Calcium biominerals in typical Argiudolls from the Pampean Plain, Argentina: An approach to the understanding of their role within the calcium biogeochemical cycle

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Abstract

The aims of this work are: (a) to describe the presence of calcium oxalate crystals from fungus and vegetal origin; (b) to determine the contribution from leaves; and (c) to assess the role they could play within the calcium biogeochemical cycle, in typical Argiudolls with different vegetal cover in the Pampean Plain, Argentina. Two plots with different vegetation were investigated: *Acacia melanoxylon–Celtis tala* and *Eucalyptus globulus–Celtis tala*. Leaves of the vegetation species were diaphanized and cleared with sodium hypochlorite (50%). Leaves and mycelial mats of the organic horizons were analyzed by optical, petrographic and scanning electronic microscopy. Soil samples were taken to determine particle size distribution and mineralogy, and to test calcium concentration by UV–vis spectrophotometry both in the soils' saturated paste extract and in leached soil. Different morphologies of weddellite (i.e., primary nuclei, rosettes and druses) in the fungus hyphae were observed. Calcium oxalate crystal production was highest in *A. melanoxylon* and *C. tala*. Ca–Na feldspars are the only minerals that could release calcium to the system, but show no features of weathering. Calcium concentration in soil solution was higher in the Eucalyptus plot than in the Acacia, and it decreased from the organic to the mineral soil horizons. Fungus and vegetal calcium biomineral production, in addition to their higher susceptibility to weathering than minerals of inorganic origin, represents an important contribution to the calcium biogeochemical cycle.
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1. Introduction

The process of biomineralization is a widespread phenomenon in nature. Biominerals are mineral structures of biogenic nature generated starting from the metabolic activity of different organisms. Since biomineralizing organisms are distributed over the globe, the process of biomineralization can be considered as a terrestrial process which acts as a global soluble ions source and sink (Jahren, 1996). Within biominerals, calcium oxalate crystals are very common, and occur in two forms: monohydrated ($CaC_2O_4 \cdot H_2O$, whewellite) and polyhydrated ($CaC_2O_4 \cdot (2+X)H_2O$, weddellite).

Calcium oxalate crystals have been observed in rocks, soils and among multiple members of all five kingdoms (Monera, Protista, Fungi, Plantae and Animalia) (Nakata, 2003). In all cases, the crystals are formed from environmentally derived calcium and from biologically synthesized oxalate (Graustein et al., 1977; Cromack et al., 1979; Ilarslan et al., 1997; Nakata, 2003).

The presence of calcium oxalates in the Fungi kingdom was first pointed out by Schmidt in 1847 (Horner et al., 1983). From then, numerous studies describing the presence of these biominerals in reproductive structures and in vegetative hyphae of different fungus groups were performed.

The fungus biomineralization process can occur intra- and/or extracellularly (Franceschi and Horner, 1980;

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Connolly and Jellison, 1995; Oyarbide et al., 2001). The first form is associated with the metabolism of vegetative growth and degrading of the organic resource, while the second one is related to the activities of mycorrhizal species (Verrecchia et al., 1993).

The importance of calcium oxalate crystals for members of the Fungi kingdom is the conservation and reutilization of calcium while the organism grows through the substrate (Connolly and Jellison, 1995). These crystals, in their turn, allow the detoxification of calcium, which can be highly toxic within cells (Tait et al., 1999), since free calcium is disruptive to enzymatic activity, which is necessary for the degradation and assimilation of organic substances (Verrecchia, 2000). Furthermore, it is thought that the formation of insoluble oxalates containing other potentially toxic metal cations also represents an important mechanism of metal immobilization with implications for tolerance (Murphy and Levy, 1983; Sutter et al., 1984; Sayer and Gadd, 1997 in Tait et al., 1999).

Calcium oxalate deposition is also common in Plantae. These biominerals have been found in members of more than 215 plant families, and they occur in different parts of the plant: flowers, leaves, stems, roots and seeds (Molano-Flores, 2001; Nakata, 2003). In whatever tissue the crystals are found, they most often accumulate within the vacuoles of specialized cells called crystal idioblasts (Nakata, 2003), as well as in the cytoplasm of the parenchymatous cells (Jones and Bryant, 1992).

Calcium oxalate crystals are important for normal plant growth and development, since (1) they allow the accumulation of calcium excess, so they represent storage forms of calcium and oxalic acid which can be resorbed under conditions of calcium depletion; (2) they represent depositories for metabolic wastes which would otherwise be toxic to the cell; (3) they act in defense against herbivores (Franceschi and Horner, 1980; Prychid and Rudall, 1999; Molano-Flores, 2001; Nakata, 2002, 2003); (4) in tissue rigidity and support; and (5) in light gathering and reflection (Franceschi and Horner, 1980).

