Todd, R. L., Cromack, K., and Stormer, J. C. (1973). Chemical exploration of the microhabitat by electron probe microanalysis of decomposer organisms. *Nature 243*: 544-546.

Togwell, A. J., and Keller, W. D. (1970). A comparative study of the role of lichens and "inorganic" processes in the chemical weathering of recent Hawaian lava flows. *Amer. J. Science 269*: 446-466.

Visser, S., and Parkinson, D. (1975a). Litter respiration and fungal growth under low temperature conditions. In *Biodégradation et humification*, G. Kilbertus, C. Reisinger, A. Mourey, and J. A. Cancela da Fonseca (Eds.). Pierron, Sarreguemines, France, pp. 88-97.

Visser, S., and Parkinson, D. (1975b). Fungal succession on aspen poplar leaf litter. *Can. J. Bot. 53*: 1640-1651.

Wagner, G. H. G. (1975). Microbial growth and carbon turnover. In *Soil Biochemistry*, E. A. Paul and A. P. McLaren (Eds.), Vol. 3. Dekker, New York, pp. 269-305.

Waid, J. S., Preston, K. J., and Harris, P. J. (1973). Autoradiographic techniques to detect active microbial cells in natural habitats. In *Modern Methods in the Study of Microbial Ecology*, Th. Rosswall (Ed.). *Bull. Ecol. Res. Comm.* (Stockholm) *17*: 317-322.

Williams, S. T., and Gray, T. R. G. (1974). Decomposition of litter on the soil surface. In *Biology of Plant Litter Decomposition*, C. H. Dickinson and G. J. F. Pugh (Eds.), Vol. 2. Academic Press, New York, pp. 611-632.

Witkamp, M. (1971). Forest soil microflora and mineral cycling. In *Productivity of Forest Ecosystems*, P. Duvigneaud (Ed.). UNESCO-PBI, Paris, pp. 413-424.

Witkamp, M., and Frank, M. (1970). Effects of temperature, rainfall and fauna on transfer of ^{137}Cs, K, Mg and mass in consumer decomposer microcosms. *Ecology 51*: 465-474.

Witkamp, M., and Ausmus, B. S. (1976). Processes in decomposition and nutrient transfer in forest systems. In *The Role of Terrestrial and Aquatic Organisms in Decomposition Processes*, J. M. Anderson and A. MacFadyen (Eds.). Blackwell Sci. Publns., Oxford, England, pp. 375-396.

Zalokar, M. (1953). Reduction of selenite by *Neurospora*. *Arch. Biochem. Biophys. 44*: 330-337.

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Chapter 31

ROLE OF LOW-MOLECULAR-WEIGHT ORGANIC ACIDS IN THE INORGANIC NUTRITION OF FUNGI AND HIGHER PLANTS

Phillip Sollins, Kermit Cromack, Jr., Robert Fogel*

Oregon State University
Corvallis, Oregon

Ching Yan Li

Pacific Northwest Forest and Range Experimental Station
U.S. Forest Service
Corvallis, Oregon

I. INTRODUCTION

Since the late 1800s, oxalate compounds have been known to be important constituents of higher plants and important by-products of fungal metabolism. Recently there has been considerable interest in the role of these compounds in elemental cycling processes. Papers by Bruckert (1970a,b), Boyle et al. (1974), and Graustein et al. (1977) have demonstrated the importance of the oxalate ligand in mineral silicate weathering, calcium immobilization, and iron and aluminum transport during soil profile development. In this chapter we give additional evidence for the ubiquity and massiveness of oxalate accumulation in natural systems and explore the role of these compounds in the inorganic nutrition of fungi and higher plants.

Some properties of oxalates are shown in Table 1. Oxalic acid is the strongest of the low-molecular-weight (LMW) organic acids containing only C, H, and O. Oxalic acid is also the most highly oxidized organic form of carbon and releases only about one-seventh as much energy as is released during combustion of an equal weight of sucrose (Table 1). Production of oxalic acid by an organism therefore constitutes a minor energy drain compared with production of other LMW acids such as citric acid. Also, oxalic acid, because of its low heat of combustion, does not encourage growth of potentially competing organisms. In fact, it cannot be used as a sole energy source by most organisms (Harder, 1973). Unlike other LMW organic acids, oxalic acid forms a sparingly soluble precipitate with Ca [pK_{sp} = 8.64 (Ringbom, 1963)]. The Mg salt is substantially more soluble [pK_{sp} = 4.07 (Ringbom, 1963)]. The oxalate ligand forms exceedingly stable complexes with the transition metals, which accounts for its ability to extract iron and aluminum from feldspars and clays and to transport the elements downward in solution through the soil profile (Graustein et al., 1977).

