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CALCIUM OXALATE ACCUMULATION AND SOIL WEATHERING IN MATS OF THE HYPOGEOUS FUNGUS *HYSTERANGIUM CRASSUM*

KERMIT CROMACK, JR*, PHILLIP SOLLINS*, WILLIAM C. GRAUSTEIN†, KAREN SPEIDEL†, ALLEN W. TODD‡, GODY SPYCHER*, CHING Y. LI§ and ROBERT L. TODD¶

* Department of Forest Science, School of Forestry; † Soil Science Department, ‡ Department of Botany and Plant Pathology, Oregon State University; § US Forestry Sciences Laboratory, Corvallis, OR 97331, U.S.A.; ‖ Department of Geology and Geophysics, Yale University, New Haven, CT 06520, U.S.A.; ¶ Department of Agronomy and Institute of Ecology, University of Georgia, Athens, GA 30602, U.S.A.

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Summary—Fungal mats of *Hysterangium crassum* (Tul. and Tul.) Fischer occupied a mean of 9.6% of the upper 10 cm of soil developed under a 40–65 yr old stand of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in Oregon. This hypogeous basidiomycete exudes large amounts of oxalic acid, some of which precipitates with Ca in microscopic crystals of calcium oxalate, resulting in a mean CaC_2O_4 content of 82 g m^{-2} for the entire soil. Soil oxalate concentration was significantly greater within fungal mats ($P < 0.01$) and soil pH was significantly lower ($P < 0.01$) than in soil adjacent to mats. The quantity of Ca present as CaC_2O_4 is 0.5 the amount of exchangeable Ca in the soil and exceeds the mass of Ca lost annually in runoff. Scanning electron micrographs show intense chemical weathering, attributable to oxalate attack, in the immediate vicinity of hyphae. X-ray diffraction patterns of clay indicate bulk weathering is more intense within the fungal mats than in adjacent uncolonized soil.

INTRODUCTION

Organic acid secretion by mycorrhizal fungi has been suggested as a chemical mechanism for solubilizing P from sparingly-soluble forms such as rock phosphate or iron and aluminum hydroxy phosphates (Bowen, 1973; Harley, 1975). Evidence of increased weathering loss of Fe and P from the upper soil profile of forest soils colonized by basidiomycete fungal mats was found by Hintikka and Naykki (1967) and Fisher (1972); Fisher also observed greater depletion of Ca, Mg and Al in the upper part of the A horizon in fungal colonized soil. These authors concluded that organic acids produced by the fungal mats could have caused the increased weathering. It has been shown in several laboratory studies that organic acids produced by microorganisms increase cation or P solubilization from soil minerals (Johnston, 1952; Bruckert and Jacquin, 1969; Silverman and Munoz, 1970; Boyle *et al.*, 1974; Berthelin *et al.*, 1974).

We present evidence that appreciable amounts of crystalline Ca oxalate accumulate in the dense fungal mats of the basidiomycete *Hysterangium crassum* (Tul. and Tul.) Fischer, a hypogeous species that we presume to be an ectomycorrhizal associate of Douglas-fire (*Pseudotsuga menziesii* (Mirb.) Franco) in the Pacific Northwest (Fogel, 1976). The existence of *H. crassum* mats as discrete entities afforded the opportunity to compare colonized and uncolonized soil for differences in pH, oxalate content and weathering.

MATERIALS AND METHODS

Study area

The study area is a 40–65 yr old second-growth Douglas-fir stand with a sparse understory located in Benton County, Oregon about 16 km west of the town of Philomath, at an elevation of 460 m (Fogel, 1976). The soil is primarily of the Slickrock series, derived from weathered sedimentary rock (Knezevich, 1975). We also found weathered colluvial igneous rock fragments to be present. Such colluvial materials are known to occur in the general area (Knezevich, 1975). Rainfall averages 191 cm a year but little occurs in the summer (Fogel, 1976).

Collection of materials

A random transect was laid out through the stand. The first three areas of soil colonized by discrete mats of *H. crassum* were collected for analysis. Intact mats, consisting of mycorrhizas and other Douglas-fir roots, sporocarps and attached soil were excavated from the A horizon to a depth of 10 cm. Samples of soil 10–50 cm away from the edges of colonized areas were also collected to the same depth. Fresh mat and soil samples were analyzed immediately upon return to the laboratory.