Oxalate concentrations in soils can range from 10^{-6} to 10^{-3} M (Allison et al., 1995 in Tait et al., 1999). Such production of oxalic acid can increase the rate of soil weathering, enhancing the availability of nutrients (Cromack et al., 1979 in Tait et al., 1999). The presence of calcium oxalate crystals, both from vegetal and fungal origin, allows the calcium removal from excessively Ca-enriched environments (Verrecchia, 2000), as well as promotes soil heterogeneity by increasing biologically available phosphorus and potassium, and detoxifying aluminum (Graustein et al., 1977; Connolly and Jellison, 1995; Tait et al., 1999).

Typical Argiudolls are the representative soils of the southeastern Pampas region. They are very important from an economic point of view, due to their high productivity and the intense agricultural and horticultural activity which is usually developed in the study area. This fact has also generated a noticeable decrease in the content of organic matter and clay fraction in the arable layer of the A horizon and the corresponding loss of structural stability and fertility (Osterrieth and Maggi, 1996; Osterrieth et al., 1998, 2001).

Keeping in mind that calcium is one of the determinative elements in the organic–mineral complexes formation necessary for soil structure recovery, the study of calcium biominerals could contribute to the understanding of soil structure weathering and evolution.

The aims of this study are (a) to describe the presence of calcium oxalate crystals from fungal and vegetal origin; (b) to determine calcium oxalate crystals' contribution starting from leaves of vegetal species; and (c) to realize an approach to the role they could play within the calcium biogeochemical cycle, in typical Argiudolls with different vegetal cover in the Pampean Plain, Argentina.

2. Materials and methods

2.1. Study site

The site under study is located in the Partido of General Pueyrredón, around Los Padres Lake, Province of Buenos Aires (38°S–58°W) (Fig. 1). The climate is mesothermic and sub-humid, with little or no water deficiency, including an annual precipitation of 809 mm. The annual average temperature is 13.7 °C, with minimum mean values (8.1 °C) in June and maximum ones (19.8 °C) in January (Mar del Plata National Meteorological Service, according to the 1920–1980 record). In agreement with the standards set by Soil Survey Staff (1996), the soil temperature is of the mesic type, and the regime of humidity is udic.

The Los Padres Lake site belongs to the geomorphological unit known as "Perinange-eolian hills", which comprises a relief of morphologically complex hills, with relative heights of up to 30 m and concave–convex profiles with intermediate straight patches and slopes between 6% and 8% (Osterrieth et al., 1998). It originated from processes of primary eolian accumulation that was modified later on by superficial wash (Osterrieth and Martínez, 1993) (Fig. 1).

The morphology of the terrain, together with the dense vegetal cover in the natural field, makes sheet runoff a predominant feature. The good permeability of the loessian sediments allows infiltration of the precipitation (Osterrieth and Maggi, 1996).

In the southeast of the Pampean Plain, predominant soils are typical Argiudolls. They originated from eolian loessian sediments linked to the latest arid cycle of the late Pleistocene–Holocene (Osterrieth and Cionchi, 1985). Their mineral fraction is of a silt loam texture. The mineralogical analysis of the fine fraction indicates enrichment in clay smectites and interstratified irregular smectite–illite on alluvial horizons. The content of organic material and nitrogen is high, as are the values in their capacity for cationic exchange. Calcium, potassium,
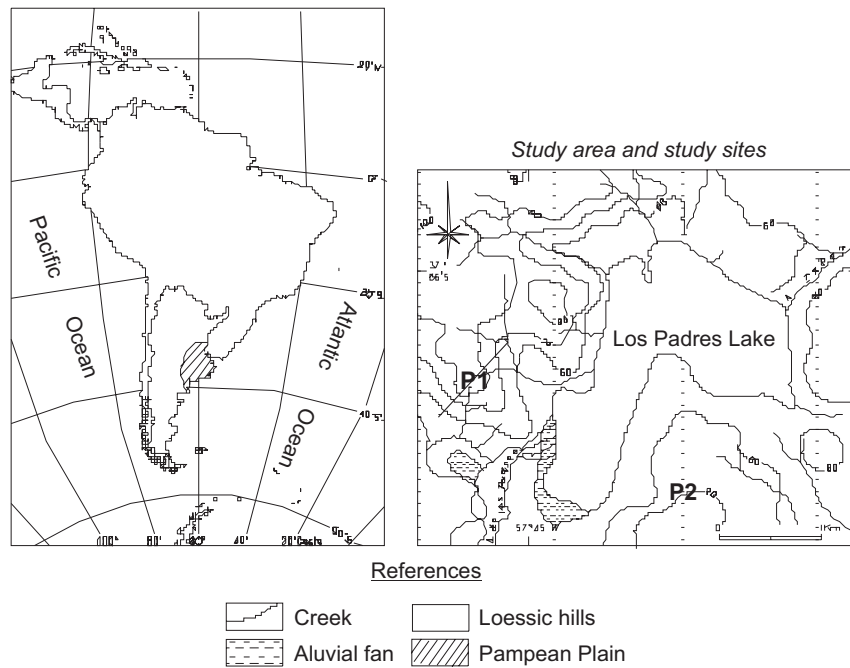
Fig. 1. Location of the study area and study sites. (P1) Plantation of *Acacia melanoxylon*, (P2) plantation of *Eucalyptus globulus*.

