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Biologically induced mineralization in the tree *Milicia excelsa* (Moraceae): its causes and consequences to the environment

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ABSTRACT

Iroko trees (*Milicia excelsa*) in Ivory Coast and Cameroon are unusual because of their highly biomineralized tissues, which can virtually transform the trunk into stone. Oxalic acid ($C_2O_4H_2$) and metal-oxalate play important roles in their ecosystems. In this study, the various forms of oxalate and carbonate mineralization reactions are investigated by using scanning electron microscopy and X-ray diffraction. Calcium oxalate monohydrate is associated with stem, bark and root tissues, whereas calcium oxalate dihydrate is found with wood rot fungi in soils, as well as in decaying wood. Laboratory cultures show that many soil bacteria are able to oxidize calcium oxalate rapidly, resulting in an increase in solution pH. In terms of *M. excelsa*, these transformations lead to the precipitation of calcium carbonate, not only within the wood tissue, but also within the litter and soil. We calculate that *c.* 500 kg of inorganic carbon is accumulated inside an 80-year-old tree, and *c.* 1000 kg is associated with its surrounding soil. Crucially, the fixation of atmospheric CO_2 during tree photosynthesis, and its ultimate transformation into calcite, potentially represents a long-term carbon sink, because inorganic carbon has a longer residence time than organic carbon. Considering that calcium oxalate biosynthesis is widespread in the plant and fungal kingdoms, the biomineralization displayed by *M. excelsa* may be an extremely common phenomena.

INTRODUCTION

The African tree *Milicia excelsa* is a complex system, in which oxalic acid and metal oxalate play important roles. Calcium oxalate in plants has been reported to have many different functions. Oxalic acid is a low-molecular-weight organic acid, which is known to play a role in the regulation of free calcium ions in cytoplasm (Robert & Roland, 1989). In addition, calcium oxalate crystals in woody tissues are thought to render trees less palatable to termites and to act as a 'combustion-retarding agent' (Prior & Cutler, 1992). In leaves, calcium oxalate crystals provide physical protection against grazing animals. More recently, it was suggested that druse calcium oxalate crystals may help to distribute light evenly to the chloroplasts lining the radial walls and surrounding the vacuole in photosynthetic palisade cells of low-light-adapted plants (Franceschi, 2001). Calcium oxalate is particularly important because it is extremely common in plants and can represent as much as 85% of a plant's dry weight (Robert & Roland, 1989).

No specific role has previously been proposed for oxalate in *M. excelsa*, because until now it has never been detected. Although its function remains unknown, metal-oxalate accumulation in *M. excelsa* must have important biogeochemical implications because oxalate minerals constitute a significant carbon source for oxalotrophic bacteria, which are numerous in both the tree and the surrounding litter. Moreover, wood-degrading fungi also influence the carbon (and some metal cation) cycles as a result of their production of large amounts of oxalic acid and other organic acids during the decay of cellulose and lignin (Cochrane, 1958; Dutton & Evans, 1996; Gadd, 1999).

The aim of this study is to investigate the role of bacteria and fungi in carbonate biomineralization within *M. excelsa*. Biomineralization (or mineralization) is defined in this study as the processes leading to inorganic mineral deposits

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by living matter. In *M. excelsa*, we are particularly concerned with how the biogeochemical cycles of carbon, oxalate and calcium lead to the storage of inorganic carbon in the tree. The consequences of the transformation of oxalate into carbonate have already been studied with regard to soil fauna in temperate forests (Cromack *et al.*, 1977) and in Mediterranean to semi-arid calcrete environments (Verrecchia, 1990; Verrecchia & Dumont, 1996). Until now, however, no studies have been conducted in either a tropical environment or on the consequences of such processes in terms of carbon cycling and storage.

Discussions regarding the carbon sink in forests are predominantly focused on organic carbon storage, whereas soil mineral carbon is generally neglected. However, owing to the longer residence time of carbonate minerals in soils (10^2–10^6 years compared with 10–10^3 years for soil organic matter), storage of large quantities represents a potentially more efficient sink. *Milicia excelsa* is an excellent example of an ecosystem acting as a net carbon sink because carbonate accumulation involves only atmospheric CO_2 and Ca^{2+} from Ca-carbonate-free sources, i.e. calcium is not provided by the dissolution of primary (inherited) $CaCO_3$. Considering the global distribution and the huge number of plants able to concentrate oxalate salts (Horner & Wagner, 1995), this potential source of carbon trapping should be clearly identified.

GEOLOGICAL SETTINGS

Samples were collected in Cameroon and Ivory Coast from trees having about the same diameter at a height of 1.20 m from the ground. In Cameroon, two sites were investigated: the first is situated 10 km west of Lolodorf (10°47′E, 3°13′N) and the second is 15 km north of Yaounde, in the Leboudy region (11°30′ E, 4°07′ N). At the first site, an evergreen forest grows on a yellowish brown lateritic (i.e. ferralitic) soil >3 m thick developed on a granitic bedrock (Tematio *et al.*, 2001). At the second site, the granite bedrock is overlain by a thin ferralitic soil (<1 m), covered by a semideciduous tropical forest. The climate at both Cameroon sites is equatorial with two wet seasons (April–July and September–December), a total precipitation 3000–5000 mm yr^{-1} and an annual mean temperature of around 25 °C.