Oxalate concentrations of up to 23% dry weight have been reported in rhizomorphs of *Hysterangium crassum*, a probable ectomycosymbiont of Douglas-fir trees. This

*Present address: Herbarium, University of Michigan, Ann Arbor, Michigan

Table 1 Properties of short-chain organic acids and other compounds[a]

Compound	Structure	pK_1	pK_2	Heat of Combustion (kcal/gm)
Oxalic acid	HO_2C-CO_2H	1.23	4.19	0.67
Malonic acid	$HO_2C-CH_2-CO_2H$	1.83	6.07	2.00
Maleic acid	$HO_2C-CH=CH-CO_2H$	2.83	5.69	2.81
Formic acid	$H-CO_2H$	3.75	--	1.37
Carbonic acid	$HO-CO_2H$	6.37	10.25	0
Acetic acid	H_3C-CO_2H	4.75	--	3.48
Malic acid	$HO_2C-CH_2-CHOH-CO_2H$	3.40	5.11	2.44
Succinic acid	$HO_2C-(CH_2)_2-CO_2H$	4.16	5.61	3.02
Citric acid	$HOC-(CH_2-CO_2H)_2-CO_2H$	3.08	4.74	2.47
Sucrose	--	--	--	3.94

[a]Based on data in the *Handbook of Chemistry and Physics*, CRC Press, Cleveland, and the *International Critical Tables*, McGraw-Hill, New York.

fungus forms dense mats in the A_1 horizon, such that the average oxalate content of the A horizon is over 850 kg/ha (Cromack et al., 1977, 1979). Maxwell and Bateman (1968) found that *Sclerotium rolfsii*, a plant pathogen, produced more than 1 g of oxalic acid for each gram dry weight of hyphal growth.

The presence of large amounts of calcium oxalate explains the discrepancy between the findings by Stark (1972), Ausmus and Witkamp (1973), and Cromack et al. (1975) of high Ca concentrations in fungal tissues and the findings of Steinberg (1948) that solutions "containing only faint spectroscopic traces of calcium" permitted maximum growth of *Aspergillus niger*, *Sclerotium rolfsii*, and other fungi. It seems reasonable to assume that Ca is required in only trace quantities but is present in large amounts because it precipitates in and on fungal tissues as the oxalate salt. The question remains, however, why so many fungi produce prodigious amounts of oxalate rather than oxidizing metabolites to CO_2. What advantages do oxalate production and release offer which compensate for the energy expended in maintaining an additional enzyme system?

In attempting to explain this, we have uncovered two other phenomena which we believe are important in the inorganic nutrition of mycorrhizae and saprophytic fungi. One is cation translocation by fungi, which we suggest is for pH equilibration. The other is a generally occurring effect of bicarbonate concentration on cation uptake by mycorrhizae and nonmycorrhizal roots.

II. METHODS

Soils colonized by the closely related basidiomycetes *Gautieria* sp. and *Hysterangium crassum* were studied in a stand of 40- to 65-year-old second-growth Douglas-fir trees with sparse understory. The stand is located about 16 km west of Philomath, Oregon, at 460 m elevation. Soils and vegetation of this Woods Creek site are described more fully by Fogel (1976) and Cromack et al. (1979).

The litter layer was removed first to locate the mats. Intact mats consisting of rhizomorphs, mycorrhizae, and other Douglas-fir roots, sporocarps, and attached soil were excavated from the A horizon to a depth of approximately 15 cm. Soil within 10 cm of the perimeter of the colonized areas was collected to the same depth.

Bulk soil samples (25 g) were taken from the A horizon of three areas colonized by *Hysterangium* and three areas colonized by *Gautieria*. Adjacent uncolonized areas were also sampled. Samples were dried at 50°C and then sieved through a 0.5-mm screen to remove roots, rhizomorph fragments, and sporocarps.

Douglas-fir roots for decay experiments were collected in the 1974-1975 winter at the Woods Creek site, which was selected because the sparse understory ensured that roots were almost purely Douglas-fir. Roots were returned to the laboratory, washed free of soil with double-distilled water while still fresh, separated into two diameter classes (2-3 mm and 3-5 mm), dried at 50°C in a forced-air oven, and stored in airtight plastic bags.