magnesium and sodium ions participate in this process with decreasing importance.

2.2. Sample units

The research was developed on two plots located in the same landscape unit and topographic position, but with different vegetal cover. The two plots under analysis are part of the Los Padres Lake Natural Reserve, and their soils have never suffered tilling.

Plot 1 (P1): The predominant vegetation corresponds to a plantation of *A. melanoxylon* R. Brown (Fabaceae: Mimosoidea).

Plot 2 (P2): The vegetation is a 50-year-old plantation of *E. globulus* Labill (Myrtaceae).

These vegetal species correspond to exotic species which were introduced by man with recreative purposes. In both plots, the associated vegetation corresponds to a native and relictual species, *C. tala* Gill (Celtidaceae).

2.3. Methodology

2.3.1. Fungi

Samples were randomly collected at the soil organic horizons, divided into five sub-samples and were preserved at low temperature (5 °C) until processed. To analyze calcium oxalate crystals, the fungus mats present in the collected material were observed under an optical microscope (OM) (Leitz Wetzlar D35780), petrographic microscope (PM) (Carl Zeiss, Jena) and scanning electron microscope (SEM) (Jeol T-100 and Hitachi), in samples with the ranking of 3×3–10×10 mm^2.

2.3.2. Plants

2.3.2.1. Calcium oxalate crystals description. Calcium oxalate crystals were analyzed in leaves of the three vegetal species studied: *A. melanoxylon*, *E. globulus* and *C. tala*. In Acacias, phyllodia were also analyzed. Leaves and phyllodia were washed with distilled water and cleaned with an ultrasound bath (Test-Lab, TBC 10 model) in order to remove any adhered material. Afterwards, the following treatments were applied: (a) diaphanization, following Dizeo de Strittmater (1973); (b) clearing of tissues with 50% sodium hypochlorite; (c) cross sectioning. The material was conserved in 70% ethanol until observed under OM (Leitz Wetzlar D35780), PM (Carl Zeiss, Jena) and SEM (Jeol JSM-6460 LV). For the latter analysis, samples were: (a) fixed in 3% glutaraldehyde, in 0.1 M sodium cacodylate buffer (pH 7.2–7.4); (b) dehydrated in ethanol (from 30% to absolute); (c) dried with HMDS (hexamethyldisilazane) in replacement of critical point dried; (d) metallized during 30″ between 80 and 100 Å.

2.3.2.2. Crystal density. Crystal density was determined at the tip, middle and base of the cleared leaves and phyllodia. In each of these regions, all crystals within the following areas were counted: 0.01 cm^2 (*Acacia* leaves); 0.0375 cm^2 (*Acacia* phyllodias and *C. tala* leaves) and 0.125 cm^2 (*Eucalyptus* leaves). Crystal density was defined as the number of crystals/area counted. For the analysis, the number of crystals in the tip, middle and base was pooled to obtain the crystal density for a leaf and phyllodia

(i.e. crystal density is the total number of crystals counted/ total areas counted).

2.3.2.3. Statistical analysis.

Crystal density was analyzed using analysis of variance (ANOVA) followed by the Tukey test. Data sets were checked for normality and homogeneity of variance. As variances were homogeneous and data passed the normality test, it was not necessary to transform crystal density data.

2.3.3. Soils

To determine soil particle size distribution and mineralogy, five sub-samples of each soil horizon were mixed, quartered and air-dried. Samples (20 g) were treated with 10% hydrochloric acid and 30% hydrogen peroxide at 70 °C to eliminate carbonates and oxidize organic matter, respectively. Particle size distribution was determined by sieve and pipette analysis (Ingram, 1971; Galehouse, 1971), and mineralogy by heavy liquid separation of light and heavy minerals with sodium polytungstate ($3Na_2WO_4 \cdot 9WO \cdot H_2O$, δ: 2.89 g/cm^3). Light and heavy minerals of the modal sand fraction were mounted with Canada balsam and 400–500 grains per slide were counted under an OM (Leitz Wetzlar D35780) and PM (Carl Zeiss, Jena).