In Ivory Coast, the study area is near the village of Biga (7°32′ E, 6°36′ N), 30 km south from Daloa. This site is located on a reddish brown lateritic soil (i.e. a moderately desaturated ferralitic soil with 1–3 mEq/100 g of exchangeable bases and a mean pH of 5.5) developed on granitic parent material. This site was investigated for the first time by Carozzi (1967), who described a carbonate-cemented sandstone formed inside the lateritic soil and associated with *M. excelsa*. The vegetation at this site is a semideciduous tropical forest bordering a cleared forest (Ministère du plan, Côte d'Ivoire, 1979). The climate at the Ivory Coast site is tropical with two wet seasons (March–July and September–November), with a total precipitation of about 2500 mm yr^{-1}. The mean annual temperature in Ivory Coast is 26.1 °C. *M. excelsa* is commonly logged in Ivorian forests.

MATERIALS AND METHODS

Milicia excelsa wood samples were tested for the presence of carbonate with 1 N hydrochloric acid. Wood, fractured bark and root samples were fixed with osmium tetroxide vapour and freeze-dried. Some of the samples were gold-coated, and then examined using Phillips XL20 and XL30 scanning electron microscopes with a 10 kV accelerating voltage to observe crystals formed in tissues and associated with wood-degrading fungi. Other parts of the samples were embedded in resin and prepared as thin sections (30 µm), and the remaining part of bark samples was crushed and analysed by X-ray diffraction (XRD) with a Scintag diffractometer.

The occurrence of oxalate-degrading bacteria in soils was monitored using soil inoculum in a 15 mL DSM 81 medium ($Na_2HPO_4{\cdot}12H_2O$ 9.0 g L^{-1}, KH_2PO_4 1.5 g L^{-1}, NH_4Cl 1.0 g L^{-1}, $MgSO_4{\cdot}7H_2O$ 0.2 g L^{-1}, ferric ammoniacal citrate 0.005 g L^{-1}, $CaCl_2$ 0.01 g L^{-1}, $ZnSO_4{\cdot}7H_2O$ 50 µg L^{-1}, $MnCl_2{\cdot}4H_2O$ 15 µg L^{-1}, H_3BO_3 150 µg L^{-1}, $CoCl_2{\cdot}6H_2O$ 100 µg L^{-1}, $CuCl_2{\cdot}2H_2O$ 50 µg L^{-1}, $NiCl_2{\cdot}6H_2O$ 10 µg L^{-1}, $NaMoO_4{\cdot}2H_2O$ 15 µg L^{-1}) also containing 4.0 g L^{-1} potassium oxalate. After the medium turned cloudy, 500 µL of the enriched culture was transferred to 15 mL of the same fresh medium. After the second enrichment, 10 µL of the culture was plated onto a solid DSM 81 medium with a sterile plastic inoculating loop (Sigma, Buchs, Switzerland). Calcium oxalate was added to this medium. The oxalate consumption by the isolated strains was controlled by observing the calcium–oxalate 4 g L^{-1} dissolution on the DSM 81 medium as described by Tamer & Aragno (1980).

In order to monitor oxalate consumption in soils associated with *M. excelsa*, two common oxalate-degrading bacteria, *Ralstonia eutropha* (DSM 428, ATCC 17699) and *Xanthobacter autotrophicus* (DSM 432, ATCC 35674; Tamer & Aragno, 1980), were inoculated into soil extract media. One to one ratio soil extracts (1 kg soil per 1 L of water) were prepared according to the method described by Parraga *et al.* (1998). Major anions and cations of this extract were analysed by liquid chromatography using a Dionex chromatograph with a conductivity detector. Columns used for anions and cations were Dionex IonPac™ AS11-HC (4 × 250 mm) and Dionex IonPac™ CS12A (4 × 250 mm), respectively. Ionic composition of the soil extract was as follows: Na^+ 13 mg L^{-1}, NH_4^+ 6 mg L^{-1}, K^+ 23 mg L^{-1}, Mg^{2+} 10 mg L^{-1}, Ca^{2+} 45 mg L^{-1}, Cl^- 18 mg L^{-1}, NO_2^- 3 mg L^{-1}, NO_3^- 150 mg L^{-1}, SO_4^{2-} 32 mg L^{-1}, and PO_4^{3-} 21 mg L^{-1}. The first medium was obtained by adding 15 g L^{-1} agar to these extracts. A second type of medium was made by adding 4 g L^{-1} calcium oxalate. Crystals produced on the different media were collected with tweezers, gold coated and observed with a Phillips XL30 SEM.

