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THE ROLE OF OXALIC ACID AND BICARBONATE IN CALCIUM CYCLING BY FUNGI AND BACTERIA: SOME POSSIBLE IMPLICATIONS FOR SOIL ANIMALS

K. Cromack, Jr., P. Sollins, R.L. Todd, R. Fogel, A.W. Todd, W.M. Fender, M.E. Crossley and D.A. Crossley, Jr.

Abstract

Fungi can accumulate Ca in excess of their apparent physiological needs by release of oxalic acid to form the sparingly soluble Ca oxalate. Fungal release of oxalic acid may also form stable complexes with other metallic cations, which would influence both soil weathering processes and release of P from Fe and Al hydroxyphosphates. Both saprophytic and mycorrhizal fungi may be utilizing similar functional nutrient cycling mechanisms with respect to Ca accumulation. Bacteria and *Streptomyces* sp. can decompose Ca oxalate, which recycles the cation and permits formation of calcium bicarbonates or carbonates. Oxalate decomposing bacteria and actinomycetes were isolated from the digestive systems of oribatid mites, earthworms, a springtail and two immature aquatic detritivores, a mayfly and a stonefly. Earthworms and oribatid mites are among soil animals known to utilize or cycle substantial amounts of Ca. A proposed Ca cycle, operative by fungi, bacteria, and soil animals in the context of the soil ecosystem, is presented.

Introduction

Microorganisms are an integral part of the decomposition process and are important in the release of nutrients from litter and soil organic matter. As the decomposer group usually having the dominant microbial biomass in terrestrial decomposer communities, fungi contribute significantly to cycling of both macronutrients and micronutrients (Harley, 1971; Stark, 1972). Fungi are important food and nutrient sources for a variety of invertebrates and vertebrates (Miller & Halls, 1969; Fogel & Peck, 1975; Mitchell & Parkinson, 1976).

Fungi are known to excrete substantial quantities of organic acids as part of their normal carbohydrate metabolism; oxalic acid in particular may accumulate in appreciable amounts as the oxalate salt of cations present in excess in the culture medium (Foster, 1949). Under natural conditions, the accumulation of oxalic acid as Ca oxalate is of widespread occurrence in fungi (De Bary, 1887; Foster, 1949). This may in part explain the high Ca concentrations occurring in hyphae and rhizomorphs of terrestrial fungi (Stark, 1972; Todd *et al.*, 1973; Cromack *et al.*, 1975). Although oxalic acid is a low energy metabolic compound having only 8.5% of the caloric value of glucose (Foster, 1949), its common occurrence in substrates colonized by fungi may be important in weathering of soils, as the oxalate anion is an extremely effective chelator of cations such as Fe and Al (Bruckert, 1970a, b). Formation of oxalate complexes with Fe and Al may also help in solubilizing P from Fe and Al hydroxyphosphates (Stevenson, 1967). The plant pathogen *Sclerotium rolfsii* Sacc., by excreting oxalic acid, chelates Ca from Ca pectate in cells, thus permitting effective polygalacturonase activity (Bateman & Beer, 1965).

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Many soil animals are characterized by utilization of substantial quantities of Ca. Earthworms are well-known users of Ca due to calciferous glands present in certain species (Robertson, 1936). Diplopods, isopods and snails utilize appreciable amounts of Ca in their exoskeleton; oribatid mites also concentrate Ca (Gist & Crossley, 1975). Soil fauna were estimated to have processed approximately 11% of Ca while ingesting 20% of litterfall biomass from the forest floor annually in a deciduous forest watershed (Cornaby *et al.*, 1975). Wallwork (1971, 1975) has reported finding considerable Ca in both macroarthropods and microarthropods during microbomb calorimeter work. J.A. Wallwork (pers. comm.) has found some heavily sclerotized adult oribatid mites to contain up to 18% Ca on a dry weight basis.