Furthermore, calcium concentration was determined in the soils' saturated paste extract and in the leached soil. The leached soil solution was gathered by means of plastic pots covered with a permeable cloth, which were introduced in the soil profile at depths of 10 and 50 cm, over 6 months approximately. At the same depths, soil samples were taken to prepare the saturated paste according to Richards (1954). Soils' saturated paste extract was extracted with a vacuum bomb Czerweny type 56MC4RA. Leached soil and the saturated paste extract were filtered through 0.45 μm mesh cellulose nitrate filters and the calcium concentration was determined by UV–vis spectrophotometry according to APHA–AWWA–WPFC (1998).

3. Results

3.1. Fungi

3.1.1. Calcium oxalate crystals description

In both plots, organic horizon development is important: Hz. Oi: 0–3 cm, Hz. Oe: 3–7.5 cm and Hz. Oa: 7.5–9 cm as well as fungus development. In the Oi horizon, fungus forms films on the surface of leaves and branches. In the Oe horizon, fungus density was higher than in the Oi horizon. In the former horizon, fungus forms dense white mycelial mats in relation to organic matter decay.

In the organic horizons of the Acacia forest, calcium oxalate crystals (weddellite) were found in all stages of development. Primary nuclei, individual dipyramids of 1–4 μm, occur within the fungus hyphae (Fig. 2A). Rosettes (2–5 μm), interlaced dipyramids, maintain a close relationship with the fungus wall but are situated externally to it, and are mainly positioned in a bead-like formation (Fig. 2B). Druses (8–12 μm), an irregular crystalline mass formed by aggregates of closely packed dipyramids and/or tetragonal dipyramids, are present in great masses, in external
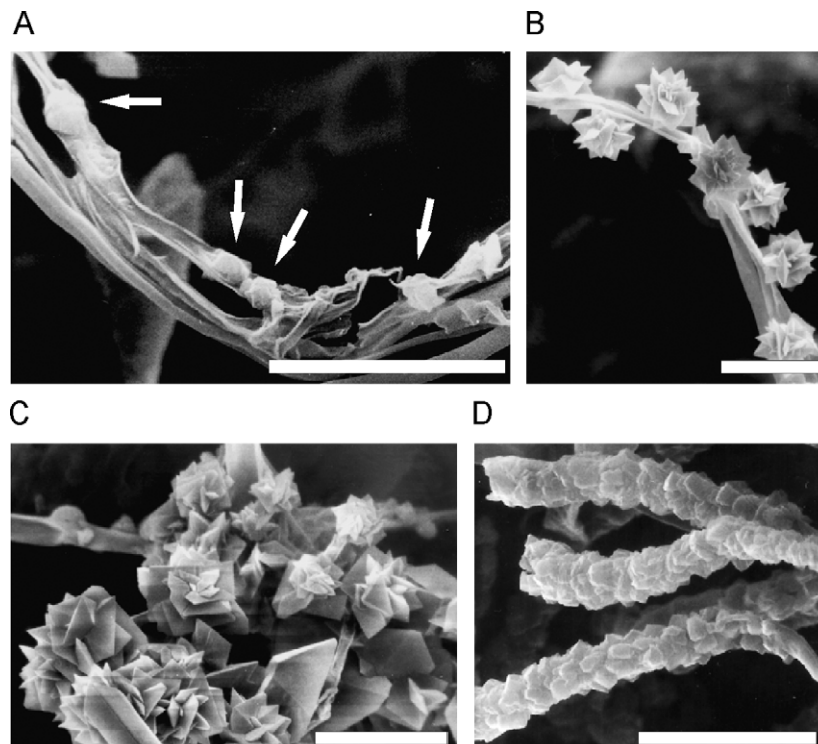

Fig. 2. SEMs of fungus mats in the organic horizons of Acacia (A–C) and Eucalyptus (D) plantations. (A) Primary nuclei (white arrows), (B) rosettes, (C) druses, (D) dipyramids. Bar: 10 μm.

position, and their relation with the hyphae is barely noticeable (Fig. 2C).