For the decay study, approximately 5 g of small Douglas-fir roots (2-3 mm diameter) and 7 g of larger roots (3-5 mm diameter) were placed in 20- X 20-cm bags of 1-mm nylon mesh. The bags were set out in May 1975 at 1-m spaces in a row at the litter-mineral soil interface on Reference Stand No. 2 of the H. J. Andrews Experimental Forest (RS-2). Reference Stand 2 is an old-growth Douglas-fir stand previously used by Fogel and Cromack (1977) for litter-decay experiments. It contains a *Tsuga heterophylla*, *Rhododendron macrophyllum*, *Berberis nervosa* understory and is typical of the Rhma-Bene community type (Dyrness et al., 1974).

Root bags were collected after 190 and 350 days, returned to the laboratory, and dried at 50°C. Roots were then removed, weighed, and ground to pass a 40-mesh sieve. Samples of the orginal undecayed roots were processed similarly. Fungal rhizomorphs colonizing the roots were removed from two of the bags collected after 350 days, dried, weighed, and analyzed separately for nutrient elements and oxalate. Several rhizomorphs were also mounted on aluminum stubs, air-dried in a desiccator, coated with gold-palladium alloy, and examined under an AMR model 1200 scanning electron microscope (SEM).

In all analyses, N was determined by the micro-Kjeldahl method (Jackson, 1958), and total C by the Walkley-Black method (Jackson, 1958). After digestion with perchloric acid, P was determined by molybdate reduction (APHA, 1971) and cations by atomic absorption spectroscopy. Lanthanum oxide was added for Ca and Mg determinations to prevent interference.

Oxalate was determined by the method of Mee and Stanley (1973) with a Microtek 2000R gas chromatograph (GC). Soil samples (200 mg) were placed in airtight tubes containing 5-ml aliquots of 5% HCl-methanol solution, which both solubilizes Ca oxalate and forms dimethyl oxalate. Commercial dimethyl oxalate samples were added to 5-ml aliquots of the HCl-methanol reagent and run as controls. We used a stainless steel coil column (0.33-cm inside diameter) which contained 15% diethylene glycol succinate on 0.17-mesh Chromosorb W washed with acid. Column life was prolonged by sample decolorization with activated charcoal for 5 min.

III. RESULTS

Oxalate concentrations were much higher in soil colonized by both fungal species than in adjacent uncolonized soil (Table 2). Soil carbon (excluding roots) was high in all areas sampled [3-8% dry weight (DW)]. It was lowest in the soil colonized by *Gautieria*. In the three *Gautieria* mats which we sampled, oxalate accounted for about 6% DW of the total soil carbon.

Concentrations of N, P, K, Ca, and Mg in undecayed roots were similar to those reported elsewhere (Table 3). Results of the root decay experiments showed about 80% loss in dry weight within the first 180 days for both root size classes (Table 4). Amounts of Ca increased significantly ($p < 0.05$ for both size classes and dates), but N remained essentially constant in small roots and increased slightly ($p < 0.05$) in the larger roots. Other elements decreased in amount as decomposition proceeded. Potassium was unusual in that it decreased during the first 190 days, then increased significantly ($p < 0.01$) between 190 and 350 days. Concentrations in rhizomorphs picked from the decayed roots were generally higher than in the bulk samples (decayed roots plus rhizomorphs). These rhizomorphs were abundant (Fig. 1) and accounted for an average of 1.14% of the bulk dry weight, 5.1% of the N, 6.4% for both P and K, 6.9% of the Ca, and 4.0% of the Mg in two root bags. These percentages are conservative because they include only those rhizomorphs which could be removed easily.

Table 2 Oxalate and carbon content of mat occupied and adjacent soil[a]

	Carbon (% DW)	Oxalate (% DW)	Oxalate-C (% total C)
Hysterangium			
Colonized	8.78 ± 2.35	0.73 ± 0.23[b]	2.30 ± 0.30
Adjacent	3.90 ± 1.76	0.02 ± 0.006[b]	0.19 ± 0.09
Gautieria			
Colonized	3.02 ± 0.73	0.65 ± 0.15	6.20 ± 2.20
Adjacent	4.27 ± 1.63	0.03 ± 0.01	0.24 ± 0.21

[a] Values are ±1 SD.