The decomposition of oxalate salts is of considerable interest. Fungi do not appear to break down Ca oxalate due to its low solubility; they can decompose soluble oxalates and oxalic acid (Foster, 1949). A number of bacteria, including *Streptomyces* can break down Ca oxalate (Jakoby & Bhat, 1958; Chandra & Shethna, 1975). Oxalate decomposing bacteria, including *Streptomyces*, have been isolated from earthworms or their casts, indicating that in these animals a possible Ca source could be the decomposition of Ca oxalate in the gut by resident microflora (Bassilik, 1913; Jakoby & Bhat, 1958). Oxalate decomposing bacteria were first isolated from casts of earthworms which had ingested plant material containing crystals of Ca oxalate (Bassilik, 1913). Robertson (1936) observed secretion of the calciferous gland in one specimen of *Lumbricus terrestris* (Linn.) which ingested Ca oxalate. Parle (1963) obtained evidence that numbers of bacteria and actinomycetes increased in the intestine of earthworms such as *L. terrestris*, *Allolobophora caliginosa* (Sav.) and *Allolobophora terrestris* (Sav.). The fact that soil arthropods such as isopods can degrade hemicelluloses and aromatic compounds such as phenols, cinnamic acid and quinic acid, possibly with specialized gut flora, makes it highly likely that simple C compounds, such as oxalate, could also be decomposed in a wide variety of soil animals' digestive systems (Reyes & Tiedje, 1976; Neuhauser & Hartenstein, 1976).

The objectives of this paper are to present further evidence of Ca oxalate accumulation in terrestrial fungi and to present preliminary evidence for presence of Ca oxalate decomposing bacteria in detritivore digestive systems.

Methods and materials

Calcium concentrations and oxalate were measured in saprophytic fungi and fungal substrates from several coniferous forests in Oregon, including soil, decomposing roots and wood as listed in Table I. An ectomycorrhizal fungus, *Hysterangium* sp., which occurs on *Pseudotsuga menziesii* (Franko) Mirb. in the Pacific Northwest, was analyzed following collection by hand separation of soil from an extensive rhizomorph and hyphal mat in an old-growth Douglas-fir stand on the H.J. Andrews Experimental Forest near Blue River, Oregon. Douglas-fir roots, which had decomposed for nearly a year in 1 mm mesh litterbags buried 5 cm deep in mineral soil in an old-growth stand on the H.J. Andrews Forest, were analyzed for Ca separately from fungal rhizomorphs attached to them.

Calcium analysis was done following dry ashing at 450°C with elemental determination by direct reading spark emission spectroscopy (Chaplin & Dixon, 1974). The root, fungal rhizomorph and *Hysterangium* sp. samples were digested in perchloric acid with subsequent Ca analysis performed using atomic absorption spectroscopy following methods given in Noonan & Holcombe (1975).

The *Hysterangium* sp. sample was extracted with 1N HCl before passing through cation exchange resin (H^+ loaded) to remove cations. The eluate was evaporated nearly to dryness, then taken up in a small volume of H_2O. Finally, an acetate loaded anion exchange resin was used to remove the free oxalate and other organic anions, eluted with 1N HCl and the eluate analyzed with gas chromatography by the methods of Horning *et al.*

(1968) and von Nicolai & Zilliken (1974). A Microtek 2000R gas chromatograph was used with a column containing 5% SE-30 on chromosorb W-HP. The column temperature was 50–200°C programmed at 5° per minute with a He carrier. An internal standard of Decane-Eicosane was added to increase precision of peak location and acid quantification.

The two earthworms from which oxalate decomposing bacteria were isolated came from Oregon. An endemic species of earthworm collected from western hemlock litter, *Tsuga heterophylla* (Rafn.) Sarg., was surface sterilized in 30% H_2O_2 for 30 seconds, then rinsed in sterile distilled H_2O before a ½ cm length from its central portion was removed and agitated in 10 cm^3 of sterile distilled H_2O to suspend the intestinal contents. Then one cm^3 of the suspension was plated out on each of three plates of Ca oxalate agar (Jayasuriya, 1955). *Lumbricus rubellus* (Hoffmeister) was collected from soil in Corvallis, Oregon, and the same procedures described above were used for isolation of oxalate decomposing bacteria.

Several adult oribatid mites (Family Pelopoidea) and springtails (*Sinella* sp., Sinellidae) were surface sterilized before crushing to make a H_2O suspension of their digestive systems. Calcium oxalate decomposers were isolated as above. Two immature insects which represent aquatic detritivores, one a stonefly (*Peltoperla* sp., Plecoptera) and the other a mayfly (*Stenonema* sp., Ephemeroptera) were collected near Franklin, North Carolina. These also were surface sterilized, their digestive systems dissected open and a H_2O suspension plated onto Ca oxalate medium.

Results and discussion

Calcium concentrations for several terrestrial fungi and their substrates, together with oxalate concentration data are given in Table 1. The *Hysterangium* sp. had the highest Ca and oxalate concentrations. Scanning electron microscope examination of a similar sample from *Hysterangium crassum* (Tul. & Tul.) Fischer, collected from a young-growth Douglas-fir stand, showed abundant crystals on hyphae. Data from X-ray diffraction analysis gave patterns most nearly matching Ca oxalate monohydrate (K. Speidel, pers. comm.).

