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The oxalate–carbonate pathway in soil carbon storage: the role of fungi and oxalotrophic bacteria

ERIC P. VERRECCHIA, OLIVIER BRAISSANT AND GUILLAUME CAILLEAU

Institute of Geology, University of Neuchatel, Switzerland

Introduction

Although fungi are generally disregarded in the biogeochemical literature, they undoubtedly constitute crucial biogeochemical factors in many elemental cycles. This fact, combined with their abundance in the soil warrants greater detailed study into their geoecological impact. The network formed by fungal filaments can represent 10 000 km of thread-like mycelia in 1 m^2 of fertile soil. Their mass is evaluated at 3500 kg ha^{-1} at a depth of 20 cm in an average continental soil, i.e. taking into account all the different terrestrial environments on the Earth (Gobat *et al.*, 2004). In comparison, bacteria and algae would represent 1500 and 10–1000 kg ha^{-1} respectively, in the same virtual average soil. Fungi are not only biologically important as saprophytes in the recycling of organic matter, but also play a geological role by excreting notable amounts of organic acids, among which oxalic acid is particularly important (Gadd, 1999), contributing to continental weathering as well as to mineral neogenesis (Verrecchia & Dumont, 1996; Verrecchia, 2000; Burford *et al.*, 2003 *a*, *b*).

The first fossil fungi have been identified in rocks dated from the Ordovician, i.e. 460 to 455 Ma ago (Redecker *et al.*, 2000). However, molecular clock estimates for the evolution of fungi have suggested a Late Precambrian (600 Ma) colonization on land (Berbee & Taylor 2000). Recent molecular studies, based on protein sequence analysis, indicate that fungi were present on continents 1 billion years ago and possibly affected (together with plants) the evolution of Earth's atmosphere and climate since 700 Ma (Heckman *et al.*, 2001). Therefore, if fungi have been present on the Earth's surface for such a long time, producing large amounts of oxalic acid able to precipitate as metal oxalates, why is there no evidence of oxalate accumulation in paleosols?

Fungi in Biogeochemical Cycles, ed. G. M. Gadd. Published by Cambridge University Press.

The question is also valid for present-day soils. The aim of this chapter is to demonstrate (1) that plants and fungi can produce high amounts of calcium oxalate polymorphs (weddellite, $CaC_2O_4.2H_2O$ and whewellite, $CaC_2O_4.H_2O$) by various processes, and (2) that oxalotrophic bacteria must have used these abundant oxalate crystals as carbon, electron and energy sources by oxidizing them into calcium carbonate ($CaCO_3$), a common polymorphic mineral (in the form of calcite, vaterite or aragonite) found in soils and surficial sediments. Thermodynamic approaches will show that spontaneous oxidation of oxalate is impossible and therefore necessitates a biomediated process, leading to secondary formation of calcium carbonate and a pH increase in the soil solution. In addition, it can be demonstrated that the oxalate–carbonate pathway constitutes a carbon sink, as the carbon source is organic and not an inherited lithogenic mineral carbon (e.g. fossil limestones).

The oxalate pool

Plants, fungi and oxalate

There are several ways by which plants modify the surrounding soil to their advantage. They may be biotic, like the accumulation of plant-protecting rhizobacteria increasing the soil's ability to fight against root disease, or the acclimation of mycorrhizal fungi. There may also be abiotic factors, like the precipitation of toxic cations and minerals. Accumulation of calcite in carbonate-poor soils may improve the soil structure and function. There is increasing evidence that the calcium oxalate cycle is a major pathway in calcite biomineralization. This was shown with different oxalate-accumulating plants, such as iroko trees (Braissant *et al.*, 2004) and Cactaceae (Garvie, 2003). Nevertheless, a question is still pending: how and why do plants form oxalate crystals? In plant metabolism, oxalic acid is produced in varying amounts, depending on plant taxon and external conditions. This oxalic acid may be either accumulated in the vacuole, or precipitated in the form of insoluble calcium oxalate crystals in the cell, called crystal idioblasts (Fig. 12.1). Calcium oxalate crystal formation in plants appears to play a central role in a variety of important functions, including tissue calcium regulation, protection from herbivores and metal detoxification (Nakata, 2003). It seems that ascorbic acid is the primary precursor for oxalate biosynthesis. The ascorbic acid can be synthesized directly within the calcium oxalate crystal-accumulating cell (Nakata, 2003). Obviously, calcium oxalate accumulated in the aerial parts of the plant can eventually be transferred to the litter after plant death, during organic matter oxidation.

As an example, iroko trees (*Milicia excelsa*, Moraceae), which are trees from the tropical African forest, can accumulate large amounts of oxalate crystals in their trunk as well as their roots (Cailleau *et al*., 2005). In the wood, calcium oxalate occupies some idioblasts, forming euhedral crystals (Fig. 12.2A), often occurring as chains inside neighbouring cells. In roots, calcium oxalate forms a mineral network of crystals between the inner root and the thin cuticle constituting the outer part of the root (Braissant *et al*., 2004). When fungi decay parts of dead roots or rotting wood (Fig. 12.2B, C), they free oxalate crystals inside the soil or litter (Fig. 12.2D), increasing their proportion outside the living tissues. This plant oxalate pool is a widespread and abundant carbon source for oxalate consumers.

Contrary to plants, whose excretion of oxalate ions is generally considered as negligible, fungi (mycorrhizal as well as saprophytic) are essentially

