Ca(COO)_2 + \frac{1}{2} O_2 \rightarrow \underline{CaCO_3} + CO_2$

6. Oxalotrophic bacteria in biomineralizing trees' ecosystems

Preliminary studies have shown a diversity of aerobic, oxalotrophic bacteria in decaying wood, litter and soils below biomineralizing trees. Interestingly, whereas the best studied oxalotrophs were Gram-negative bacteria like *Cupriavidus necator* (=*Ralstonia eutropha*) and *Methylobacterium extorquens,* most isolates were Gram-positive actinobacteria related to the genus *Streptomyces* (BRAISSANT et al., 2004). Even if most oxalotrophic bacteria are otherwise versatile organisms occurring in environments devoid of oxalate as well, the abundance of the *frc* gene, a gene coding for formyl-CoA transferase and characteristic of oxalate catabolism by oxalotrophs, was shown to be significantly higher in soils under a biomineralizing tree than in a distant soil (KHAMMAR et al., 2008).

7. Importance of fungi

Fungi may participate to the biomineralization process in different ways. First, a number of saprophytic and mycorrhizal Eumycetes produce calcium oxalate crystals, in general as dihydrated wedellite (fig. 2, GUGGIARI et al., 2009). In some cases, it was shown that fungi were able to dissolve and oxidize calcium oxalate. Therefore, the importance of the fungal component in the oxalate-carbonate pathway has to be studied in depth.

Otherwise, fungi may be agents of the weathering of calcium-containing minerals and of calcium translocation to the tree roots. By degrading plant cell wall materials, saprophytic fungi release oxalate crystals from plant webs and expose them to bacterial attack.

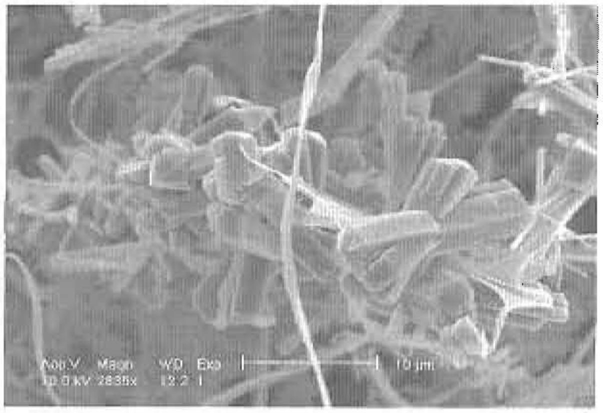

Fig. 2: Ca-oxalate crystals (dihydrate, weddellite) on fungal hyphae. Photo E. Verrecchia

8. Quantitative estimation of the biomineralizing activity

From our studies on Iroko trees in Ivory Coast, it was estimated that one adult tree induces the accumulation of 5.6 kg·yr^{-1} C as calcium carbonate in soil. With the present rate of atmospheric CO_2 increase (2 ppm/yr, that is, 1 mg/m^3.yr C), one such tree would stabilize the CO_2 concentration in 5'600'000 m^3 air, that is, in the whole air column above a ca. 1000 m^2 surface.

9. Extent of the phenomenon

By no means is the operation of oxalate-carbonate pathway limited to the Iroko tree species. A systematic survey of its occurrence under different tree species, in different regions of the world and under different climatic conditions has still to be performed. However, after a limited number of investigations, several other plant species, from different families, were shown to induce this phenomenon:

In Africa (Burkina Faso):
Kapok tree *(Bombax costatum,* Malvaceae)
Lingué *(Afzelia africana,* Leguminosae-Caesalpinaceae*)*

In Bolivia (Alto Beni, Amazona):
Ajipa *(Pentaplaris davidsmithii,* Tiliaceae)
Flor de Mayo *(Ceiba speciosa,* Malvaceae)
Verdolago colorado *(Terminalia amazonica,* Combretaceae)
Quina Quina (*Myroxolon balsamum,* Leguminosae-Caesalpinaceae)

Therefore, this phenomenon could be much more widespread than initially suspected. Even if no quantitative estimation of its global importance is yet possible, it could be at least a partial explanation to the "lack of carbon" in the carbon cycle of tropical forest, as pointed out by GIFFORD (1994).

10. Prospects for applications

Even if the precise conditions (climate, soil, landscape characteristics, tree species, time span) for obtaining an optimal biomineralizing activity are still to be investigated, the existing estimations are encouraging enough to look forward potential applications:

1. The observed widespread occurrence of this phenomenon in tropical forests gives a new importance to their conservation, as an efficient long-term CO_2 trapping system.

2. Encouraging the planting of biomineralizing trees in sustainable reforestation could be done at governmental level by mobilizing carbon incentive credits

3. The use of biomineralizing trees in three-level agroforestry practices could combine the benefits of the CO_2 sink with the delay of crops and fruit maturation and the improvement of soil fertility through neutralization and calcium carbonate accumulation.

11. Literature

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