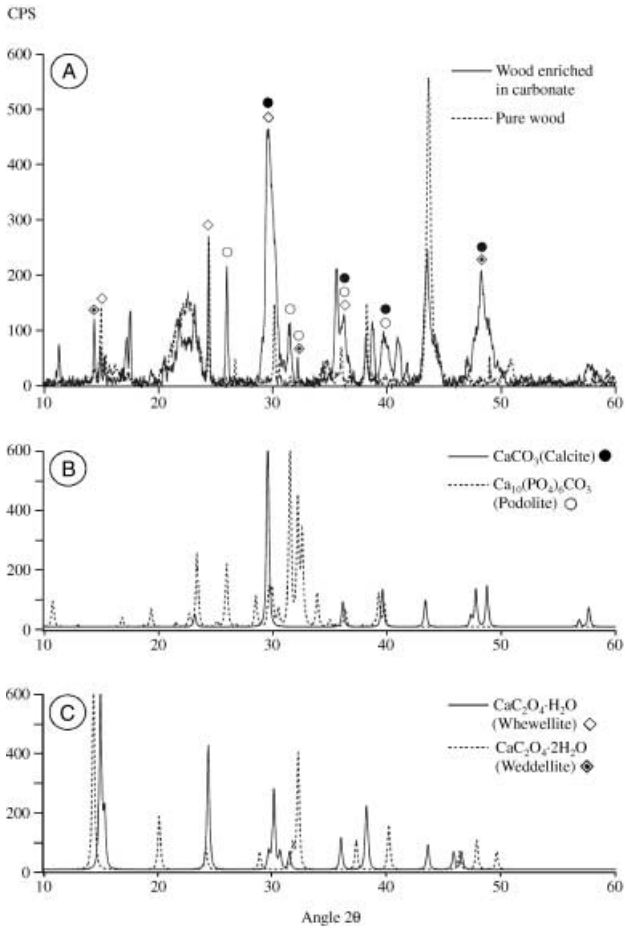

Fig. 1 X-ray diffractograms. A: wood samples enriched in carbonate and pure wood (symbols indicate characteristic peaks). B: reference calcite and calcium phosphate carbonate. C: reference calcium oxalate monohydrate and calcium oxalate dihydrate. Diffractogram A shows the strong differences between pure and carbonate-enriched wood.

RESULTS

X-ray diffraction analysis of *M. excelsa* wood and bark revealed the presence of calcium oxalate monohydrate [$CaC_2O_4 \cdot H_2O$: whewellite], calcium oxalate dihydrate [$CaC_2O_4 \cdot 2H_2O$: weddellite], calcium carbonate [$CaCO_3$], calcium phosphate carbonate [$Ca_{10}(PO_4)_6CO_3$: podolite] and amorphous silica (Fig. 1). Scanning electron microscopy (SEM) showed the presence of euhedral whewellite crystals in the bark, as confirmed by X-ray analysis. These crystals, up to 50 µm in size, seem to be principally located near or in the conductive vessels of the wood (Fig. 2A,B). At the Ivory Coast site, large amounts of whewellite were also found forming a crust around *M. excelsa* roots (Fig. 2C,D).

On decayed or rotting wood, SEM indicated the presence of weddellite associated with the hyphae of some of the wood rot fungi (Fig. 2E,F). Monohydrate oxalate crystals were observed in stem tissues in both the Cameroon and Ivory Coast samples, whereas large amounts of monohydrate oxalate associated with roots were only found in Ivory Coast *M. excelsa*. These roots do not form rhizoconcretions, but instead they are former living mineralized tissues that are found under the outer part of the root cuticle (Fig. 2C). In the latter case, it seems likely that whewellite is mainly associated with the living plant tissues, whereas weddellite is mainly related to decaying wood resulting from fungal activity. This is consistent with observations by Arnott (1995), who noted weddellite associated with wood rot fungi.

In both living and decaying wood, a large part of carbonate mineralized tissues is easily visible when thin sections of *M. excelsa* wood are observed by optical microscopy under polarized light (Fig. 3A,B). Moreover, anhedral to subhedral calcite crystals were observed filling conductive tissues using SEM (Fig. 3C,D). In addition, calcite rhombohedra and micritic aggregates were found in sap. Some of these micritic aggregates form spheres (Fig. 3E). Other carbonate features are found in sap and in decaying wood in the form of calcified tissues, including calcified cellulose fibres (Fig. 3F) and calcified cells (Fig. 3G), in which a calcified spheroid can be seen in the vacuole ghost (Fig. 3H). There is a striking difference between the Cameroon and Ivory Coast samples. In the former only traces of carbonate are found, whereas in the latter large parts of wood are replaced by carbonate.