[b] After Cromack et al. (1979).

Table 3 Mean elemental concentrations in live roots and decaying roots of Douglas-fir trees and in fungal rhizomorphs colonizing decayed roots

	Number of samples	N (% DW)	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)
Live roots						
Woods Creek (3-5 mm)	1	0.28	0.07	0.19	0.28	0.09
Woods Creek (2-3 mm)	1	0.42	0.08	0.28	0.48	0.11
Dinner Creek[a] (<2 mm)	3	0.48	0.09	0.21	0.46	0.07
WS-10, H. J. Andrews[b] (<5 mm)	243	0.62	0.10	0.17	0.69	--
Decaying roots[c]						
RS-2, H. J. Andrews (3-5 mm)	14	0.42	0.04	0.13	0.52	0.03
RS-2, H. J. Andrews (2-3 mm)	5	0.55	0.06	0.14	0.64	0.05
Fungal rhizomorphs[d]						
RS-2, H. J. Andrews	2	2.48	0.31	0.63	3.80	0.13

[a] Site near Woods Creek (R. Fogel, unpublished data).

[b] Includes hardwood roots (Santantonio et al., 1977).

[c] After 350 days of decomposition.

[d] Rhizomorphs attached to 3-5 mm roots after 350 days of decomposition.

From the root-decay results, we infer that oxalate precipitation reduces loss of Ca during root decomposition but does not affect loss of other metallic cations:

Fig. 1 Rhizomorphs attached to decayed Douglas-fir roots (X2).

Table 4 Accumulation and loss of dry weight and elements from decaying roots of Douglas-fir trees (*Pseudotsuga menziesii*)[a]

Days elapsed	Weight (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Small roots (2-3 mm)						
190 (n = 5)	21.7 ± 0.9	102.4 ± 13.0	72.5 ± 11.2	26.3 ± 8.8	126.9 ± 19.5	64.0 ± 5.9
350 (n = 5)	24.8 ± 1.1	101.6 ± 11.4	54.6 ± 9.7	37.6 ± 5.5	100.4 ± 11.0	31.5 ± 13.0
Large roots (3-5 mm)						
190 (n = 10)	20.6 ± 2.7	115.8 ± 13.0	61.4 ± 8.7	33.3 ± 6.2	169.7 ± 16.4	54.2 ± 10.0
350 (n = 14)	21.3 ± 1.5	119.2 ± 10.5	48.8 ± 5.8	54.2 ± 5.8	144.1 ± 14.6	26.0 ± 7.0

[a]Data are mean ± 95% confidence interval.

Fig. 2 Crystals (presumably Ca oxalate) adhering to hyphae of rhizomorphs shown in Fig. 1 (X2100). (SEM photograph by R. B. Addison, U.S. Forest Service.)

Ca, which forms a sparingly soluble oxalate salt [pK_{sp} = 8.64 (Ringbom, 1963)], accumulates during root decay; Mg and K, both of whose solution activity is essentially unaffected by the presence of oxalate anions, are leached rapidly from the dead roots. Although x-ray diffraction patterns were not run on the root specimens, SEM photographs (Fig. 2) show crystals which are similar in morphology to those identified definitively as Ca oxalate by Graustein et al. (1977). Gas chromatographic analysis of the rhizomorph samples showed presence of large amounts of oxalate, so large in fact that the columns became overloaded and no precise estimate could be obtained. There is little doubt that the Ca accumulated as the oxalate salt.

Also from the root decay results, we can infer that fungal colonization accounts for much of the accumulation of elements in the decaying roots. This does not mean necessarily that fungi translocated elements into the root bags. We believe that water percolating downward through the bags may have provided the input. The Ca accumulated because it precipitated as the oxalate salt; other elements, because they were efficiently absorbed and immobilized by the colonizing fungi. Translocation may in fact have occurred (see Sec. IV, below), but water percolation through the bags would also account for the increases.