In the organic horizons of the Eucalyptus forest (P2), the presence of dipyramids ($2.5 \times 2.5\,\mu m$) in a close relationship with the fungus wall was observed; they were either separate or forming complex associations between them (Fig. 2D). These crystals resemble the morphologies cited by Klappa (1979) as calcite or the morphologies cited by Verrecchia et al. (1993) as mono- to di-pyramid forms, typical of the weddellite tetragonal system.

3.2. Plants

3.2.1. Calcium oxalate crystals description

In leaves and phyllodia of *A. melanoxylon*, calcium oxalate crystals are arranged in files along vascular bundles (Fig. 3A, B). Only individual crystals were observed with the following morphologies: (a) dipyramids of about $8\,\mu m$ (Fig. 3C); and (b) prismatic crystals of $12\,\mu m$ length by $4\,\mu m$ width, with square ends (Fig. 3C, D).

In leaves of *E. globulus*, calcium oxalate crystals appear randomly distributed when they are far from vascular bundles and in files when associated with them (Fig. 3E). Two crystal morphologies were observed: (a) druses (one per cell) of about $15\,\mu m$ (Fig. 3F, G), randomly dispersed in the palisade parenchyma; and (b) prismatic crystals in a letter envelope of about $10\,\mu m$, closely associated with vascular bundles (Fig. 3H, I).

In *C. tala* leaves, calcium oxalate crystals of druse type were observed with an average size of 7.5–$10\,\mu m$. These crystals (one per cell) are randomly dispersed in the leaf's mesophyll (Fig. 3J) and arrange in files along vascular

bundles (Fig. 3K). In the epidermal tissue, a high siliceous cystolith density (Fernández Honaine et al., 2005) was registered.

3.2.2. Crystal density

Besides differences in calcium oxalate crystal morphologies, significant differences were found for crystal density (CD) between vegetal species ($F = 74.86$, df $= 3$, $P = 0.000$, Fig. 4). Phyllodia of *A. melanoxylon* had greater CD than its leaves (Tukey test, $P = 0.000$) and leaves of *E. globulus* (Tukey test, $P = 0.000$, Fig. 4). In the same way, leaves of *C. tala* had greater CD than leaves of
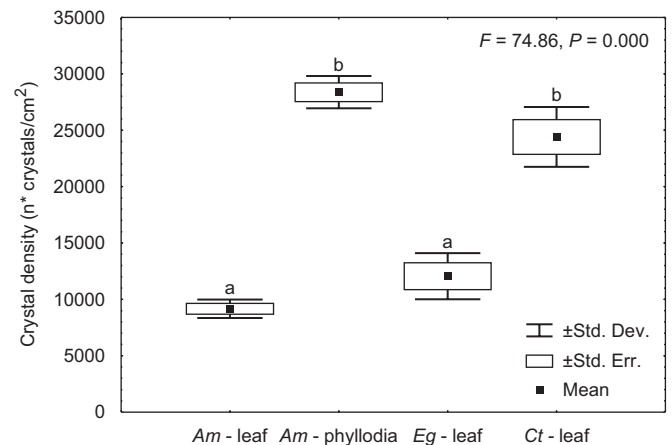

Fig. 4. Mean crystal density in leaves (*Ac*—leaf) and phyllodia (*Ac*—phyllodia) of *Acacia melanoxylon*, leaves of *Eucalyptus globulus* (*Eg*—leaf) and leaves of *Celtis tala* (*Ct*—leaf). Means with different letters differ significantly (Tukey test, $P < 0.05$).
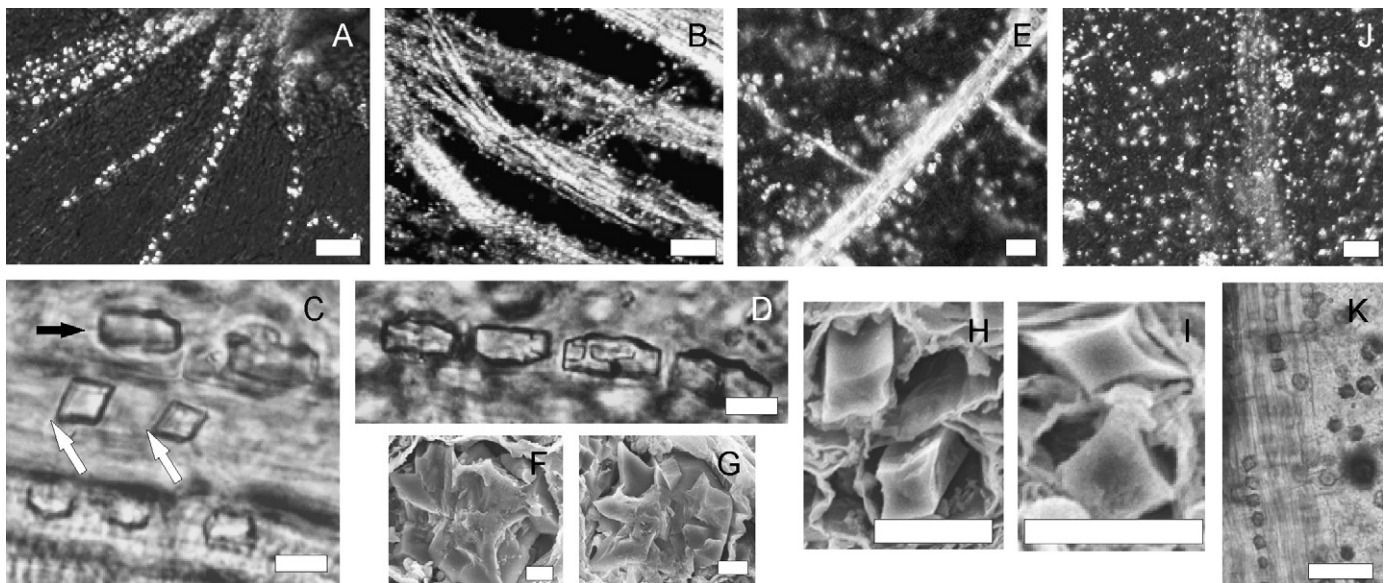