More than 20 different morphotypes of oxalate-degrading bacteria were found in the soils surrounding *M. excelsa* (Fig. 2B). Some of these were identified as *Streptomyces* and other Actinomycetes, Pseudomonads and putative *Variovorax* (Sahin, 2003). All isolated strains quickly dissolved calcium-oxalate on the DSM 81 medium, and the resulting precipitation of calcium-phosphate-carbonate was identified by XRD, optical and electron microscopy, coupled with energy-dispersive X-ray (EDAX) analysis (Fig. 4A,B; Braissant *et al.*, 2002). For example, a rapid consumption of oxalate has been observed during the growth of a *R. eutropha* culture in a DSM 81 medium. After 48 h, oxalate is no longer present in the medium (Fig. 5). In addition, rapid dissolution of calcium oxalate was observed on a soil-extract medium to which calcium oxalate was added. On a 1 : 1 soil extract (with or without the addition of calcium oxalate) *R. eutropha* and *X. autotrophicus* show normal growth, demonstrating that an oxalate molecule is preferred to other carbon sources present in the soil extract. As the consumption of calcium oxalate by oxalotrophic bacteria progresses, the pH of the medium increases to a point at which calcium carbonate deposition is favourable. Spheroid micritic aggregates of calcite were also found in soil extract media associated with gypsum, showing that calcium oxalate associated with *M. excelsa* is rapidly consumed and transformed into carbonate (Fig. 4C,D). The precipitation of one or the other of the two minerals, calcite or podolite (a calcium-phosphate-carbonate), is mainly related to the concentration of phosphate in the different media (Stumm & Morgan, 1981). In DSM 81, phosphate is abundant because it is used as a buffer. Finally, one fungal strain was

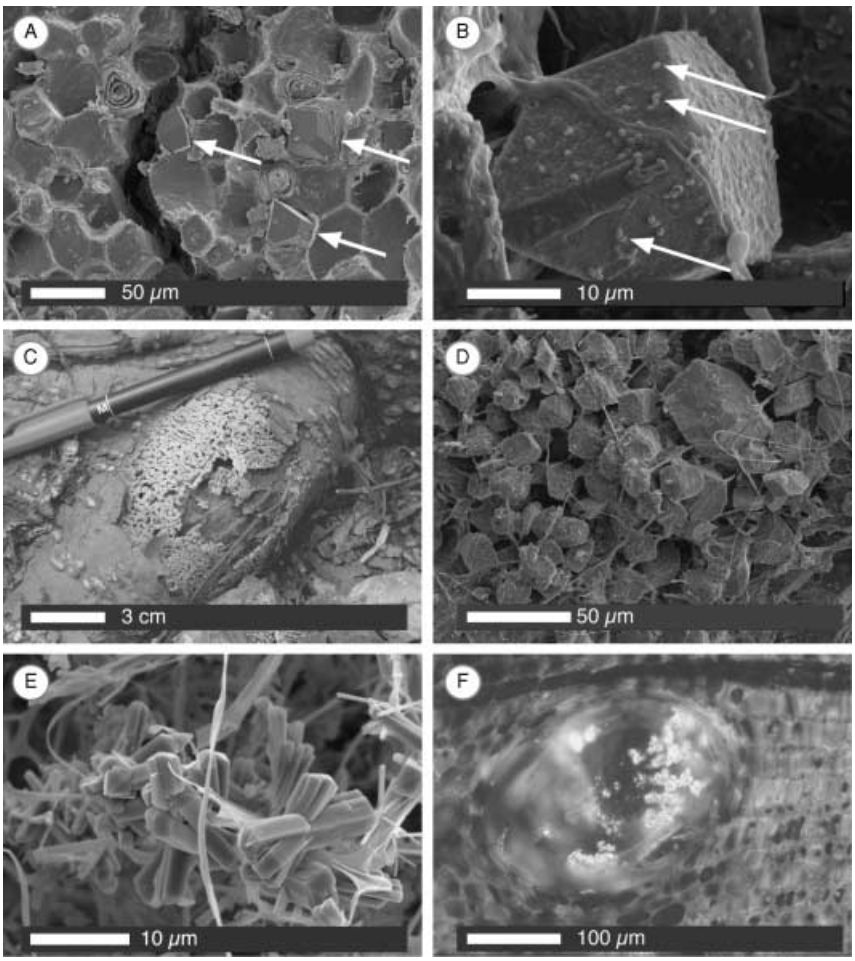

Fig. 2 SEM images of calcium oxalate crystals associated with *M. excelsa*. A: view of fractured bark showing calcium oxalate monohydrate encased in the wood tissues (arrows). B: bacteria (arrows) on a calcium oxalate monohydrate crystal. C: *M. excelsa* root encrusted with calcium oxalate monohydrate. D: details of C showing the whewellite crystals encrusting *M. excelsa* roots. E: weddellite associated with fungal hyphae on decayed wood. F: weddellite associated with fungi in *M. excelsa* conductive tissues.

co-isolated with bacteria on the DSM 81 medium. This strain is able to grow on oxalate as the sole carbon source, resulting in the dissolution of calcium oxalate and production of a mixed calcium-phosphate-carbonate phase.