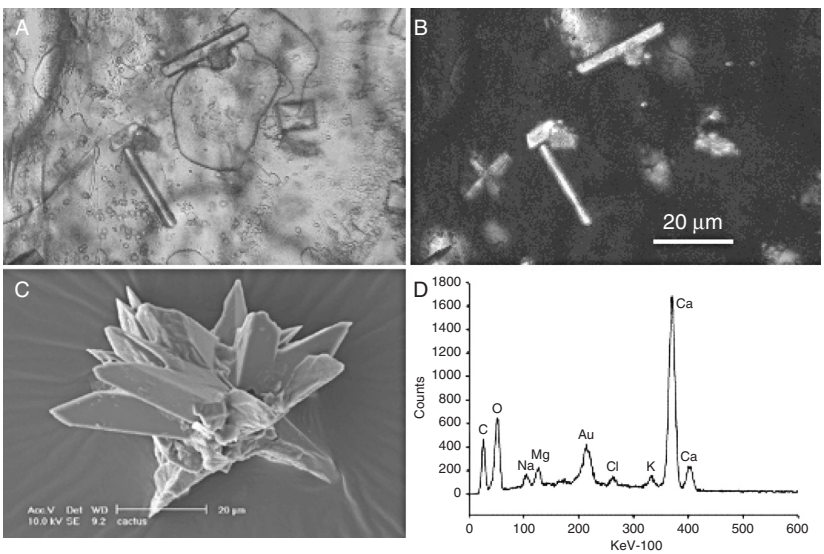

Fig. 12.1. (A) Plane-polarized light microphotograph of a cross-section inside a *Cereus* sp. succulent stem: prism and styloids of calcium oxalate are visible as crystal idioblasts. Scale bar shown on image B. (B) The same view in cross-polarized light showing the mono-crystalline nature of the biominerals. (C) Scanning electron micrograph showing the structure of a drusic agglomerate of calcium oxalate inside an *Opuntia* sp. (D) Electron dispersive energy spectrum for druse: elements found are mainly carbon, oxygen and calcium (for the calcium oxalate), magnesium, potassium, sodium and chlorine as accessory salts, and traces of phosphorus. The gold peak is due to sample coating. The identification of the mineral (calcium oxalate monohydrate or COM) was confirmed by X-ray diffraction analysis.

oxalate excreters. Metal oxalates, essentially calcium oxalates (Lapeyrie *et al*., 1990; Gadd, 1999; Tait *et al*., 1999), then crystallize at the mycelium surface or in the nearby soil, mainly as monohydrate (COM – calcium oxalate monohydrate or whewellite) and dihydrate (COD – calcium oxalate dihydrate or weddellite; Fig. 12.3A, B). Each of them belongs to a different crystallographic system, monoclinic and tetragonal, respectively (see synthesis in Verrecchia *et al*., 1993). An extensive review of oxalate biosynthesis in fungi has been produced by Gadd (1999). Oxalic acid production by fungi appears to depend on whether glucose or citrate is used as the carbon source (Fig. 12.3C). When glucose is used, oxalate is produced through oxidation of glucose to pyruvate (Verrecchia, 1990; Wolschek & Kubicek, 1999):

$$C_6H_{12}O_6 + O_2 \rightarrow 2CH_3COCOOH + 2H_2O \quad (12.1)$$

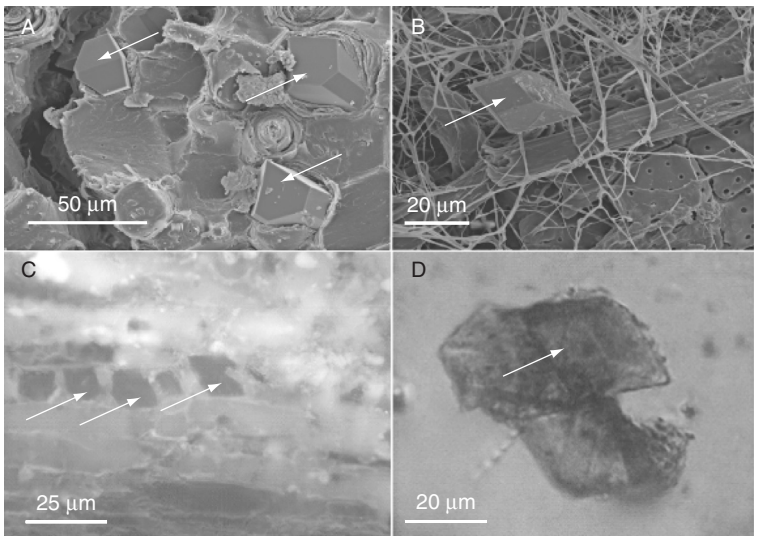

Fig. 12.2. (A) Scanning electron micrograph showing euhedral crystals (arrows) of COD (calcium oxalate dihydrate or weddellite): the other part of the image is mainly cellulose. (B) COD crystal observed with scanning electron microscope. The crystal (arrow) is surrounded by fungi decaying iroko wood whose cellulose fibrous structure is still observable. (C) Ultraviolet-fluorescent light microphotograph of a cross-section inside decaying wood. Between the wood fibres, a chain of dark euhedral crystals of COD has almost been freed in the medium. (D) View with optical binoculars of free crystals of COD inside a soil aggregate. The shape (habitus) of the crystal shown with the arrow is exactly the same as the one of the crystals shown in B.

Then, carboxylation of pyruvate yields oxaloacetate:

$$2CH_3COCOOH + 2CO_2 \rightarrow 2HOOC.CH_2CO.COOH \qquad (12.2)$$

The hydrolysis of oxaloacetate allows the formation of oxalate and acetate:

$$2HOOC.CH_2CO.COOH + 2H_2O \rightarrow 2(COOH)_2 + 2CH_3COOH \qquad (12.3)$$

Oxalic acid reacts with calcium, forming calcium oxalate crystals, either COM or COD. Although calcium oxalate crystals are easily observed