The area and volume of mats were mapped in three 5 × 5 m randomly-selected plots from which the litter layer had been removed. Although mats often were visible at the litter–soil interface, excavation to a depth of 10 cm was necessary to determine the total

Tabele 1. Surface area and soil occupancy of *H. crassum* mats in 5 × 5 m plots in a second-growth Douglas-fir forest (means ± standard error)

Plot	No. of mats	Average mat area (m^2)	Average mat depth (cm)	% Surface area occupied by mats	% soil volume occupied (10 cm depth)
1	6	0.18 ± 0.04	4.7 ± 0.6	4.3	2.0
2	5	0.66 ± 0.18	7.6 ± 1.3	13.2	10.0
3	14	0.49 ± 0.13	6.1 ± 0.53	27.4	16.7

number of mats and the volume of soil that they occupied. Duplicate measurements were made of depth and diameter; areas and volumes were calculated from mean dimensions.

Methods of analysis

Attached soil was removed with stainless steel forceps from the moist fungal mat samples, which were then rinsed with distilled water. Sporocarps and roots, except for some mycorrhizal fragments, were removed from the rinsed mats, leaving predominantly rhizomorph tissue for chemical analysis. Both mat and soil were dried at 50°C. Fresh A horizon samples were mixed with water in a 1:10 ratio and the pH of the resulting slurry was measured.

All mat samples separated for nutrient analyses were ground (<0.41 mm) in a Wiley mill. N was determined by the micro-Kjeldahl method (Jackson, 1958). After digestion with perchloric acid, P was assayed by the molybdate-blue technique (Taras *et al.*, 1971) and cations were determined by atomic-absorption spectrophotometry. Exchangeable Ca was extracted with ammonium acetate at pH 7 and determined spectrophotometrically.

The oxalate content of mat rhizomorphs was assayed by titration with $KMnO_4$ (Baker, 1952). Oxalate was extracted by boiling 0.5 g of dry *H. crassum* in 1 M HCl; the remainder of Baker's procedure was not changed. Based on the stoichiometry of the reaction, 5 moles of $H_2C_2O_4$ react with 2 moles of $KMnO_4$ and Baker's formula must be corrected to read:

$$1\text{ ml } 0.02\text{ M } KMnO_4 = 4.5\text{ mg } H_2C_2O_4$$

All data have been reported as the oxalate anion, $C_2O_4^=$.

Total oxalate concentrations of A horizon material were determined for both uncolonized soil and the total mass of soil and rhizomorph that comprise the fungal mats. Four subsamples (25 g) of dried material were taken from each soil sample, ground (<0.41 mm) and mixed together. Soil oxalate was determined by gas chromatography (g.c.) (Mee and Stanley, 1973). A 200 mg soil subsample was placed in an airtight screw-top tube containing a 5 ml aliquot of 1 part 12.4 M HCl to 19 parts absolute methanol (v/v). This solution dissolves CaC_2O_4 and methylates the oxalate to form dimethyl oxalate. Samples prepared by adding known quantities of dimethyl oxalate to the HCl-methanol reagent were used as standards. A Microtek 2000R g.c. was employed with a 1.6 mm i.d. stainless steel column 183 cm long containing 15% diethylene glycol succinate on 0.17 mm acid-washed Chromosorb W. Column life was prolonged by deco-

lorizing the sample with activated charcoal for 5 min. Subsequent to the work reported here we have found the g.c. method of Mee and Stanley (1973) to be suitable for fungal tissue.

Standard techniques were used for scanning electron microscope (SEM) and X-ray diffraction examination of rhizomorphs (Graustein *et al.*, 1977).

The <2 μm size fraction was separated from each of the three pairs of colonized and uncolonized soil samples by centrifugation (Jackson, 1958). Oriented specimens of Mg- and K-saturated clay were prepared for X-ray diffraction analyses by the paste method of Theisen and Harward (1962). The Mg-saturated specimens were equilibrated for 24 h and then X-rayed in an atmosphere of 54% relative humidity. K-saturated samples were X-rayed in dry air after heat treatments at 105 and 300°C. A Phillips Norelco diffractometer equipped with a focussing monochromator using CuK α-radiation was used to obtain the diffraction patterns.