DISCUSSION

The presence of whewellite in the *M. excelsa* tissues, as well as weddellite associated with fungi, correlates well with previous studies that have documented oxalate biosynthesis and occurrence in plants and fungi (Pobeguin, 1943; Franceschi & Horner, 1980; Robert & Roland, 1989; Khan, 1995; Dutton & Evans, 1996; Gadd, 1999). In *M. excelsa*, whewellite is often found in conductive tissues and is able to encrust roots. It is likely that this form of calcium oxalate is a result of the mineral nutrition of *M. excelsa* (see Jones, 1998, for a review) or in aluminium detoxification, as suggested for bacteria by Hamel *et al.* (1999). The presence of whewellite and amorphous silica in *M. excelsa* bark could also be related to termite protection, as suggested for acacia trees (Prior & Cutler, 1992).

Weddellite is commonly associated with fungi in decaying wood. The role of oxalic acid in fungal pathogenicity is well documented (see Dutton & Evans, 1996, for a review). However, wood rot fungi, which are not typically associated with calcium oxalate minerals, do appear to play a role in the oxalate cycle. During their consumption of cellulose, they probably help to release euhedral whewellite crystals encased in the wood tissues.

Both whewellite and weddellite are abundantly consumed by bacteria (see Eqs 1 and 2, modified from Harder *et al.*, 1974) and other micro-organisms because no macroscopic accumulation of calcium oxalate was observed in the soil and litter surrounding *M. excelsa*:

$$CaC_2O_4 + nH_2O \rightarrow C_2O_4^{2-} + Ca^{2+} + nH_2O \rightarrow C_2H_2O_4 + Ca^{2+} + (n-2)H_2O + 2OH^- \quad (1)$$

$$1000C_2H_2O_4 + 372O_2 + 32NH_4^+ \rightarrow 32C_4H_8O_2N^+ + 1872CO_2 + 936H_2O. \quad (2)$$

This is consistent with the rapid organic acid consumption observed for malate by Jones *et al.* (1996). Regarding *M. excelsa*, the results of bacterial growth on soil extract media indicate that such consumption is also true for oxalate. There are two