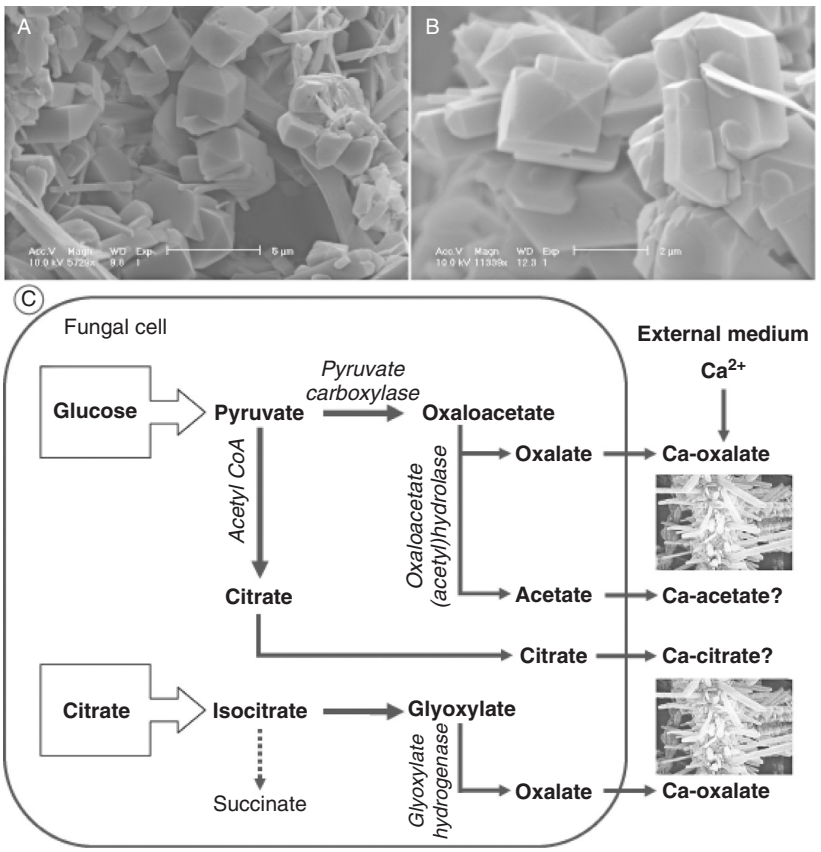

Fig. 12.3. (A) Scanning electron micrograph showing euhedral crystals of COD (calcium oxalate dihydrate or weddellite) associated with fungal filaments in decaying iroko wood (Ivory Coast). Scale bar = 5 µm. (B) Detail of COD crystal shown in A. The COD crystals belong to the tetragonal system. Scale bar = 2 µm. (C) Diagram of various pathways for calcium oxalate production by fungi. Biosynthesis of calcium oxalate is often accompanied by other low-molecular-weight organic acids, such as citrate or acetate.

associated with fungi, calcium acetate or calcium citrate crystals have never been identified. On the one hand, it is true that they constitute extremely soluble salts. But, on the other hand, organic substrates such as acetate or citrate can easily be used as carbon sources by bacteria. The culture of oxalatrophic bacteria (e.g. *Ralstonia eutropha* and *Xanthobacter autotrophicus*) for a few days on acetate-rich and citrate-rich media results in the production of calcite (calcium carbonate) crystals at the expense of the low-molecular-weight organic acids (Fig. 12.4; Braissant *et al*., 2002). Therefore, these by-products can also be a source of secondary calcium carbonate in soils, even if they are highly soluble, and probably rapidly leached. In contrast, calcium oxalate crystals precipitate and may constitute almost 25% of soil hyphae and rhizomorphs dry weight in some ecosystems (Cromack *et al*., 1977). When citrate is the source, oxalate is produced through the isocitrate and glyoxylate cycle involving glyoxylate hydrogenase (Dutton *et al*., 1993).

The accumulation of oxalate crystals by fungi has also unexpected consequences: calcium oxalate can be disseminated inside the soil and

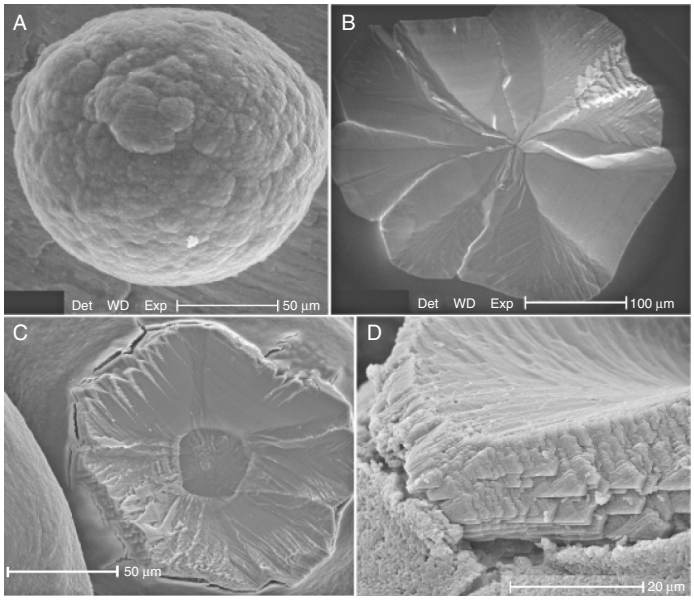

Fig. 12.4. Scanning electron micrograph showing calcium carbonate crystals found (A) associated with a *Xanthobacter autotrophicus* culture on an acetate-rich medium, (B) on the same medium with *Ralstonia eutropha*, (C) with the same bacterium on a citrate-rich medium and (D) on the same medium with *Xanthobacter autotrophicus*.

the litter through oribatid mites. Such mites feed on calcium oxalate crystals from the producing fungi and then reprecipitate the mineral in their cuticular hardened parts (Norton & Behan-Pelletier, 1991). They are thought to process a significant portion of the calcium pool in some ecosystems (Gist & Crossley, 1975). In conclusion, calcium oxalate is a common product of the biosphere–lithosphere interface, it can be found in numerous environments and it results from biomineralization under the control of organisms. So why does it not accumulate in surficial environments and soils?

Calcium oxalate stability

In order to solve the problem of missing calcium oxalate accumulation, the first question to ask is whether or not the mineral is stable, i.e. is calcium oxalate able to spontaneously oxidize when in contact with the atmosphere? If this is the case, the explanation is simple: all the oxalate produced is rapidly oxidized as CO_2, and therefore can be neither accumulated in the surficial environment nor in the fossil record. This assumes that the transformation of oxalate into CO_2 must be complete and rapid in normal conditions, i.e. at 25 °C (298.15 K) and a pressure of 1 atm. This complete oxalate oxidation in solution is given by the following reaction:

$$C_2O_4^{2-} + \frac{1}{2}O_2 + 2H^+ \rightleftharpoons 2CO_2 + H_2O \tag{12.4}$$

This equation can be divided into two redox equations:

$$C_2O_4^{2-} \rightleftharpoons 2CO_2 + 2e^- \tag{12.5}$$

$$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O \tag{12.6}$$

For each of the redox couples, the potential can be calculated using the Nernst equation. This equation correlates Gibb's free energy, known as ΔG, and the electromotive force provided by an oxido-reduction reaction (such a reaction acts as a galvanic cell). Given the following equation due to a chemical reaction:

$$aA + bB \rightleftharpoons cC + dD \tag{12.7}$$

reaction coefficient Q is calculated using the following ratio:

$$Q = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b} \tag{12.8}$$

At equilibrium in the solution, $Q = K_{eq}$, K_{eq} being the equilibrium constant associated with the reaction. The Gibb's law is expressed as:

$$\Delta G = \Delta G^0 + RT \ln Q \text{ and } \Delta G = -n\mathcal{F}\Delta E \tag{12.9}$$

This last equation gives the relationship between Gibb's free energy and the electromotive force, ΔE. Consequently, by expressing ΔG and ΔG^0 (standard free energy) in terms of ΔE and ΔE^0 (standard electrode potential), the following equation is obtained:

$$\begin{aligned} -n\mathcal{F}\Delta E &= -n\mathcal{F}\Delta E^0 + RT \ln Q \Leftrightarrow \Delta E \\ &= \Delta E^0 - \frac{RT}{n\mathcal{F}} \ln \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b} \end{aligned} \tag{12.10}$$