IV. DISCUSSION

Massive accumulation of calcium oxalate in habitats colonized by fungi is now well documented, as are the effects of this on calcium, iron, and aluminum dynamics (Cromack et al., 1975, 1977, 1979; Graustein et al., 1977). What advantage the fungi derive by maintaining an extra enzyme system to excrete oxalate rather than bicar-

bonate or CO_2 remains unknown. Bacterial solubilization of "rock phosphate" has been clearly demonstrated in the laboratory (Sperber, 1957) and in the field (Azcon et al., 1976). Rose (1957) showed that both *Aspergillus niger* and *Sclerotium rolfsii* solubilized various "insoluble" phosphates by releasing oxalic acid. Various studies have shown that *Endogone* and several vesicular-arbuscular (V-A) mycorrhizal fungi are unable to solubilize rock phosphate (Sanders and Tinker, 1971; Hayman and Mosse, 1972), but there is no evidence that these species produce oxalic acid. Nevertheless, P release from so-called insoluble inorganic forms cannot be the entire explanation for oxalic acid production because P is not generally present in inorganic forms in the organic substrates frequented by wood-rotting fungi and other accomplished oxalate producers. Oxalic and other organic acids may help release P from resistant organic compounds. Bateman and Beer (1965) found that oxalic acid produced by *S. rolfsii* facilitated polygalacturonase digestion from cell walls of a host plant by removing Ca from the cell wall pectin and precipitating it as Ca oxalate. Malic acid has been observed to solubilize Ca phytate under laboratory conditions (K. Cromack, Jr., unpublished data). This P compound constitutes an important fraction of organic P in soils (Russell, 1973). However, a definite role for oxalic acid in P release from organic substrates remains to be demonstrated.

In vascular plants, factors regulating oxalate production are much better understood. Oxalate (or malate) is synthesized in response to a decrease in intracellular acidity caused, for example, by nitrate assimilation (Osmond, 1967; Raven and Smith, 1976; Kirkby and Knight, 1977). The H^+ used when NO_3^- is converted to organically bound NH_3 is supplied by conversion of neutral carbohydrate to organic acids (usually malic or oxalic acid). If malate is produced, then it must be excreted from the plant to prevent an endless increase in osmotic potential. Excretion of large amounts of malate by roots has been demonstrated by Smith (1976).

Excessive osmotic potentials will also be prevented if the organic anion precipitates in an inert form. Many plants in which nitrate is assimilated in the foliage maintain constant pH by producing oxalic rather than malic acid. The oxalate then precipitates as the inert Ca salt in the foliage (Raven and Smith, 1976). Calcium oxalate thus serves not to sequester excess Ca but rather to dispose of oxalate. In fact, it is easy to induce Ca deficiency in plants accumulating calcium oxalate (Brumagen and Hiatt, 1966; Lötsch and Kinzel, 1971)--hardly what one would expect if the oxalate served to dispose of excess Ca.

The majority of elements required for plant growth (N, P, K, Ca, and Mg) occur in the soil solution as cations. In meeting only nutritional requirements plants would absorb more cations than anions, but because electrical neutrality must be maintained either other cations must be excreted or other anions absorbed. This phenomenon, unfortunately termed "excess" cation absorption, was first studied by Ulrich (1941), who showed that either the plant releases H^+ in exchange for the "excess" absorbed cations or the plant absorbs HCO_3^- to balance the "excess" cation uptake. This causes not only an increase in cell pH but also a corresponding decrease

in the pH of the external solution. To supply the H^+ which is either excreted or used to neutralize the absorbed HCO_3^-, malic acid (or oxalic acid in a few plants) is synthesized from carbohydrate. This mechanism operates in fungi also (Shere and Jacobson, 1970) but is not as well studied as in higher plants.

In fungi, the oxalate is apparently secreted through the cell membrane and precipitates externally as the Ca salt. The function, i.e., maintenance of electrical neutrality, could be the same as in vascular plants, though this apparently has not been studied. If the function is the same, then oxalate yields should be greater when N is supplied as NO_3^- than when it is supplied as NH_4^+. *Aspergillus niger* produces large amounts of oxalic acid when KNO_3 is the sole N source (Müller, 1965), but the effect of different N sources does not appear to have been compared directly.

Also interesting is the possibility that fungi release oxalate rather than bicarbonate as a respiratory end product to avoid reabsorbing an unwanted anion. The oxalate precipitates and cannot compete with other more useful anions for uptake sites on the hyphal surfaces.

Another curious phenomenon documented several times and perhaps relevant to this discussion is that decayed wood, particularly rotten portions of standing live trees, often shows high concentrations of certain cations, particularly K (Rennerfelt and Tamm, 1961; Johansson and Theander, 1974; Safford et al., 1974). The amounts of K per unit volume appear to be much greater in the decay zones than in uninfected wood, which indicates that the fungi must have translocated K into the decay zone. Amounts of H^+ typically decrease during decay (increase in pH), which suggests that H^+ is translocated out of the zone by the fungus as K^+ is translocated in.