Table 1. Calcium concentrations and oxalate content in terrestrial fungal habitats. Values are mean ± standard error.

Tissue or substrate	Calcium (% dw)	Oxalate (% dw)	No. of samples
Soil hyphae and rhizomorphs from Douglas-fir mycorrhizae (*Hysterangium* sp.)	10.8 ± 0.2	22.8 ± 0.4	2
Decaying Douglas-fir log	0.1	NA[a)]	1
Fomitopsis pinicola – rhizomorphs	0.5	1.0	1
Undecomposed Douglas-fir roots	0.3	NA	1
Decomposing roots	0.6	NA	1
Root-colonizing rhizomorphs	3.8	b)	1
White fir heart rot *Echinodontium tinctorium*:			
Uninfected sapwood[c)] (infected tree)	0.09 ± 0.0	0.14 ± 0.01	2
Wet wood	0.10 ± 0.01	0.12 ± 0.01	2
Incipient decay zone	0.46 ± 0.01	0.80 ± 0.0	2
Advanced decay zone	0.32 ± 0.15	0.38 ± 0.18	2
Sapwood (uninfected tree)	0.08 ± 0.01	0.07 ± 0.02	2
Heartwood (uninfected tree)	0.21 ± 0.03	0.09 ± 0.02	2

a) Not analyzed.

b) Crystals, presumably of Ca oxalate, observed on hyphae.

c) Aho, P., Li, C.Y. & Hutchins, A., unpublished data.

Calcium and oxalate were present in both *H. crassum* and *Fomitopsis pinicola* (Swarz. & Franz) samples in an approximate 2:1 weight ratio of oxalate to Ca, which is consistent with the compound's chemical structure. It seems reasonable to assume that most of the Ca present was in the form of Ca oxalate. The incipient and advanced wood decay zone samples from *Echinodontium tinctorium* (Ell. & Ev.) exhibited greater Ca and oxalate concentrations than an uninfected control tree of *Abies concolor* (Gord. & Glend.) Lindl. Calcium matched oxalate stoichiometrically only in the incipient decay zone.

It may be concluded from the kinds of data presented in Table 1, together with previously published data (Stark, 1972; Cromack *et al.*, 1975), that substantial concentrations of Ca can exist in fungal hyphae and rhizomorphs colonizing a diverse set of terrestrial habitats. Evidence to date indicates that the Ca is likely to be present as Ca oxalate. There may be many fungi, including other mycorrhizal species, which synthesize little or no oxalic acid under natural conditions. Hence, a more thorough explanation of the role of fungi in organic acid production and Ca cycling is to be encouraged. Although there is less literature on bacterial production of oxalic acid, it is possible for bacteria of the genus *Thiobacillus* to produce oxalic acid under certain conditions (Magne *et al.*, 1973). With regard to litter substrates ingested by soil animals, significant amounts of Ca oxalate should also be present in fresh leaf litter as a result of foliar deposits of the compound (Chandler, 1937; Osmond, 1967).

Preliminary results for oxalate decomposers isolated from digestive systems of both terrestrial and aquatic detritivores were all positive (Table 2). These data provide

Table 2. Oxalate decomposers in soil animals.

Source, organism or substrate	Oxalate decomposer	Reference
Earthworm cast	*Pseudomonas extorquens*	Bassilik (1913)
Earthworms *Pheretima* sp.	*Pseudomonas oxalaticus*	Khambata & Bhat (1953)
Common Indian earthworm	*Streptomyces* sp.	Khambata & Bhat (1954)
Common Indian earthworm	*Mycobacterium lacticola*	Khambata & Bhat (1955)
Lumbricus rubellus (earthworm)	Bacteria & actinomycetes	This study
Endemic earthworm species – Oregon	Actinomycetes	This study
Cryptostigmata (Oribatid mite) Pelopoidea	Actinomycetes	This study
Collembola (springtails) *Sinella* sp.	Actinomycetes	This study
Insecta (stonefly) *Peltoperla* sp.	Actinomycetes	This study
Insecta (mayfly) *Stenonema* sp.	Actinomycetes	This study

circumstantial evidence for the existence of oxalate decomposers as a component of the terrestrial flora in these animals, as well as extending the previously published work on Ca oxalate decomposers present in earthworms. Decomposition of organic acid salts such as Ca oxalate in the guts of soil animals may contribute to an increase in pH as a moderately strong acid is converted into a much weaker one:

$$2CaC_2O_4 + O_2 \longrightarrow 2Ca^{++} + 2CO_3^{--} + 2CO_2$$

Jayasuriya (1955) observed pH to increase from 7.0 to 9.5 during bacterial decomposition of K oxalate. Both van der Drift & Witkamp (1960) and McBrayer (1973) found higher pH in litter detritivore faeces than in leaf litter prior to ingestion, results which could have been due in part to organic acid salt decomposition.