RESULTS

H. crassum mats were common throughout the stand. They ranged from 0.25 to 1.0 m in dia (Table 1) and occupied *ca.* 9.6% of the volume of the top 10 cm of soil. Although mats generally were restricted to 0–10 cm of the A horizon, smaller pockets of *H. crassum* occurred locally in association with roots to depths of 22 cm.

The Ca content of rhizomorph tissue ranged from 36 to 79 mg g^{-1} while oxalate content ranged from 61 to 121 mg g^{-1} (Table 2). The ratio by weight of oxalate to Ca in CaC_2O_4 is 2.20:1. We measured ratios of 1.69, 1.51 and 1.54:1, indicating that most of the Ca was present as Ca oxalate. Ca was by far the most abundant cation (Table 2).

The mean concentration of oxalate within the A horizon of colonized soil, 7.3 ± 1.34 mg g^{-1} (mean ±

Table 2. Chemical composition of *H. crassum* rhizomorphs

	Mat 1	Mat 2	Mat 3
	(mg g^{-1} dry weight)		
N	5.5	5.1	5.1
P	1.3	1.5	1.3
Ca	72.6	36.4	79.0
K	3.0	2.3	2.7
Mg	3.0	3.2	3.3
Na	0.1	0.2	0.2
Oxalate	109.3	61.4	121.3

Mat	pH	oxal (mg g^{-1}
1	4.80	6.
2	4.86	9.
3	4.96	5.

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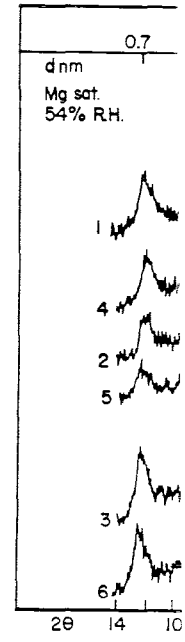

Fig. 1. X-ray diffracti

Table 3. Chemical properties of A horizon soil

Mat	Colonized soil pH	oxalate (mg g^{-1} dry wt.)	exchangeable-Ca (meq 100 g^{-1} dry wt.)	Uncolonized soil pH	oxalate (mg g^{-1} dry wt.)	exchangeable-Ca (meq 100 g^{-1} dry wt.)
1	4.80	6.4	3.3	6.15	0.3	3.3
2	4.86	9.9	3.5	6.22	0.2	2.8
3	4.96	5.5	3.0	6.07	0.2	2.0

standard error), was significantly different ($P < 0.01$) from, and 31 times higher than the mean, 0.23 ± 0.03 mg g^{-1} oxalate, in the uncolonized soil 10 cm distant (Table 3). The difference between the mean pH of the colonized soil, 4.9, and that of the uncolonized soils, 6.1, also was significant ($P < 0.01$). Amounts of exchangeable-Ca in colonized and uncolonized soil were not significantly different.

SEM showed rosettes of crystals of the mineral weddellite ($CaC_2O_4 \cdot 2H_2O$) adhering to the surface of *H. crassum* hyphae in the interstitial spaces of the soil. The rosettes were 2–5 μm dia and spaced *ca.* 10 μm apart along the hyphae (Graustein *et al.*, 1977).

The X-ray diffraction patterns obtained from Mg-saturated clay samples separated from the fungal mats (1, 2 and 3) were similar to those obtained from clays in uncolonized soil (4, 5 and 6, Fig. 1). Adjacent samples (1 and 4; 2 and 5) yielded different patterns after K saturation and heat treatment. The 1.4 nm peaks were less intense in K-saturated than Mg-saturated samples, indicating that the d spacing of some of the clay crystals had decreased to less than 1.4 nm. The difference between clays from uncolonized soil and those from mats was most pronounced in the K-saturated samples heated to 300°C. Slight shoulders appeared at 1.0 nm in the former; all peaks were reduced in intensity in the latter.

DISCUSSION

Oxalic acid has long been known to be a common product of fungal metabolism (De Bary, 1887; Foster, 1949), but the accumulation of large amounts of Ca oxalate in forest litter and soils appears to have been noticed previously only in a study of an apple orchard soil by Shorey (1913). Assuming a bulk density of 0.9 g cm^{-3} (R. Fogel, pers. comm.) and given a mean oxalate concentration of 7.3 ± 1.34 mg g^{-1}, the mats we sampled contained 417 ± 130 g of oxalate m^{-2} to a depth of 10 cm. The mean oxalate content of the uncolonized soil was 21 ± 3.0 g m^{-2}.

