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Detection of active oxalate–carbonate pathway ecosystems in the Amazon Basin: Global implications of a natural potential C sink

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ABSTRACT

The oxalate–carbonate pathway (OCP) is a biogeochemical process, which has been described in *Milicia excelsa* tree ecosystems of Africa. This pathway involves biological and geological parameters at different scales: oxalate, as a by-product of photosynthesis, is oxidized by oxalotrophic bacteria leading to a local pH increase, and eventually to carbonate accumulation through time in previously acidic and carbonate-free tropical soils. Former studies have shown that this pedogenic process can potentially lead to the formation of an atmospheric carbon sink. Considering that 80% of plant species are known to produce oxalate, it is reasonable to assume that *M. excelsa* is not the only tree that can support OCP ecosystems.

The search for similar conditions on another continent led us to South America, in an Amazon forest ecosystem (Alto Beni, Bolivia). This area was chosen because of the absence of local inherited carbonate in the bedrock, as well as its expected acidic soil conditions. Eleven tree species and associated soils were tested positive for the presence of carbonate with a more alkaline soil pH close to the tree than at a distance from it. A detailed study of *Pentaplaris davidsmithii* and *Ceiba speciosa* trees showed that oxalotrophy impacted soil pH in a similar way to at African sites (at least with 1 pH unit increasing). African and South American sites display similar characteristics regarding the mineralogical assemblage associated with the OCP, except for the absence of weddellite. The amount of carbonate accumulated is 3 to 4 times lower than the values measured in African sites related to *M. excelsa* ecosystems. Still, these secondary carbonates remain critical for the continental carbon cycle, as they are unexpected in the acidic context of Amazonian soils. Therefore, the present study demonstrates the existence of an active OCP in South America. The three critical components of an operating OCP are the presence of: i) local alkalinization, ii) carbonate accumulations, and iii) oxalotrophic bacteria, which were identified associated to the oxalogenic tree *C. speciosa*.

If the question of a potential carbon sink related to oxalotrophic–oxalogenic ecosystems in the Amazon Basin is still pending, this study highlights the implication of OCP ecosystems on carbon and calcium biogeochemical coupled cycles. As previously mentioned for *M. excelsa* tree ecosystems in Africa, carbonate accumulations observed in the Bolivian tropical forest could be extrapolated to part or the whole Amazon Basin and might constitute an important reservoir that must be taken into account in the global carbon balance of the Tropics.

1. Introduction

At the turn of the last century, tropical forest lumberjacks discovered unexpected “stones” in tissues of iroko trees (*Milicia excelsa*). These mineral inclusions were identified as calcium carbonate ($CaCO_3$; Campbell and Fisher, 1932). A few decades later, calcite-cemented sandstones were described in wounds on trunks, as well as associated with the rhizosphere of a *M. excelsa* tree in Ivory Coast (Carozzi, 1967). This $CaCO_3$ accumulation is totally unexpected as the soils in these tropical areas are acidic, with pH values varying between 4.3 and 6.0 (Leneuf, 1959). Investigations in the domains of microbiology and geology have recently been conducted in order to understand how such a biomineralization process can occur. These studies aimed at testing the hypothesis that a specific pathway, the oxalate–carbonate pathway (OCP), is at the origin of such pedogenic carbonate accumulations (e.g. Bravo et al., 2011; Cailleau et al., 2011; Martin et al., 2012). This pathway was first proposed as a model, based on chemical equilibrium between oxalate and carbonate species, in the context of chalk weathering (Verrecchia, 1990; Verrecchia and Dumont, 1996). Historically, this model is linked to the isolation of oxalate-oxidizing bacteria in soils, as well as to the demonstration that this bacterial metabolism

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leads to an increase in pH and a concomitant release of carbon dioxide in the aqueous medium (Jayasuriya, 1955). Indeed, studies based on *M. excelsa* trees in Ivory Coast and Cameroon clearly demonstrated that pedogenic carbonates, accumulated in the *M. excelsa* rhizosphere, constitute the final product of the oxalate consumption by soil oxalotrophic bacteria (Braissant et al., 2002, 2004; Cailleau et al., 2005).

Under appropriate geological settings, the OCP acts as an atmospheric C sink. This is the case when calcium-sequestering carbon (C) as pedogenic $CaCO_3$ originates from a different source than an inherited $CaCO_3$ according to Elbersen et al. (2000). An amount of 8 tons of $CaCO_3$ (around 1 ton of pure carbon) was quantified in a soil associated with a 170 year-old *M. excelsa*, constituting an important C sink (Cailleau et al., 2004, 2011). Considering the geographical distribution of *M. excelsa* in Africa and their potential of C trapping through the OCP, it is reasonable to consider that this type of C sink should not be ignored in the global C cycle of the Tropics. Results obtained in the past few years have allowed temporal (Cailleau et al., 2011) and theoretical models (Verrecchia et al., 2006) of the processes involved in the *M. excelsa* ecosystem to be developed.

All the studies carried out so far have only focused on the *M. excelsa* tree (Braissant et al., 2004; Bravo et al., 2013; Cailleau et al., 2004, 2005, 2011). However, oxalate is a common photosynthetic by-product in the Plant Kingdom (Khan, 1995; Pobeguin, 1943). It can be assumed that oxalotrophic ecosystems associated with other oxalogenic trees must exist elsewhere on Earth. In order to identify an active oxalogenic–oxalotrophic system, several parameters must be considered: i) alkalinization of the soil close to the studied oxalogenic plant, which is the first consequence of the OCP; ii) the presence of carbonate, the ultimate result of OCP, if the local environmental or micro-environmental conditions reach the stability pH for calcite; and, iii) the presence of the biological agents as well as the initial products (i.e. oxalotrophic bacteria and oxalate-producing organisms). All these elements support a site with an effective OCP. As a consequence, two specific initial conditions should exist in the field. First, the investigated area must present acidic soils on which the alkalinization due to oxalotrophy can be identified. Secondly, the absence of inherited carbonate in the soil and the basement is fundamental to accurately determine the final consequence of an active OCP ecosystem, i.e. the presence of $CaCO_3$.