Large quantities of LMW organic acids are released from roots (Smith, 1976; one must consider the effects on the rhizosphere. Malic acid in particular is a high-energy intermediate of the tricarboxylic acid cycle and can act as the sole carbon and energy source for many microorganisms (Palmer and Hackskaylo, 1970). Recently it has been shown that malic acid is a necessary and sufficient carbon source for at least one strain of the N_2 fixer *Rhizobium* in soybean nodules (Tjepkema and Evans, 1975). Carbohydrate alone is not sufficient, although it can be utilized if LMW organic acids (such as malic or succinic acid) are also supplied (Postgate, 1975).

Malic acid released by roots is unquestionably used as a carbon and energy source by a wide range of rhizosphere bacteria and fungi. An interesting speculation is whether any rhizosphere organisms have evolved which can increase the rate at which roots release these high-energy compounds into the rhizosphere. This process would create half of the requirement for a symbiosis; the remaining requirement would be that the process be beneficial for the rooted plant.

Such a mechanism not only is easily hypothesized, but many of the links have been demonstrated in laboratory experiments. Several studies have shown that an increase in HCO_3^- concentration in the external solution causes increased cation absorption (Carrodus, 1966, 1967) and synthesis of organic acids (Lee and Woolhouse, 1971). Jacoby and Laties (1971) showed that increased absorption of "excess" cations

results in higher HCO_3^- levels inside the root cells and that this elicits organic acid synthesis. The presence of large amounts of respiring organisms in the rhizosphere almost certainly increases the HCO_3^- concentration near the root surfaces. The only remaining evidence required, other than a demonstration of all of these processes for a single species, is that an increase in external HCO_3^- concentrations increases release of organic acids from the roots, an experiment which is easily performed.

The other half of the symbiosis, the microbially mediated process which benefits the rooted plant, is also easily demonstrated. Heterotrophic bacteria, able to release nutrients from detrital material, provide an obvious example. They produce cations in excess of needs, which are absorbed by the plant roots and cause synthesis of malic acid. The malic acid could be stored in vacuoles or released into the external solution. The latter would of course be more beneficial to the heterotrophs because it would provide them with a high-energy carbon source.

Fungi extending from root surfaces into the litter or soil matrix could also take advantage of this mechanism by absorbing cations in regions far from the roots and transporting them to the root surfaces where their release would cause a flow of high-energy carbon compound from the root to the fungus. The mechanism is intriguing because we have seen that some fungi translocate cations into regions of low pH, perhaps as a means of increasing the pH. The release of malic acid by the roots certainly results in a low pH, which would be a further link in an elegant symbiosis.

It may be unnecessary to add that we believe that this symbiosis exists and is called the mycorrhizal condition. Although many links have not been demonstrated, and although we can raise many objections, we feel that this scheme's simplicity and elegance call for serious thought and experimentation. Too many questions are unanswered by other hypotheses. Why do plant roots release high-energy substrate to their mycorrhizal associate? Pathogenic fungi obtain their energy by destroying root cell walls and actively removing the energy substrate, but how does a mycorrhizal fungus obtain its food? Why does a mycorrhizal fungus release its hard-won nutrients to the plant roots? Obviously the root efficiently removes any nutrient released from the fungus, perhaps even creating a concentration gradient greater than exists at other points along the hyphae. Possible effects of the fungus on the membrane structure of the host plant and effects of substrate availability on active transport of P across the host cell membrane have been considered (Woolhouse, 1975). But a mechanism whereby hyphae would actively release nutrients and thereby obtain some advantage over other nonmycorrhizal fungi seems at least as plausible an explanation.

We began this chapter by presenting data on cation and LMW organic anion accumulation in fungal-colonized habitats and argued from this for the existence of mechanisms whereby inorganic nutrition is intimately tied to problems of pH regulation. Other authors have noticed the importance of H^+ creation or absorption in mineral cycles (Likens et al., 1969; Sollins et al., 1974; Reuss, 1975, 1976) and in biochemical processes (Raven and Smith, 1976). Sollins et al. (1974, 1980) show how information on carbon, water, and nutrient cycles can be integrated, summarized, and better interpreted by constructing an overall H^+ budget for the system. Many, if not most, biological and physical processes either cause fluxes of H^+ or are affected by pH. We have shown that careful consideration of such pH interactions, usually viewed as a complicating nuisance, can provide insights into a wide range of biochemical and ecological questions. We hope that this paper prompts further examination of these questions as well as a careful testing of our hypotheses.