Equation (12.10) is known as the Nernst equation, where R is the gas constant (8.314 J mol^{-1} K^{-1}), T the temperature in Kelvin, Q the reaction quotient as defined in Eq. (12.8), $\mathcal{F}$ the Faraday constant (9.65×10^4 C mol^{-1}) and n being the number of electrons involved during the oxido-reduction reaction. By using log (to the base 10) instead of the natural logarithm, replacing the variables with their numerical values and fixing the temperature at 25 °C (298 K), the Nernst equation becomes:

$$\Delta E = \Delta E^0 - \frac{0.059}{n} \log \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b} \tag{12.11}$$

Therefore, by using Eq. (12.11), the potential E_1 for Eq. (12.5) is given by:

$$E_1 = -0.49 + \frac{0.059}{2} \log \frac{(\mathrm{pCO_2})}{\left|\mathrm{C_2O_4}^{2-}\right|} \tag{12.12}$$

where $E_0 = -0.49$ is obtained via experimental measurement. For Eq. (12.6), the Nernst equation is written as:

$$\begin{aligned} E_2 &= E^0_{water} + \frac{0.059}{2} \log\left((\mathrm{pO_2})^{\frac{1}{2}}\,|\mathrm{H^+}|^2\right) \Leftrightarrow \\ E_2 &= 1.23 - \frac{0.059}{2} \times 2 \times \underbrace{(-\log|\mathrm{H^+}|)}_{pH} + \frac{0.059}{2} \log\sqrt{(\mathrm{pO_2})} \Leftrightarrow \\ E_2 &= 1.23 - 0.059 \times \mathrm{pH} + \frac{0.059}{2} \log\sqrt{(\mathrm{pO_2})} \end{aligned} \tag{12.13}$$

At equilibrium in the solution, $E_2 = E_1$. By combining Eqs. (12.12) and (12.13) and by replacing variables with numerical values, such as $\mathrm{pO_2} = 0.2095$ atm., $\mathrm{pCO_2} = 3.3 \times 10^{-4}$ atm, the equilibrium condition yields:

$$-0.49 + \frac{0.059}{2}\log\frac{(pCO_2)^2}{\left|{C_2O_4}^{2-}\right|} = 1.23 - 0.059 \times pH + \frac{0.059}{2}\log\sqrt{(pO_2)} \Leftrightarrow \underbrace{\frac{-1.72 \times 2}{0.059}}_{-58.305} + 2 \times pH + \underbrace{\log\frac{(pCO_2)^2}{\sqrt{(pO_2)}}}_{-6.623}$$

$$= \log\left|{C_2O_4}^{2-}\right| \Leftrightarrow -64.928 + 2 \times pH = \log\left|{C_2O_4}^{2-}\right| \qquad (12.14)$$

By varying the pH between 7 and 14 in Eq. (12.14), the concentration of oxalate ions is:

$$7 < pH < 14 \Rightarrow 1.18 \times 10^{-51} < \left|{C_2O_4}^{2-}\right| < 1.18 \times 10^{-37}$$

From these calculations, it appears that there is effectively no ${C_2O_4}^{2-}$ in solution. But in fact, this reaction occurs in the solution at an almost infinitely low rate under normal conditions of pressure and temperature. Indeed, many oxalate salts have a low solubility index. However, this point cannot explain why a solution of potassium oxalate, which is soluble, will not oxidize spontaneously into CO_2. The problem is linked to a lack of *activation energy*. Such an oxidation needs a certain initial amount of energy to occur. If a solution of calcium oxalate is left in contact with the atmosphere, it will never be oxidized into CO_{2aq}, ${HCO_3}^-$, ${CO_3}^{2-}$ and ${Ca}^{2+}$. Nevertheless, if oxalatrophic bacteria are in contact with the solution, they will provide energy to start the oxidation and feed on the oxalate carbon source.

In conclusion, any metal oxalate can be considered as a compound in a metastable equilibrium. The only sub-spontaneous diagenetic evolution of calcium oxalate is a possible transformation of weddellite into whewellite by dehydration (Frey-Wyssling, 1981; Verrecchia *et al.*, 1993). Consequently, activation energy has to be provided for a complete oxidation of COM or COD, and life is the best and the most obvious candidate. Therefore, biogenic activity could be the key explanation for the absence of oxalate in paleosols as well as in the geological sedimentary record.

Oxalate oxidation

Oxalotrophic bacteria

The fate of oxalate in natural systems remains unclear, although oxalate catabolism by bacteria is a well-recorded phenomenon (Tamer & Aragno, 1980; Allison *et al.*, 1995). Oxalate is used as an energy, electron

and carbon source by bacteria belonging to diverse taxonomical groups (Tamer & Aragno, 1980; Jenni *et al*., 1987, 1988; Sahin, 2003). These bacteria may occupy different oxalate-containing niches, like the rhizosphere (Knutson *et al*., 1980), the litter, or the gut of soil animals (Cromack *et al*., 1977). It seems that, after oxalate is transported into the bacterial cell, its fate is determined by either the glyoxylate or formate pathway (Fig. 12.5). If oxalate is reduced into glyoxylate by the enzyme oxalyl-CoA reductase, biosynthesis can take one of two routes: the glycolate pathway (Allison *et al*., 1995) and a variant of the serine pathway (Sahin, 2003). The glycolate pathway is common in soil oxalotrophic bacteria such as *Ralstonia oxalatica* or *Ralstonia eutropha*. The serine pathway is mainly used by oxalate-using pink-pigmented facultatively methylotrophic bacteria, which contain L-serine glyoxylate aminotransferase and hydroxypyruvate reductase, key enzymes of the serine pathway (Sahin, 2003). If, however, oxalate is transformed into formate through oxalate decarboxylation, the formate is then used for cell energy and finally results in the production of CO_2 (Dijkhuizen *et al*., 1977). Carbon dioxide is usually transported and excreted as bicarbonate ions (HCO_3^-) through the bacterial membrane. Its combination with calcium ions leads to precipitation of calcium carbonate and concomitantly generates a proton motive force (Fig. 12.5). Therefore, oxalotrophic bacteria perform a complete oxidation of oxalate to CO_2, leading to bicarbonate ion excretion, and finally carbonate deposition. But are soil conditions favourable for calcium carbonate storage?