The weighted average oxalate content of all the soil under the Douglas-fir stand was 0.91 ± 0.22 mg g^{-1} or 82 ± 29.0 g m^{-2} of oxalate to a depth of 10 cm. This value is close to Shorey's estimate of 88 g m^{-2}.

In forming CaC_2O_4, 82 g of oxalate precipitates 37 g of Ca. At our site, 59.0 ± 0.7 g m^{-2} to 48.6 ± 4.5 g m^{-2} of Ca were present as exchangeable-Ca in mat-colonized and uncolonized soil to a depth of 10 cm, respectively. The proportion of the soil Ca present as oxalate in mat-colonized soil was 3.2 times that present as exchangeable-Ca. In uncolonized soil, the ratio was 0.2, whereas, for the stand as a whole, the ratio was 0.5. Total Ca present in the A horizon, as the sum of oxalate plus exchangeable-Ca, was 87 ± 17 g m^{-2} for the whole stand.

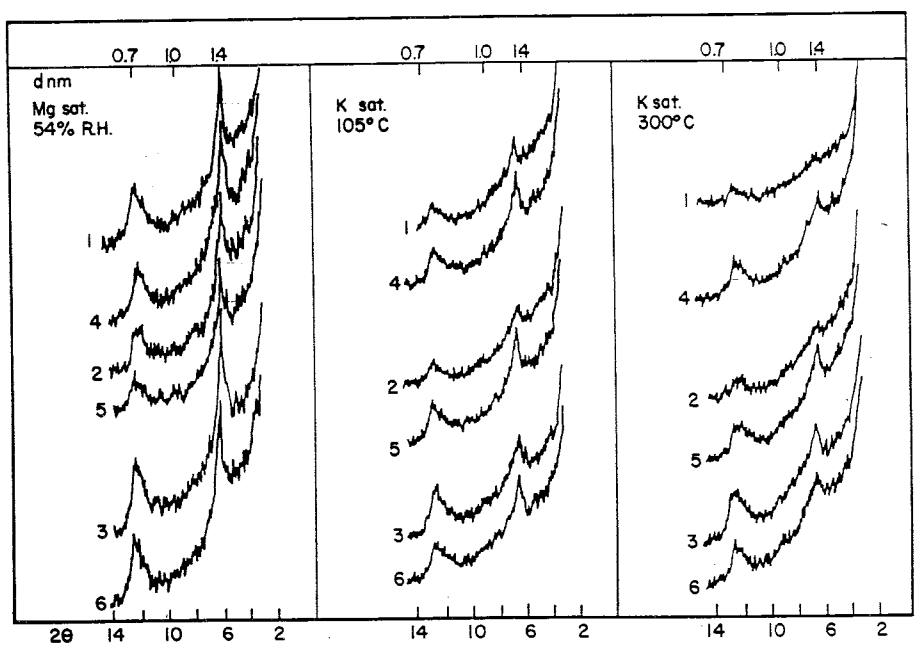

Fig. 1. X-ray diffraction patterns for the clay fraction of mat-colonized soil (1, 2, 3) as paired comparisons with diffraction patterns of uncolonized soil (4, 5, 6).