ACKNOWLEDGMENTS

Chemical analyses (other than gas chromatographic) were performed by the U.S. Forest Service Chemical Laboratory, Corvallis, Oregon. The SEM photograph was prepared by R. G. Addison, U.S. Forest Service. Research was supported by National Science Foundation Grants DEB 77-06075 and DEB 74-20744A06. This is contribution No. 319 from the Coniferous Forest Biome and paper 1254 from the Forestry Research Laboratory, Oregon State University, Corvallis.

REFERENCES

American Public Health Association (1971). *Standard Methods for the Examination of Water and Waste Water*, 13th ed. APHA, Washington, D.C.

Ausmus, B. S., and Witkamp, M. (1973). Litter and soil microbial dynamics in a forest stand. USAEC Rep. No. EDFB-IBP-73-10, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Azcon, R., Barea, J. M., and Hayman, D. S. (1976). Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate-solubilizing bacteria. *Soil Biol. Biochem. 8*: 135-138.

Bateman, D. F., and Beer, S. V. (1965). Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. *Phytopathology 55*: 204-211.

Boyle, J. R., Voight, G. K., and Sawnney, B. L. (1974). Chemical weathering of biotite by organic acids. *Soil Sci. 117*: 42-45.

Bruckert, S. (1970a). Influence des composés organiques solubles sur la pédogénese en mileu acide. I. Etudes en terrain. *Ann. Agron. 21*: 421-452.

Bruckert, S. (1970b). Influence des composés organiques solubles sur la pédogénese en mileu acide. II. Experiences de la laboratoire modalites d'action des agents complexants. *Ann. Agron. 21*: 725-757.

Brumagen, D. M., and Hiatt, A. J. (1966). The relationship of oxalic acid to the translocation and utilization of calcium in *Nicotiana tabacum*. *Plant Soil 24*: 239-249.

Carrodus, B. B. (1966). Absorption of nitrogen by mycorrhizal roots of beech. I. Factors affecting the assimilation of nitrogen. *New Phytol. 65*: 358-371.

Carrodus, B. B. (1967). Absorption of nitrogen by mycorrhizal roots of beech. II. Ammonium and nitrate as sources of nitrogen. *New Phytol. 66*: 1-4.

Cromack, K., Jr., Todd, R. L., and Monk, C. D. (1975). Patterns of basidiomycete nutrient accumulation in conifer and deciduous forest litter. *Soil Biol. Biochem. 7*: 265-268.

Cromack, K., Jr., Sollins, P., Todd, R. L., Fogel, R., Todd, A. W., Fender, W. M., Crossley, M. E., and Crossley, D. A., Jr. (1977). The role of oxalic acid and bicarbonate in calcium cycling by fungi and bacteria: Some possible implications for soil animals. *Ecol. Bull.* (Stockholm) *25*: 246-252.

Cromack, K., Jr., Sollins, P., Graustein, W. C., Speidel, K., Todd, A. W., Spycher, G., Li, C. Y., and Todd, R. L. (1979). Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. *Soil Biol. Biochem. 11*: 463-468.

Dyrness, C. T., Franklin, J. F., and Moir, W. H. (1974). A preliminary classification of forest communities in the central portion of the western Cascades in Oregon. *Coniferous For. Biome Bull. No. 4*. University of Washington, Seattle.

Fogel, R. (1976). Ecological studies of hypogeous fungi. II. Sporocarp phenology in a western Oregon Douglas-fir stand. *Can. J. Bot. 54*: 1152-1162.

Fogel, R., and Cromack, K. (1977). Effect of habitat and substrate quality on Douglas-fir litter decomposition in Western Oregon. *Can. J. Bot. 55*: 1632-1640.

Graustein, W. C., Cromack, K., and Sollins, P. (1977). Calcium oxalate: Occurrence in soils and effect on nutrient and geochemical cycles. *Science 198*: 1252-1254.

Harder, W. (1973). Microbial metabolism of organic C1 and C2 compounds. *Antonie van Leeuwenhoek J. Microbiol. Serol. 39*: 650-652.