Fig. 3. PMs (A, B) and OMs (C, D) of *Acacia melanoxylon*; PM (E) and SEMs (F–I) of *Eucalyptus globulus*; PM (J) and OM (K) of *Celtis tala* leaves. (A, B) Prismatic crystals arrange in files along vascular bundles of leaf and phyllodia, respectively, (C) dipyramids (white arrows) and prismatic crystals (black arrows), (D) prismatic crystals, (E) calcium oxalate crystals dispersed at random in the mesophyll and arrange in files along vascular bundles, (F, G) druses in idioblasts of the mesophyll, (H, I) Prismatic crystals in a letter envelope associated to vascular bundles, (J) druses dispersed at random in the mesophyll, (K) druses arrange in files along vascular bundles. Bars: $50\,\mu m$ (A, B, E, J, K), $10\,\mu m$ (C, D, H, I), $5\,\mu m$ (F, G).
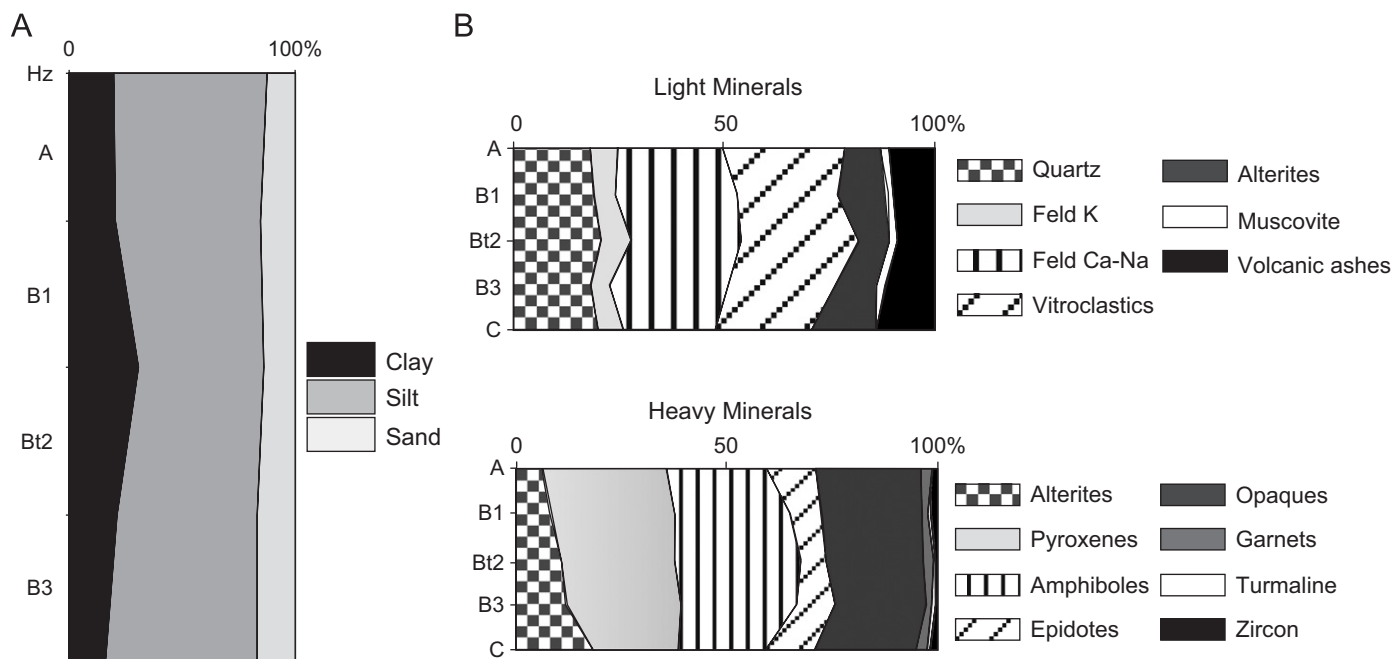
Fig. 5. Soil analysis. (A) Particle size distribution. Percentage of clay, silt, and sand. (B) Mineralogy. Percentage of light and heavy minerals in the modal sand fraction.