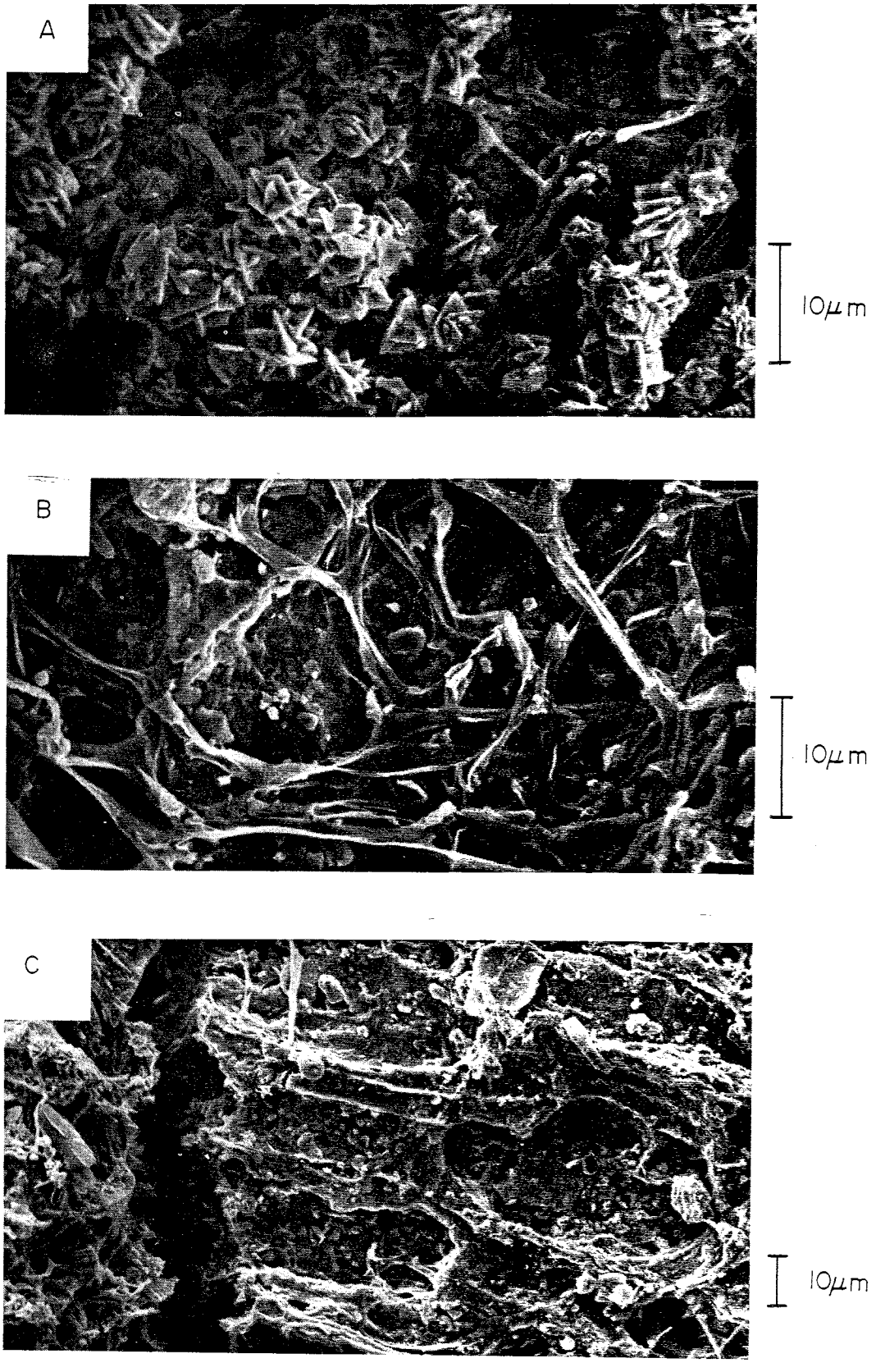

Fig. 2. Colonization of an andesite fragment by *H. crassum*. A. Dense network of hyphae covered by Ca oxalate crystals. B. Bare hyphae in contact with surface of andesite grain. C. Surface of andesite grain showing pitted and cracked surface suggesting intense chemical weathering.

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Because weddellite is a pure phase that excludes cations other than Ca^{2+} and is appreciably soluble only in acid solution, techniques for measuring exchangeable ions that involve treatment of the sample with a neutral or alkaline solution should not detect Ca that is present in weddellite. Our data for exchangeable-Ca show clearly that this is true, since there was no significant difference in exchangeable-Ca between *H. crassum* mats and adjacent soil areas. Treatment with a chelating agent as strong or stronger than EDTA should cause the dissolution of small crystals of CaC_2O_4.