A proposed Ca cycle operative in fungi, bacteria, and soil animals in the context of the soil ecosystem, is presented in Fig. 1. In this diagram, Ca is depicted as cycling either within the soil animal digestive system or externally within the soil ecosystem. As depicted in Fig. 1, calcium may exist in several forms: as Ca on an exchange site in soil or litter; as Ca bicarbonate; as Ca oxalate; or as Ca^{++} in solution. In this simplified diagram, we omit comprehensive detail of other Ca compounds existing in soil or litter or internally within the soil animal. A point worth emphasizing is that waste products of the C cycle may profoundly influence cycling of elements such as Ca, and in a more general context, influence cycling of elements such as P, Fe, Al and others.

Further studies are needed on microflora in soil animal digestive systems such as the one by Parle (1963) to confirm a resident oxalate decomposer flora. Radioisotope tagging, using ^{45}Ca and ^{14}C labeled oxalate would be useful in confirming oxalate decomposition in digestive systems of a variety of soil animals.

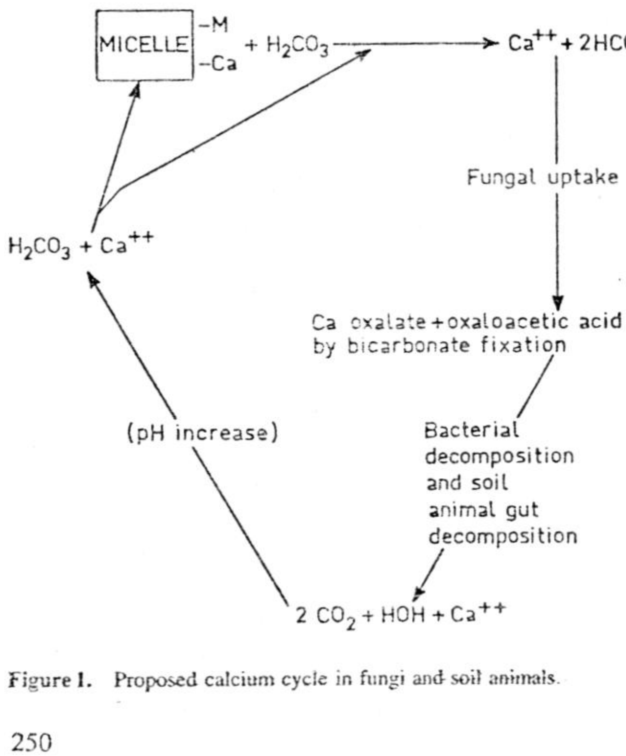

Figure 1. Proposed calcium cycle in fungi and soil animals.

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DISCUSSION

J. P. Curry: Is there evidence of Ca contribution by litter fungi in very acid soils? If so this might be of considerable significance for saprophagous microarthropods which otherwise might be severely Ca limited.

K. Cromack: Evidence from very acid coniferous litter (pH 4.5) has not yet been obtained. Our data to date are from coniferous forest soils in Oregon at pH 5–5.5.

J. Wallwork: It is a comment more than a question on the deposition of Ca in the exoskeleton of oribatid mites. I have come across this in a number of groups within the oribatid mites and I am particularly struck by it in one group, Phthiracaridae, which in fact have as much as 45% of the dry weight as $CaCO_3$ in the exoskeleton. It would be interesting to carry on this kinds of investigation on this group of mites.

K. Cromack: A survey of several oribatid mites has been started.

D. Reichle: Are there any differences in the Ca-concentration in the soil fauna between different trophic levels?

K. Cromack: The Ca-concentration seems to be higher in lower trophic levels. A further interesting question is what the fungi is doing with Na.

D. Reichle: Na-concentration is high in the first link of the food-chain.

W. Block: Do you have any explanation for the rather high concentration of the deposited Ca in the head-end of the oribatid mite you showed? Would it in any way be connected with the moulting?

K. Cromack: We don't know.

Soil Organisms as Con
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THE ROLE OF
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D. A. Krivolutzky a

Abstract

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