The fact that protons are used as a motive force can contribute to an increase in the pH of the soil solution, due to the H^+ uptake by bacteria. Moreover, the pH can also be increased due to the fact that reactions involve the transformation of oxalic acid into carbonic acid, i.e. of a strong ($pK_1 = 1.25$, $pK_2 = 4.27$) to a weak ($pK_1 = 6.35$, $pK_2 = 10.33$) acid (Braissant *et al*., 2002). This alkalinization facilitates precipitation of calcium carbonate ($CaCO_3$). By modifying a general equation for oxalate metabolism by oxalatrophic bacteria (Harder *et al*., 1974), the following balanced equation can be proposed:

$$\begin{aligned} 1000CaC_2O_4.nH_2O + 372O_2 + 32NH_4^+ \rightarrow \\ 32C_4H_8O_2N^+{}_{biomass} + 936CaCO_3 + 64Ca(OH_2)_{aq} + \cdots \\ \cdots + (1000.(n-2) + 1872)H_2O + 936CO_2 \end{aligned} \tag{12.15}$$

It is easy to see in this balanced equation that 93.6% of the organic carbon is transformed into mineral carbon (as precipitated $CaCO_3$ in addition to

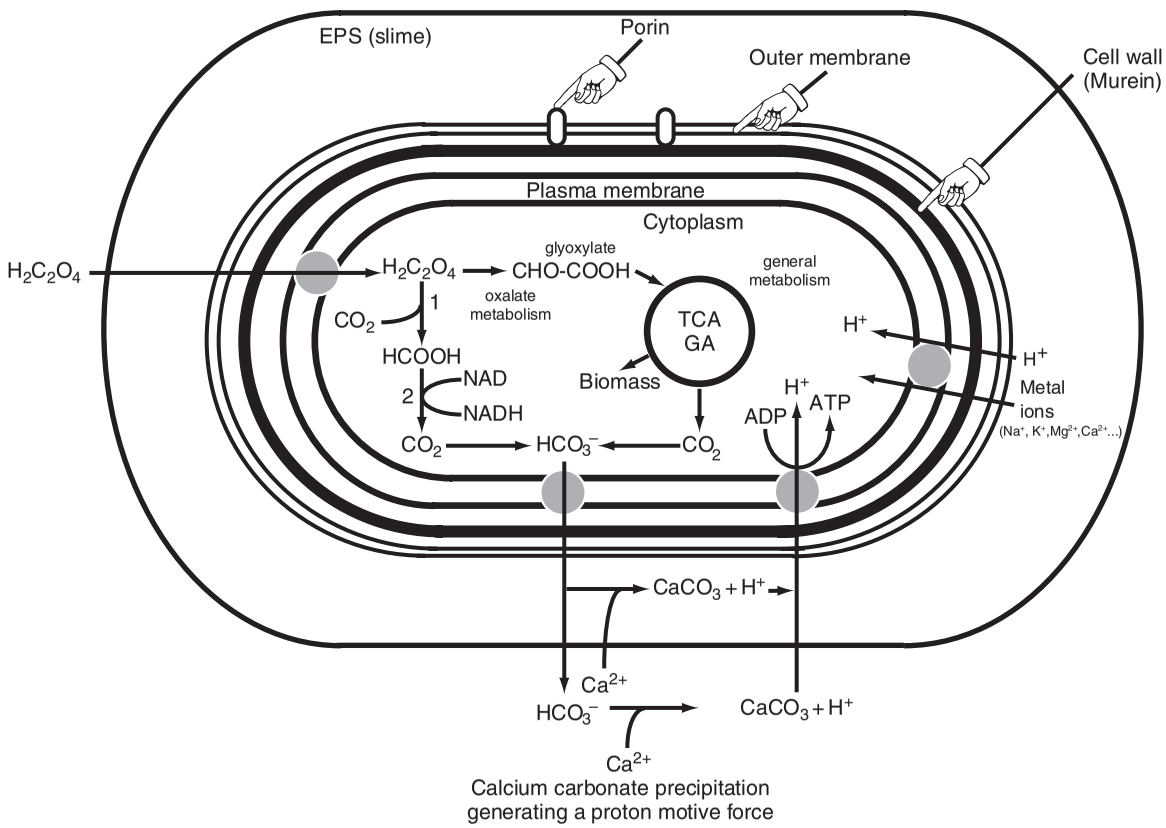

Fig. 12.5. The two main processes identified that lead to the precipitation of calcium carbonate from an oxalate source in oxalotrophic bacteria, the formate and the glyoxylate pathways. TCA, tricarboxylic acid cycle; GA, glyoxylic acid cycle; EPS, exopolysaccharides; 1, oxalate decarboxylation into formate; 2, formate dehydrogenation; ● permease/transporters.

CO_2, the latter being dispersed in the environment and eventually degassed to the atmosphere), whereas the other part of the carbon (i.e. only 6.4%) is used to increase the biomass. These figures demonstrate the far-reaching impact of such processes on organic carbon mineralization and sequestration.

Finally, it has to be noted that oxalate oxidation by oxalate oxidase is a different means of degrading oxalate. By this mechanism, oxalate is completely oxidized to CO_2 with concomitant production of H_2O_2. It has no trophic significance and its importance in oxalate cycling is not known.

All these results are related to observations and measurements in the field as well as from oxalotrophic bacterial cultures in the laboratory (Braissant *et al.*, 2004). There is no theoretical model available to explain the oxalate–carbonate transformation and its consequences on the soil solution properties, i.e. alkalinization facilitating precipitation of calcium carbonate ($CaCO_3$) and CO_2 release into the atmosphere. This kind of model should be able to explain the process of oxido-reduction reactions, pH regulation and the evolution of the various phase concentrations involved in the system, i.e. oxalate, carbonate, water and CO_2. This is the aim of the next section.

A theoretical analysis of oxalate–carbonate transformation

The objective of this theoretical approach is to try to compare the equilibrium diagrams of the $CaCO_3-CaC_2O_4-CO_2-H_2O$ system with the biogeochemical data available on the oxalate–carbonate transformation. The initial hypothesis is that the oxido-reduction reaction of oxalate–carbonate occurs biochemically, i.e. is due to bacterial activity. In other words, activation energy is present and high enough to start the reactions. The theoretical system studied is defined by three different phases present:

1. The aqueous solution, which may or may not be associated with the two mineral species, calcium oxalate (CaC_2O_4) and calcium carbonate ($CaCO_3$). These two species are defined by their solubility products. Concentrations of the various chemical species in solution (including carbonates – HCO_3^-, CO_3^{2-}; oxalates – $C_2O_4^{2-}$, $C_2O_4H^-$; Ca^{2+} and CO_2) can vary in relation to the outside environment. Therefore, the system is considered as open.
2. Inside the system, the acido–basic equilibria are always reached. Nevertheless, solutions can be supersaturated regarding the two mineral species, calcite ($CaCO_3$) and calcium oxalate (CaC_2O_4).
3. Gas exchange is assumed to be fast enough to reach equilibrium between $CO_{2(dissolved)}$ and $CO_{2(gas)}$.