A. melanoxylon (Tukey test, $P = 0.000$) and leaves of *E. globulus* (Tukey test, $P = 0.000$, Fig. 4). No significant differences were found between vegetal species with the lowest CD (leaves of *A. melanoxylon* and *E. globulus*) (Tukey test, $P = 0.302$, Fig. 4), and between vegetal species with the highest one (phyllodia of *A. melanoxylon* and leaves of *C. tala*) (Tukey test, $P = 0.116$, Fig. 4).

3.3. Soils

3.3.1. Particle size distribution and mineralogy

Profiles were homogeneous in their particle size distribution. Silt fraction was predominant (55–70%), clay fraction reached values of about 20–30% and sand fraction represents about 12–16% (Fig. 5A). Predominant light minerals in the modal sand fraction were Ca–Na feldspars (25–30%), which show no features of weathering and appear clear to partially coated by oxides and weathered or coated vitroclastics (25–30%, Fig. 5B). Quartz showed a regular distribution along the profile with values around 20% (Fig. 5B). Volcanic ashes reached values around 10% (Fig. 5B) and usually appear clear, translucid and weathered. Alterites (10%, Fig. 5B) appeared as clay amorphous aggregates. K feldspars were in lesser proportions (5%) and very weathered (Fig. 5B). Heavy minerals represent only 0.9–4 %wt of the sand fraction. Among them, pyroxenes (20–30%), amphiboles (20–30%), opaques (20–25%), alterites (7–17%) and epidotes (5–10%) were predominant (Fig. 5B). Garnets, tourmalines and zircons did not exceed 2% (Fig. 5B).

Table 1
Calcium concentration (ppm) in the soil's saturated paste extract and in the leached soil of Acacia (P1) and Eucalyptus plantations (P2)

Plot	Horizon	Depth (cm)	Ca^{2+} (ppm) (extract)	Ca^{2+} (ppm) (leached)
Acacia plantation (P1)	O	5	–	20.6
	A	10	15.2	8.8
	B1	50	9.2	7
Eucalyptus plantation (P2)	O	5	–	29
	A	10	92	15.5
		50	22	10.4

3.3.2. Calcium in soil solution

In both parcels, calcium content in the soils' saturated paste extract and in leached soil decreases from the mollic epipedon (10 cm) to the argilic endopedon in the Acacia plot (P1), or to the bottom of the mollic epipedon in the Eucalyptus plot (P2) (50 cm) (Table 1). Calcium content in the soils' saturated paste extract is higher than in the leached soil (Table 1). In the Eucalyptus plot (P2), calcium concentration is higher than in the Acacia plot (P1) (Table 1).

4. Discussion

Organic horizon development in both plots is favorable to fungal biomineralization processes. Moisture retention and the presence of calcium ions due to organic matter

decay allow both fungi development and calcium oxalate crystal production. This was indicated by the presence of weddellite crystals (polyhydrated form) in all stages of development, from individual dipyramids to crystals with more complex spatial arrangements, including rosettes and druses. The observation of the primary nuclei within a fungus covering could support the hypothesis of an intracellular formation. However, extracellular formation cannot be discounted, as fungi secrete a large quantity of oxalate into the atmosphere, which can react with the available calcium and precipitate in crystalline shapes.

In the three vegetal species, calcium oxalate crystals were observed in files along vascular bundles. This could be related to the suggestion of Prychid and Rudall (1999), that crystals often occur in idioblasts near vascular bundles, possibly due to calcium being transported through the xylem.