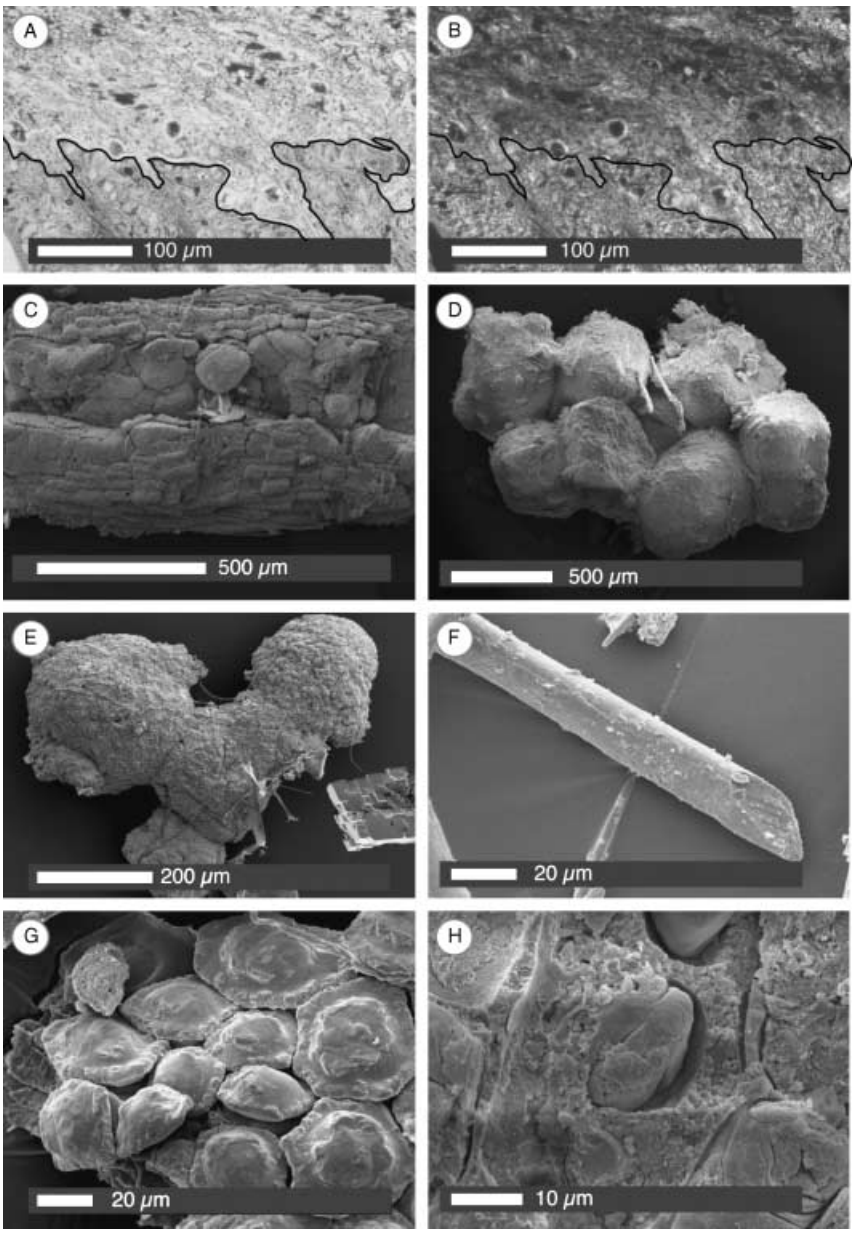

Fig. 3 Calcium carbonate crystals and carbonate-enriched tissues found in mineralized *M. excelsa*. A,B: thin section of *M. excelsa* woody tissues observed in plane (A) and cross-polarized light (B) showing large sections of carbonate mineral precipitation. Above black line, calcified woody tissues; below black line, non-mineralized woody tissues. C: SEM view of a conductive vessel infilled with anhedral to subhedral calcium carbonate crystals. D: SEM view of calcium carbonate crystals found in decaying wood. E: SEM view of calcium carbonate as micritic aggregates forming spheres and rhombohedron found in *M. excelsa* sap. F: SEM view of a cellulose fibre entirely replaced by calcium carbonate. G: SEM view of cells totally replaced carbonate. H: SEM view of carbonate-enriched tissues showing ghosts of the cell vacuole infilled by calcium carbonate and carbonate-mineralized cell walls.

principal consequences of oxalate consumption. First, metal ions are released in soils and are probably more available for other organisms, and particularly for plant and soil animals as proposed by Cromack *et al.* (1977). Second, there is a pH increase owing to the transformation of oxalate into bicarbonate, because oxalic acid (pk1 = 1.25, pk2 = 4.27) is 'stronger' than carbonic acid (pk1 = 6.35, pk2 = 10.33). This pH increase provides the required conditions for carbonate precipitation in *M. excelsa* environments (Eq. 3, with degassing of carbon dioxide):

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + H_2O + CO_2. \quad (3)$$

During bacterial growth on DSM 81 and soil extract media, biologically induced changes in chemical conditions also resulted in the precipitation of calcium-phosphate-carbonate (Braissant *et al.*, 2002), along with calcite. Therefore these experimental results seem to explain the relationship between the oxalotrophic bacteria and these minerals found in the natural environment (Fig. 1A). They might also help to explain some of the contributory factors leading to the precipitation of calcite-cemented sandstones that are observed in the tropical forested ecosytems of Ivory Coast (Carozzi, 1967; Cailleau *et al.*). Only gypsum has neither been detected in the tree nor in the soil, although it has been precipitated in laboratory experiments; the tropical conditions probably result in its dissolution.

Based on our results, a model for the calcification of *M. excelsa* is as follows (Fig. 6):

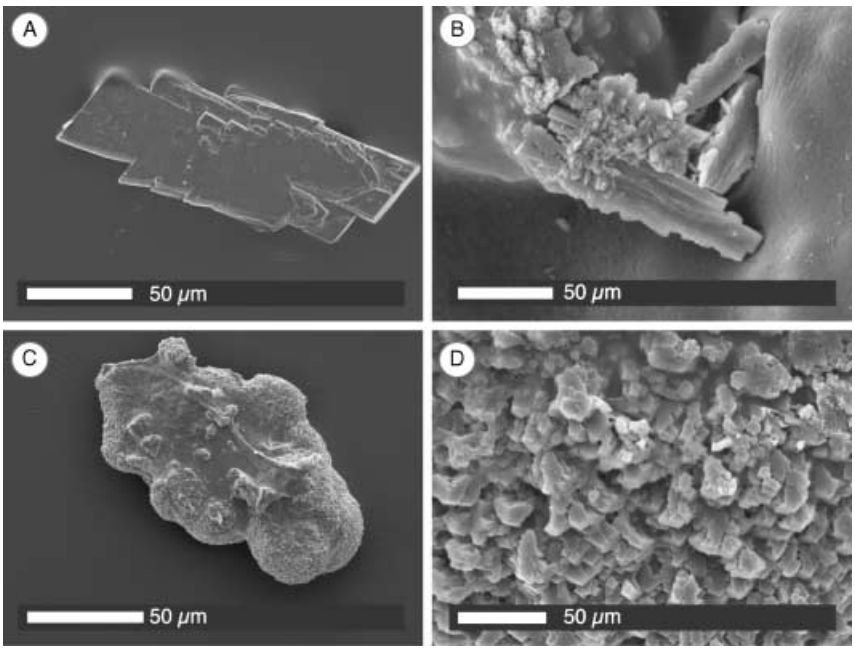

Fig. 4 SEM view of crystals resulting from calcium oxalate consumption by bacteria on Petri dishes. A: calcium-phosphate-carbonate crystal produced on DSM 81 medium. B: calcium-phosphate-carbonate crystal produced on soil extract media containing calcium oxalate. C: calcite micritic aggregates forming spherulites produced on soil extract media containing calcium oxalate. D: detail of a micritic aggregate showing the calcitic habit of individual crystals.