The fact that the system is open is justified because atmospheric oxygen is needed for the transformation (oxidation) of oxalate into carbonate by bacteria. In addition, the gas exchange with the atmosphere must be fast enough to meet the condition given in point 3. The system is described in Fig. 12.6. This system is supposed to evolve reversibly, i.e. the system is in permanent equilibrium. Therefore, at each moment, there is chemical equilibrium in the solution between the carbonate species (carbonates, bicarbonates, dissolved CO_2) and the two oxalate species (oxalate salt and oxalic acid). Nevertheless, equilibrium between solution and solids (i.e. minerals) could not be reached when the solution is supersaturated regarding one or the other of the minerals. Concerning the exchange kinetics between dissolved species inside the system and the exterior environment (Fig. 12.6), it is considered as extremely slow because equilibria between dissolved species inside the system are assumed to be continuously reached. Consequently, transformation of oxalate into carbonate is described as an exchange between dissolved species, the amount of oxalate and carbonate being variable. In other words, the transformation of oxalate into carbonate is an input of the model and considered to occur outside this theoretical system, i.e. through oxalotrophic bacterial activity (Fig. 12.6). Species' activities inside the system will be considered as equal to concentrations to simplify calculations. Except for H^+, the only cation present in the system is Ca^{2+}. This point is also only a means to simplify the calculations, but is true in laboratory bacterial cultures. The anions are the carbonate and the oxalate species, as well as OH^-. Therefore, the alkalinity can be defined as:

$$|Alc| = 2\left|Ca^{2+}\right| \tag{12.16}$$

The various limits of the variables in the system are given as follows. The values taken for the total quantity of oxalate $A = |C_2O_4{}^{2-}| + |C_2O_4H^-|$ are $0 < A < 10^{-1}$ mol l^{-1}. The concentration of calcium ions in the model is $10^{-6} < |Ca^{2+}| < 10^{-1}$ mol l^{-1}. The partial pressure of CO_2 will vary between 0 and 1 atm. Finally, the pH ranges between 4 and 11. The equilibrium constants at 1 atm and 25 °C (298 K) are the following:

$$k = \frac{\left(C_2O_4{}^{2-}\right)(H^+)}{(C_2O_4H^-)} = 10^{-4.3} \tag{12.17}$$

$$K_1 = \frac{(CO_3H^-)(H^+)}{(CO_2)} = 10^{-6.4} \tag{12.18}$$

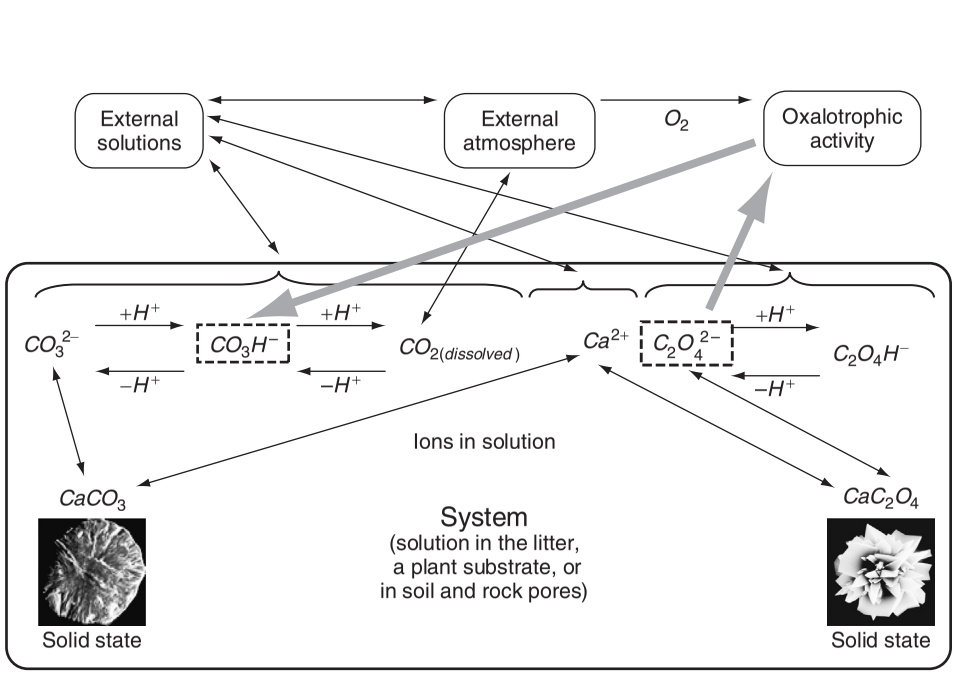

Fig. 12.6. Sketch of the system upon which is based a theoretical model of oxalate transformation into carbonate through bacterial oxalotrophic activity. The system is defined as open and therefore, exchanges are possible with the external environment.

$$K_2 = \frac{(CO_3^{2-})(H^+)}{(CO_3H^-)} = 10^{-10.2} \tag{12.19}$$

$$K_w = (H^+)(OH^-) = 10^{-14} \tag{12.20}$$

The mineral solid phases (calcium carbonate and calcium oxalate) are characterized by their solubility products:

$$\Pi_1 = \left(Ca^{2+}\right)\left(CO_3^{2-}\right) = 10^{-8.3} \tag{12.21}$$

$$\Pi_2 = \left(Ca^{2+}\right)\left(C_2O_4^{2-}\right) = 10^{-8.7} \tag{12.22}$$

The model is built using equations that follow two laws. The mass action law is applied to acido–basic reactions of carbonate and oxalate species with water. The equilibrium between the CO_2 concentrations in the gas and the solution is described by Henry's law:

$$|CO_3H_2| \text{ or } |CO_2| = k_H.pCO_2 \tag{12.23}$$

Finally, the electro-neutrality of the solution is given by:

$$\begin{aligned} |C_2O_4H^-| + 2\left|C_2O_4^{2-}\right| + |CO_3H^-| + 2\left|CO_3^{2-}\right| + |OH^-| \\ = 2\left|Ca^{2+}\right| + |H^+| \end{aligned} \tag{12.24}$$