The aim of the present study is twofold: i) to make an inventory of trees in the Amazon Basin presenting some of the characteristics associated to an active OCP, such as the presence of unexpected alkaline soil conditions among acidic tropical soils and the presence of $CaCO_3$ on the tree and/or in the soil; and ii) to validate a field methodology for the screening and characterization of OCP systems that can be applied worldwide.

2. Material and methods

2.1. Site settings

During this study, an important exploration phase was carried out in the Alto Beni province of Bolivia (Fig. 1) in order to find oxalogenic trees. As part of the Tertiary Andean intracratonic range, this area constitutes the western boundary of the sub-Andean zone, located between the Brazilian shield and the Hercynian range of the Eastern Cordillera. The valley of the river Río Alto, where the exploration phase was carried out, has its southwestern flank aligned with a reverse fault oriented SE–NW. This fault divides the southwestern flank of the valley, composed of Palaeozoic formations of the Eastern Cordillera, from the northeastern Cretaceous deposits. The studied sites, mainly northwards of the Sapecho village, are located on late Cretaceous to early Cenozoic formations, mainly composed of sandstones, considered as carbonate-free deposits according to Elbers (1995). The only soil map of the area describes three types of soils with the exception of soils developed on young terraces of river Río Alto Beni (Elbers, 1995). According to the WRB classification (IUSS, 2006), Cambisol, Lixisol, and Acrisol are present in the studied area. Following Köppen's climate classification, Sapecho corresponds to an Aw type, i.e. a tropical wet and dry or savannah climate. The climate becomes of type Af, i.e. a tropical rainforest climate as at Entre Ríos, at altitudes higher than 550 m. At Sapecho, precipitations range from 1300 to 1600 mm/yr.

2.2. Field screening

Four sites in the area around Sapecho and along the Río Quendeque in Bolivia (Fig. 1; sites A, B, C, and D) were included in the sampling strategy during the exploration phase in order to find mineralizing trees. In this first step, a systematic screening for the presence of the OCP was conducted around large trees found in the studied area. A simple set of tests was carried out, consisting of an acid test with 10% HCl on bark, top-, and 10 cm-deep soil samples in order to detect the presence of carbonates, as well as the measurement of the topsoil pH using a pH determination kit (Hellige pH meter) for soils, near and at distance from the tree trunk. The second test was used to determine if an unexpected alkaline pH was present near a tree compared to the distant soil, where the pH should be acidic, considering the type of soil normally occurring in this area (tropical oxisols).

Plant tissue samples collected during this first phase were air-dried in order to prevent any decay process. An ajipa tree (*Pentaplaris davidsmithii*) was selected at site A (approximately 15°33′00.00″S; 67°20′28.00″W). It had a diameter at breast height (i.e. DBH) of 1.20 m and the tree itself was about 30 m high. It was growing on a slope of roughly 20° and two soil profiles were dug, one close to the trunk (A1) and the other 13 m away (A2), on the same isohypse (contour line). At site B (approximately 15°33′15.00″S; 67°20′00.00″W), a flor de mayo tree (*Ceiba speciosa*) was selected (DBH 1.30 m, height about 30 m). The tree was located on a roughly 5 to 10° slope. Two soil profiles were investigated, one near the trunk (B1) and the other 15 m away (B2), at approximately the same altitude. Samples were collected in each identified soil horizon. In addition, rock samples were collected at site C, a spot called *Cumbre de Marimonos*, in order to determine the nature of the bedrock. Various plant samples were harvested during the exploration phase at site D, near the confluence of Río Quendeque and Río Alto Beni to build a non-exhaustive database of plant mineralogical content.

2.3. Soil analyses

Approximately 500 g of soil were collected from each soil horizon, air-dried in the field, and sieved to 2 mm for further laboratory analyses. When needed, soil samples were powdered using a mortar grinder RM 100 (Retsch). All plant samples were air-dried in the field and powdered using a rotor mill pulverisette 14 (Fritsch).

Preliminary analyses focused on confirming the presence or absence of OCP in Bolivia. One of the major changes induced by the OCP is an increase in pH of upper soil horizons: consequently, pH_{H2O} was measured using a Metrohm 827 pH lab. In addition, carbonate content was evaluated using a back titration method. Briefly, 0.5 N H_2SO_4 was added to 1 g of a 2 mm sieved soil sample and 0.5 N NaOH was used to back-titrate the resulting solution until a pH of 7. Carbonate content is expressed in percentage of dry weight.

The oxalate content was determined using an enzymatic method for plant and soil samples (adapted from Certini et al., 2000). Twenty-five milliliters of HCl 1 N were added to 10 g of dried powdered sample for an overnight reaction. Then, pH was adjusted to 2 with a 6 N HCl solution. The resulting solution was filtered to 2 μm with a Nalgene syringe. The filtrate was analyzed using an oxalate determination kit (Sigma). This test works with the coupled action of an oxalate oxydase and a peroxydase in order to obtain a colorimetric measurement of the oxalate concentration. Measurements were carried out using a spectrophotometer (Perkin-Elmer) at 590 nm. Final results are expressed in mg of oxalate per kg of soil.