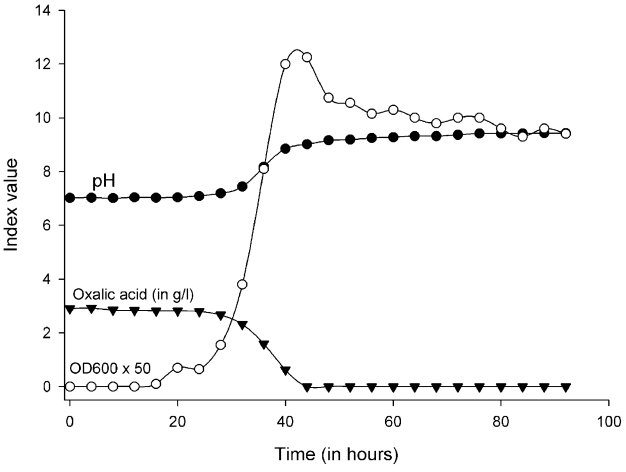

Fig. 5 Evolution of index values (cell density measured turbidimetrically at 600 nm, pH and oxalate concentration in g L^{-1}) during the culture of *R. eutropha* in a DSM 81 liquid medium.

1 Whewellite forms in the tree wood and bark because *M. excelsa* produces large amounts of oxalic acid as a metabolic product, which reacts with calcium ions (see 1 in Fig. 6).

2 Weddellite forms in rotting wood because, during cellulose and lignin degradation, oxalic acid is produced in large amounts by fungi. Weddellite formation results from calcium-ion chelation from the plant cell wall (Dutton & Evans, 1996; see 2 in Fig. 6).

3 Oxalotrophic bacteria present in the soil and the tree tissues (introduced through wounds) transform oxalates (Ca-oxalate and oxalic acid) into carbonate (see 3 in Fig. 6).

4 Other minerals, such as gypsum and podolite, also form as a result of local pH changes and carbonate ion production (see 4 in Fig. 6).

5 Calcite produced in the soil by bacteria (see 3 in Fig. 6) can be partially dissolved by rain (pH = 4.7). Bicarbonate ions can be pumped by the tree leading to $CaCO_3$ deposition, even in undersaturated conditions as described by Wollast (1971; see 5 in Fig. 6).

The differences in carbonatation between Cameroon and Ivory Coast samples are probably due to differences in hydrological conditions. It is possible that carbonate ions produced in Cameroon are leached and diluted as a result of the higher rainfall. Therefore, the accumulation of calcium carbonate inside the soil, as well as in the tree, is less likely to occur. However, all the processes described in Fig. 6 result in the accumulation of calcium carbonate in alkalinized soils associated with *M. excelsa* in Ivory Coast. In summary, calcium carbonate is derived from the oxidation of carbon originally fixed during tree photosynthesis from atmospheric CO_2 through an oxalate step. This may constitute an important potential carbon sink.

During the last century, deforestation in Ivory Coast has led to the disappearance of 12.5 million hectares of the original rainforest. *Milicia excelsa* has been extensively logged and now it only remains in protected areas (Braissant *et al.*, 2003). Nevertheless, calculations using soil and tree carbonate titrations suggest that at least 1500 kg of $C_{mineral}$ is trapped during the life of a single *M. excelsa* tree (*c.* 500 kg inside the tree and *c.* 1000 kg in surrounding soils). This value is equivalent to a sequestration of 18.25 kgC yr^{-1}.

Typically, the forestry carbon sequestration component of the global carbon cycle is linked to soil organic matter, not to soil mineral carbon (Prentice *et al.*, 2001). However, as we