By applying these laws to the data given in Eqs. (12.18) to (12.24), a general equation describing the relationship between the different variables of the system can be found. The key equation describing the model is expressed as:

$$\begin{aligned} 2\left|Ca^{2+}\right| = &- |H^+| + \frac{K_w}{|H^+|} + \cdots \\ &\cdots + \left(\left|C_2O_4^{2-}\right| + |C_2O_4H^-|\right)\left\{1 + \frac{1}{1 + \frac{|H^+|}{k}}\right\} \\ &+ K_1 k_H \frac{pCO_2}{|H^+|}\left\{1 + 2\frac{K_2}{|H^+|}\right\} \end{aligned} \tag{12.25}$$

In a two-dimensional plot, only three variables can be put in relationship to one another. Therefore, in Eq. (12.25), three variables have to be chosen among $|H^+| = 10^{-pH}$, $|C_2O_4H^-| + |C_2O_4^{2-}|$, $|Ca^{2+}|$ and pCO_2. For example, curves can be drawn for a variety of pH and concentrations of $|Ca^{2+}|$. In this example, curves represent the function $pH = f(\log(|Ca^{2+}|), pCO_2$ (Fig. 12.7). The next step consists of calculating the saturation curves related to the two mineral species: calcium oxalate and calcium carbonate.

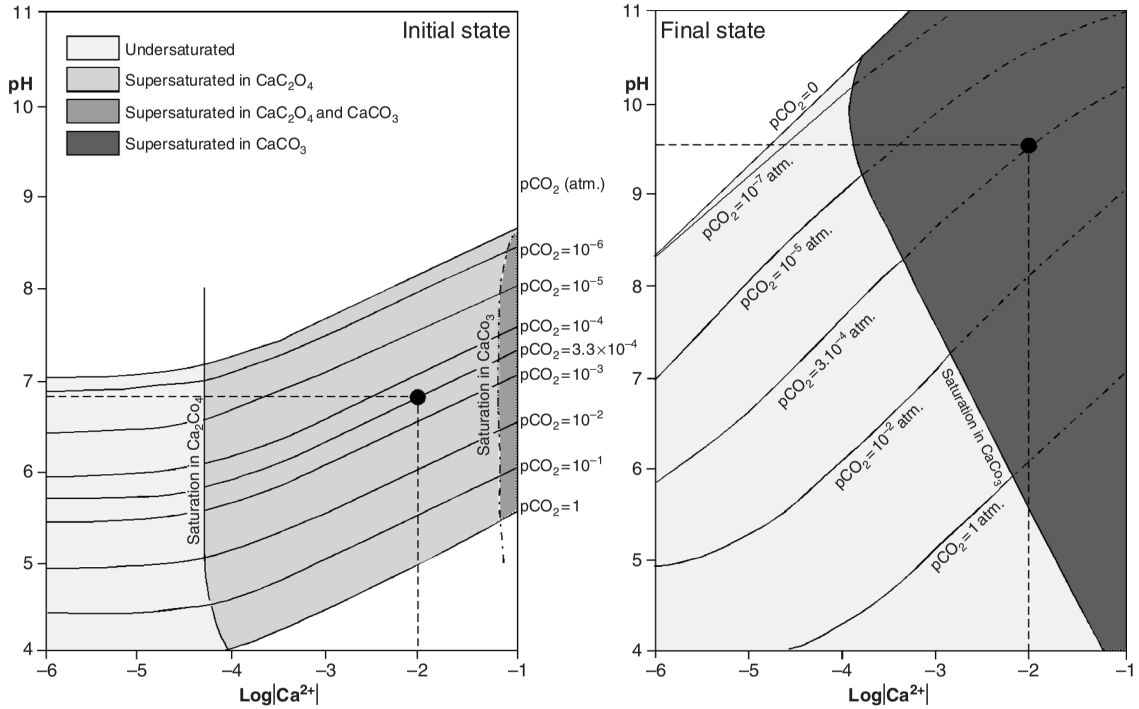

Fig. 12.7. Curves of the function $pH = f(\log|\ Ca^{2+}\ |, pCO_2)$ obtained using the model given in Fig. 12.6. Two states are described: the initial state, in which oxalate is present and begins to be oxidized by oxalotrophic bacteria, and the final state, showing the evolution of pH and calcium concentration after total disappearance of oxalate. The dotted lines refer to observed conditions. Measurements are in agreement with the model. All the oxalate (intersection inside the area related to oxalate supersaturation in the initial state) has been transformed into carbonate (intersection inside the area corresponding to calcite supersaturation in the final state) accompanied by an increase in pH compared with the initial state.

If we consider that the oxalate concentration is known: the saturation curve (i.e. solution in the presence of solid calcium oxalate) is represented by the following equation obtained by using Eqs. (12.25), (12.22) (the solubility product), (12.18) (the value of k) and the concentration in oxalate species ($|C_2O_4H^-| + |C_2O_4{}^{2-}|$):

$$2\left|Ca^{2+}\right| = -|H^+| + \frac{\Pi_2}{\left|Ca^{2+}\right|}\left(2 + \frac{|H^+|}{k}\right) + K_1 k_H \frac{pCO_2}{|H^+|}\left(1 + \frac{2K_2}{|H^+|}\right) + \frac{K_w}{|H^+|} \tag{12.26}$$

The pCO_2 values are given by the intersection of the saturation curve with the isobar. The result is given in Fig. 12.7 when the only source of calcium is considered to be the oxalates, i.e. $|Ca^{2+}| = |C_2O_4H^-| + |C_2O_4{}^{2-}|$. The same approach can be used to calculate the saturation curve regarding calcite. By combining Eqs. (12.19), (12.23) and (12.21), the following relationship is obtained:

$$\left|Ca^{2+}\right| = \frac{\Pi_1}{k_H K_1 K_2}.\frac{|H^+|^2}{pCO_2} \Rightarrow pH = -\frac{1}{2}\log\left(\left|Ca^{2+}\right|\right) - \frac{1}{2}\log(pCO_2) + 4.86 \tag{12.27}$$

Once again, the result given in Fig. 12.7 assumes that oxalates are the only source of calcium. To check this hypothesis, the model can be compared with an in vitro culture of oxalotrophic bacteria in the presence of calcium oxalate as the sole source of carbon, energy and calcium. Such an experiment has been conducted by Braissant *et al.* (2004). They show that, in a liquid medium, calcium oxalate consumption is followed by an increase in pH of the solution, accompanied by precipitation of calcium carbonate. During the experiment, the initial pH of the solution was 6.9. At the end of the experiment, i.e. after total consumption of oxalate, the final pH was 9.6. The concentration of $|Ca^{2+}|$ does not change during the experiment: it can be used as a probe during the transformation of calcium oxalate into calcium carbonate. The initial concentration of calcium is around 10^{-2} mol l^{-1}, i.e. $\log(|Ca^{2+}|) = -2$. Fig. 12.7 (initial state) shows that, for such a concentration of calcium in the presence of calcium oxalate crystals and at a $pCO_2 = 3.10^{-4}$ atm, the theoretical pH should be between 6.8 and 6.9, a range corroborated by the measurements. At the end of the experiment (Fig. 12.7, final state), there is no more oxalate. Calcium carbonate can precipitate because the intersection of the $pCO_2 = 3.10^{-4}$ atm curve and a concentration of calcium around 10^{-2} mol l^{-1} is situated

inside the area of supersaturation regarding $CaCO_3$. In addition, the pH should be between 9.5 and 9.6, which is also consistent with the experiment.