Youngberg (1966) reported 19.3–35.1 g m^{-2} total Ca and 7.2–22.8 g m^{-2} exchangeable-Ca in the forest floor of Oregon Coast Range stands of second-growth Douglas-fir. At our site the reservoir of Ca present in the soil as oxalate is greater than the total quantity of Ca reported in the forest floor of several Douglas-fir forests. This reservoir of Ca (37 g m^{-2}) is also large with respect to the annual loss of Ca in runoff from watersheds that have been studied in the region. The mean annual Ca flux from an old-growth Douglas-fir watershed on the H. J. Andrews Experimental Forest in Oregon is 5.2 g m^{-2} yr^{-1} (Fredriksen, 1972); loss of Ca from the rooting zone of a second-growth Douglas-fir stand in Washington is 0.45 g m^{-2} yr^{-1} (Cole *et al.*, 1967).

The large surface area per unit mass of CaC_2O_4 crystals suggests that they dissolve readily if the soil solution becomes undersaturated with respect to CaC_2O_4. Calcium oxalate does not react with alkaline solution, but H^+ does compete with Ca^{2+} for the oxalate anion, forming $HC_2O_4^-$, so that Ca oxalate becomes increasingly soluble as the pH drops below 4.5. Thus, Ca in Ca oxalate crystals probably is available to any organism that can reduce the Ca^{2+} activity or raise the H^+ activity in the vicinity of weddellite crystals.

X-ray diffraction patterns similar to those of the K-saturated clay samples have been observed frequently in Pacific Northwest soils whose clay fraction is dominated by chloritic intergrades coated with amorphous inorganic material (Singleton and Harward, 1971). The intergrade is created by the fixation of Al or Fe in the space between the 2:1 layers of chlorite or smectite. The crystal structure of such a clay does not change greatly with K-saturation and heat treatment. If the interlayer Fe and Al atoms are completely removed, K-saturation and heat treatment will cause the 1.4 nm d spacing of the crystal to collapse to 1.0 nm. The reduction in intensity of the 1.4 nm peak, and the appearance of a weak shoulder observed in the treated clays from the fungal mats, is interpreted as the result of a partial removal of the interlayer cations from these clays. We attribute this removal of the interlayer material to the more acidic environment and the presence of oxalate, a strong chelator of Fe and Al, within the fungal mats.

Calculations based on equilibrium constants for reactions of oxalate with Ca^{2+}, H^+, Fe^{3+} and Al^{3+} indicate a more complicated interaction exists between these species than is generally acknowledged in the mycological literature (cf. Tansey, 1977). Over a range of Ca^{2+} and H^+ activities that are reasonable for soil solution, Ca oxalate will dissolve in the presence of Fe^{3+} or Al^{3+}, and the oxalate will form soluble complexes with these trivalent ions (Graustein, 1976; Graustein *et al.*, 1977). SEM indicates that this weathering reaction takes place within the fungal mats. Figure 2a shows a dense network of hyphae covered by weddellite crystals. Figure 2b shows bare hyphae in contact with a small grain of andesite, an igneous rock type common in the parent material of Pacific Northwest soils. We infer that oxalate produced by these hyphae has reacted with Fe and Al in andesite, thus accelerating weathering. The pitted and cracked appearance of the andesite grain (Fig. 2c) suggests intense chemical weathering. The fields of view of these three micrographs are separated by only 0.5 mm.

The elemental composition of *H. crassum* mats is similar to that of rhizomorphs collected from forest floors in North Carolina (Cromack *et al.*, 1975) except for Mg which is *ca.* 10 times more abundant in samples of *H. crassum*. Concentrations of Ca oxalate in *H. crassum* rhizomorphs and in mat-colonized soil varied considerably, indicating substantial variation in oxalate production within individual mats. S. Bruckert and F. Toutain (pers. comm.) have observed dense deposits of Ca oxalate crystals on fungal hyphae colonizing forest floor litter in France. High concentrations of Ca also are found in fungal rhizomorphs of tropical forests (Stark, 1972) and in rhizomorphs growing on decayed Douglas-fir roots (Sollins *et al.*, 1979). These commonly-observed large accumulations of Ca, combined with X-ray identification of CaC_2O_4 on fungal hyphae in several temperate forest sites (Graustein *et al.*, 1977), indicate that CaC_2O_4 frequently is present in soils, although most probably not in as great abundance as it is at this study area.

Fisher (1972) suggested that organic acids could be responsible for Fe and Al transport from fungal mats. We have found that oxalate is produced abundantly by similar mats which strongly suggests that it is in large part responsible both for accelerated weathering of primary minerals and clays and for Fe and Al transport.

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