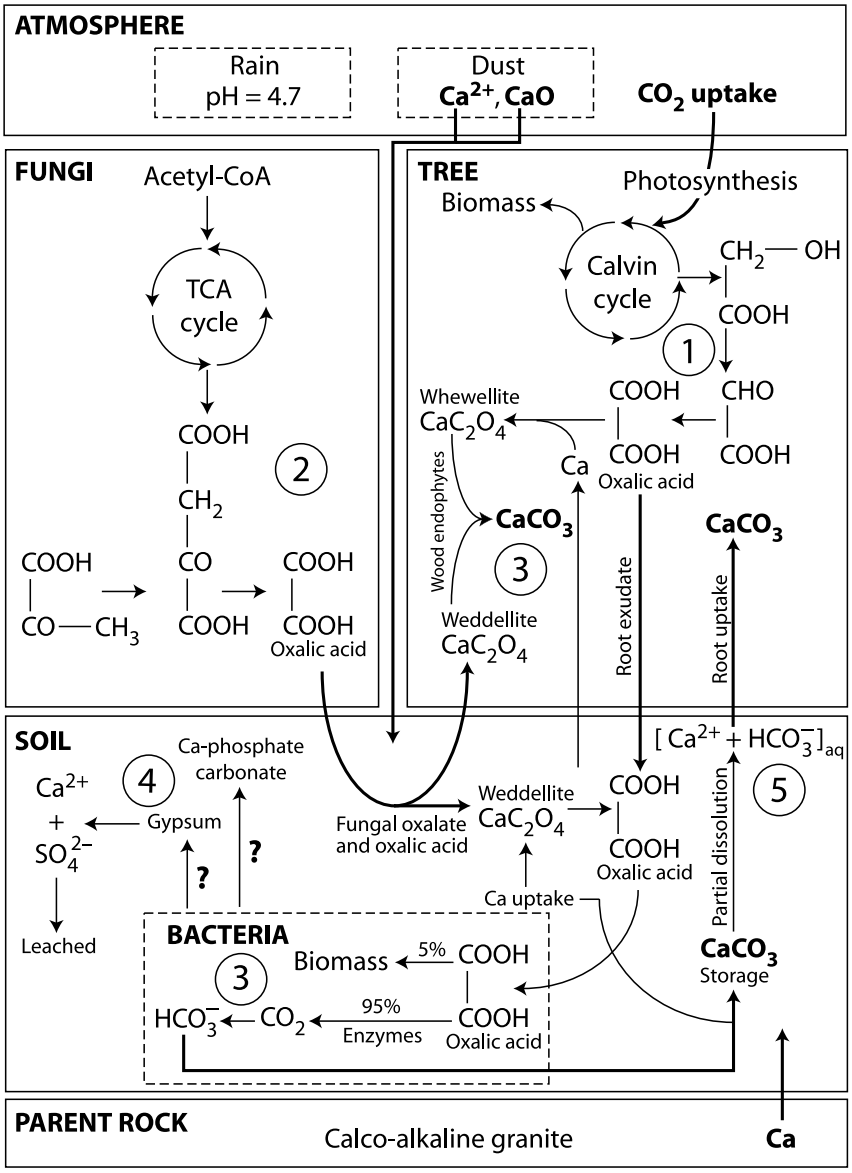

Fig. 6 Sketch showing possible mechanisms leading to the precipitation of carbonate in *M. excelsa* tissues; calcium ions are mainly provided by rain and dust and (to a much lesser extent) by granite. (1) Whewellite is produced inside the tree through atmospheric CO_2 uptake and the Calvin cycle. (2) Fungi also produce large amounts of weddellite through different pathways [including the tricarboxylic acid (TCA) cycle]. (3) Oxalic acid and both forms of calcium oxalate found in the tree and its litter can be transformed into calcite by the action of oxalate-degrading bacteria. Wood pathogens (fungi and bacteria) are able to release whewellite crystals, generating a pool of free oxalate crystals. Oxalate-degrading bacteria enter the tree through wounds, and also transform oxalate into carbonate inside the tree itself. In the *M. excelsa* rhizosphere and litter, oxalate-degrading bacteria can use fungal Ca-oxalate and oxalic acid excreted by the tree roots. This biological process leads to the formation of calcite concretions inside the wood and calcite accumulation in the *M. excelsa* rhizosphere. (4) Gypsum and calcium-phosphate-carbonate are produced as a result of a local pH increase and bicarbonate ion concentration. Gypsum is not stable in the *M. excelsa* environment and is easily leached. However, calcium-phosphate-carbonate is found in the soil but at lower concentration than calcite. (5) The acidity of the rain (pH = 4.7) allows some of the calcium carbonate formed in the soil to be dissolved. Bicarbonate ions may be reabsorbed by the tree during water absorption. These anions reprecipitate in the tree even from an undersaturated solution because of strong capillary action. This process probably leads to secondary calcium carbonate deposition inside the tree through vessels.

have shown, the abundance of carbonate minerals within *M. excelsa* testifies to the fact that the inorganic sink is of great significance to the terrestrial carbon cycle. As mentioned above, the sustainability of a carbon pool is also an important point: time residence for organic matter and mineral carbon in soil are at least 10^1–10^3 years and 10^2–10^6 years, respectively (Retallack, 1990; Prentice *et al.*, 2001). Biologically induced mineral carbon sinks are undoubtedly more efficient for carbon sequestration than soil organic matter.

CONCLUSIONS

Milicia excelsa trees in Ivory Coast and Cameroon are unusual because of their highly biomineralized roots and trunk. Moreover, around these trees, calcium carbonate accumulations occur in acidic ferralitic soils, making these soils locally alkaline. This change in pedochemical conditions is made possible by the presence of oxalate in the soil and tree, which creates favourable conditions for $CaCO_3$ precipitation. At least three sources of oxalate contribute to the budget: the *M. excelsa* tree, fungi and bacteria. The soil microflora includes bacteria that oxidize oxalate into carbonate, increasing the pH, and enhancing calcium carbonate accumulation. It seems that a period of low rainfall is necessary to allow $CaCO_3$ accumulation in the upper soil. To summarize, three conditions are necessary for calcium carbonate accumulations in ferralitic soils: (i) a large amount of oxalate, (ii) appropriate bacteria for oxalate oxidation into carbonate and (iii) a dry season. These conditions exist in many areas of tropical Africa. Consequently, carbon storage as inert calcium carbonate in soils from atmospheric CO_2 through a Ca-oxalate step constitutes a newly identified and neglected carbon sink.

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