The morphologies and distribution of calcium oxalate crystals in *E. globulus* differ from the findings of O'Connell et al. (1983) in *E. diversicolor*. *E. globulus* has druses in palisade parenchyma cells and prismatic crystals in idioblasts inside vascular bundles. In contrast, O'Connell et al. (1983) found only prismatic crystals in the palisade parenchyma cells at the margins of the bundle sheath. Ilarslan et al. (1997) and Nakata (2003) proposed that, usually, the morphology of a crystal, as well as its spatial distribution, is conserved within specific taxa. The differences found between these two *E.* species could be related to differences in the pattern of calcium oxalate precipitation at a specific level within this genus.

In Acacias, only prismatic crystals were observed. This is in agreement with Franceschi and Horner (1980), Nakata (2002) and Nakata and Mc Conn (2003), who have described the presence of prismatic calcium oxalate crystals in different vegetal species of the Fabaceae family.

Calcium cations could be released from minerals and/or biomineral weathering. In accordance with mineralogical analysis and due to their chemical composition, minerals which could be releasing calcium to the system are Ca–Na feldspars (plagioclases) and the pyroxenes–amphiboles group. The former showed no features of weathering probably because oxide coatings act as a protective layer, diminishing their dissolution capacity. Pyroxenes and amphiboles produce an insignificant contribution to the soil due to their scarcity. Furthermore, Kelly et al. (1997) proposed that plagioclase, pyroxene and amphibole weathering mainly occurs in C soil horizons with slow reaction rates, due to the types of proton sources and low biological activity. Although there are no specific studies about mineralogy and calcic aluminosilicates weathering in the analyzed soils, these results could demonstrate the limited calcium contribution to the soil from minerals, and, accordingly, the significance of calcium biominerals in these processes.

Calcium concentration in soil solution, both in the soil saturated paste extract and in the leached soil, is higher in the Eucalyptus plot (P2) than in the Acacia (P1). Both parcels are in the same topographic position and have the same soil type. Therefore, differences found in soil calcium content might be due to vegetal cover. This is not only due to the nature of the organic source, but also is shown by calcium oxalate crystals production and their morphology. Although *Acacia* phyllodias (persistent photosynthetic organ in *Acacia* sp.) had greater crystal density than for Eucalyptus, the higher calcium concentration in soil solution of the Eucalyptus plot could be explained by crystal morphology. A druse-type crystal (as found in eucalyptus) has a higher area/volume ratio than the prismatic crystals typical of Acacias, which could directly degrade due to a higher dissolution rate. Furthermore, the monohydrated crystals (whewellite) are certainly more stable (Arnott, 1995); hence, prismatic crystals found in acacias that belong to the monoclinic system and correspond to whewellite (Verrecchia et al., 1993) could be more stable than druses characteristic of Eucalyptus and *C. tala* which belong to the tetragonal system and correspond to weddellite (Verrecchia et al., 1993). *In vitro* experimentations are being carried out to test this hypothesis.

According to O'Connell et al. (1983), calcium oxalate crystals in freshly fallen litter disintegrate rapidly on the forest floor and most of the calcium released during the breakdown of plant-derived oxalate appears to be retained on exchange sites within the organic material. This could explain higher calcium concentration in soil solutions of organic horizons compared to the mineral ones. Calcium that is free in the soil solution of organic horizons could be either remineralized by fungi or leached to the mineral horizons. In mineral horizons, calcium concentration is higher in surface levels than in subsurface ones. Therefore, this calcium could be re-absorbed by plants or utilized for clay mineral formation, which could be leached to the argilic horizons. The higher concentration of calcium in soil extract than in leached soil makes it evident that the presence of an important proportion of calcium is in direct relation with the soil's mineralogical components.

The results of this study agree with Cromack et al. (1979) and Pinilla et al. (1997), who claimed that the high values of exchangeable calcium in soils would not be understood unless the contribution of vegetal and fungal calcium biominerals to the soil is considered. Furthermore, Graustein et al. (1977), Cromack et al. (1979), and Pinilla et al. (1997) proposed that calcium oxalate crystals are generated from vegetal and fungal species and liberated to the soil function both as a calcium source and as mineralogical weathering agents. In this sense, calcium released to the soil solution by means of calcium biomineral weathering could either remain in the soil as part of the soil mineral assemblage or re-enter into their biogeochemical cycle through absorption by plants or remineralization by fungus.

5. Conclusion

In typical Argiudolls of the Pampean Plain, vegetal cover and fungus development affects calcium presence and availability within the system. *Acacia melanoxylon* and *Celtis tala* generates a greater contribution of calcium oxalate crystals to the soil than does *Eucalyptus globulus*. The morphology and hydration state of the crystals could affect their dissolution rate and, therefore, the calcium content of the soil solution.

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