In conclusion, such a model is convenient to get an idea of the calcium oxalate concentration, CO_2 pressure and conditions for potential precipitation of secondary calcium carbonate through oxalotrophic bacterial activity. It demonstrates that as long as calcium is available and oxalotrophic bacteria are present, transformation of oxalate into carbonate can occur under normal conditions found in soils and surficial sediments. Therefore, an oxalate–carbonate cycle, or at least pathway, must exist at the surface of continents (Verrecchia & Dumont, 1996), explaining the absence of calcium oxalate accumulation in soils and the fossil record.

The oxalate–carbonate pathway

A synthetic coupled cycle of calcium and carbon through the oxalate–carbonate pathway is shown in Fig. 12.8. Atmospheric CO_2 is fixed by the plants through photosynthesis to produce biomass. Inside the plant, oxalate crystals form. In addition, fungal mycelium may also accumulate oxalate. Mainly in the form of calcium oxalate (COM or COD), this carbon pool is used by oxalotrophic bacteria as a carbon, energy and electron source. The transformation of oxalate can occur in the soil

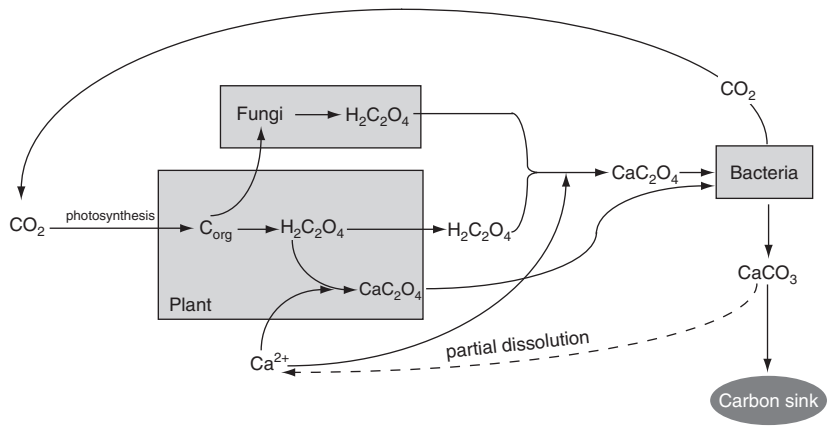

Fig. 12.8. Simplified sketch showing main relationships inside the coupled calcium and carbon cycles of the oxalate–carbonate pathway in a hypothetical ecosystem. Plants and fungi are oxalate producers. Oxalotrophic bacteria (in the soil or animal guts) use oxalate as carbon, energy and electron sources, leading to CO_2 and calcium carbonate production. Calcium carbonate can accumulate inside the soils. Because the carbon of the carbonate originates from organic carbon, its fossilization in the soil constitutes a carbon sink.

solution inside the pores, or in soil animal guts. The oxalate oxidation results in CO_2 production and calcium carbonate precipitation. Released CO_2 can be used for photosynthesis, forming a loop in the cycle. Calcium carbonate precipitation is enhanced by increasing the pH during oxalotrophy, as observed by Jayasuriya (1955) and Braissant *et al.* (2002) and demonstrated in this chapter (see p. 000). Now, if we suppose that all the oxalate available in the soil is oxidized in order to measure the potential occurrence of a carbon sink, the overall reactions can be summarized by the following two equations:

$$CaC_2O_4.nH_2O + \frac{1}{2}O_2 \rightarrow Ca^{2+} + 2CO_2 + (n-1)H_2O + 2OH^- \quad (12.28)$$

$$Ca^{2+} + 2OH^- + CO_2 \rightarrow CaCO_3 + H_2O \quad (12.29)$$

the first one describing the oxidation, the second taking advantage of alkalization to enhance calcium carbonate precipitation as stipulated above. Therefore, the final balance is the following:

$$CaC_2O_4.nH_2O + \frac{1}{2}O_2 \rightarrow CaCO_3 + CO_2 + (n)H_2O \quad (12.30)$$

The initial two moles of organic carbon are both transformed into inorganic carbon. One mole is precipitated as a mineral, with carbon being trapped inside the calcium carbonate crystals, i.e. stored on a long-term time scale. The other mole can be released into the atmosphere and reused for phototrophy. Therefore, the oxalate–carbonate pathway constitutes a true carbon sink because one out of two moles of organic carbon is stored in a mineral state with a long residence time, whereas the other one returns to the atmospheric reservoir.

Conclusions

Consequences of the oxalate–carbonate cycle have been investigated in different environments. In surficial formations, this cycle is believed to participate in the genesis of calcrete (indurated carbonate soils) in semi-arid environments (Verrecchia 1990; Verrecchia & Dumont 1996). In temperate forests, it is related to mineral nutrient cycles including calcium, and more generally, other elements such as iron, aluminium and phosphorus (Cromack *et al.* 1977). The possible oxidation of calcium oxalate pools by oxalotrophic bacteria probably means that a large amount of the secondary calcium carbonate found in soils and surficial sediments should originate in the transformation of low-molecular-weight organic acids (including citrate and acetate) into carbonates. Calcium

carbonate (as various $CaCO_3$ polymorphs) may then accumulate, modifying the soil conditions. Theoretical studies have demonstrated that soil solutions can be alkalinized enough to enhance calcium carbonate accumulation and storage. Therefore, when associated with plant biomineralization, fungi and bacteria contribute to long-term carbon storage by potentially transforming half of the organic carbon from oxalate (or low-molecular-weight organic acids) into mineral carbon (carbonate), which has a much longer residence time in soils than organic substrates (Cailleau *et al*., 2004). The other half is released as CO_2 into the atmosphere. In conclusion, because oxalate salts are organic in origin, the oxalate–carbonate pathway represents a potentially major carbon sink (Cailleau *et al*., 2005) and probably acts as a regulator of atmospheric pCO_2.

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