Hayman, D. S., and Mosse, B. (1972). Plant growth responses to vesicular-arbuscular mycorrhiza. III. Increased uptake of labile P from soil. *New Phytol. 71*: 41-47.

Jackson, M. L. (1958). *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs, N.J.

Jacoby, B., and Laties, C. G. (1971). Bicarbonate fixation and malate compartmentation in relation to salt-induced stoichiometric synthesis of organic acid. *Plant Physiol. 47*: 525-531.

Johansson, M., and Theander, O. (1974). Changes in sapwood of roots of Norway spruce attacked by *Fomes annosus*. Part I. *Physiol. Plant. 30*: 218-225.

Kirkby, E. A., and Knight, A. H. (1977). Influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation, and cation-anion balance in whole tomato plants. *Plant. Physiol. 60*: 349-353.

Lee, J. A., and Woolhouse, H. W. (1971). The relationship of compartmentation of organic acid metabolism to bicarbonate-ion sensitivity of root growth in calcicoles and calcifuges. *New Phytol. 70*: 103-111.

Likens, G. E., Bormann, F. H., and Johnson, N. M. (1969). Nitrification: Importance to nutrient losses from a cutover forested ecosystem. *Science 163*: 1205-1206.

Lötsch, B., and Kinzel, H. (1971). Zum Calciumbedarf von Oxalatpflanzen. *Biochem. Physiol. Pflanz. 162*: 209-219.

Maxwell, D. P., and Bateman, D. F. (1968). Oxalic acid biosynthesis by *Sclerotium rolfsii*. *Phytopathology 58*: 1635-1642.

Mee, J. M. L., and Stanley, R. W. (1973). A gas chromatographic method for determination of oxalic acid in biological material. *J. Chromatogr. 76*: 242-243.

Müller, H. (1965). Untersuchungen zum Sauerstoffwechsel von *Aspergillus niger*. *Arch. Mikrobiol. 52*: 251-265; *53*: 77-91, 277-287, 303-316.

Osmond, C. B. (1967). Acid metabolism in *Atriplex*. I. Regulation of oxalate synthesis by the apparent excess cation absorption in leaf tissue. *Aust. J. Biol. Sci. 20*: 575-587.

Palmer, J. G., and Hacskaylo, E. (1970). Ectomycorrhizal fungi in pure culture. I. Growth on single carbon sources. *Physiol. Plant. 23*: 1187-1197.

Postgate, J. (1975). *Rhizobium* as a free-living nitrogen fixer. *Nature 256*: 363.

Raven, J. A., and Smith, F. A. (1976). Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. *New Phytol. 76*: 415-431.

Rennerfelt, E., and Tamm, C. O. (1961). The contents of major plant nutrients in spruce and pine attacked by *Fomes annosus* (Fr.) Cke. *Sond. Phytopathol. Z. 43*: 371-382.

Reuss, J. O. (1975). Chemical/biological relationships relevant to the ecological effects of acid rainfall. U.S. Environmental Protection Agency Rep. No. EPA-660/3-75-032, National Environmental Research Center, Corvallis, Ore.

Reuss, J. O. (1976). Chemical and biological relationships relevant to the effect of acid rainfall on the soil-plant system. In *Proceedings of the First International Symposium on Acid Precipitation and the Forest Ecosystem*, U.S. Dept. of Agriculture, For. Serv. Gen. Tech. Rep. NE-23, L. S. Dochinger and T. A. Seliga (Eds.), Northeast For. Exp. Sta., Upper Darby, Pa., pp. 791-813.

Ringbom, A. (1963). *Complexation in Analytical Chemistry*. Wiley (Interscience), New York.

Rose, R. E. (1957). Techniques for determining the effect of micro-organisms on insoluble organic phosphates. *N.Z. J. Sci. Technol. 38B*: 773-780.

Russell, E. W. (1973). *Soil Conditions and Plant Growth*, 10th ed. Longman, London.

Safford, L. O., Shigo, A. L., and Ashley, M. (1974). Gradients of cation concentration in discolored and decayed wood of red maple. *Can. J. For. Res. 4*: 435-440.

Sanders, F. E., and Tinker, P. B. (1971). Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. *Nature 233*: 278-279.

Santantonio, D., Hermann, R. K., and Overton, W. S. (1977). Root biomass studies in forest ecosystems. *Pedobiologia 17*: 1-31.

Shere, S. M., and Jacobson, L. (1970). The influence of phosphate uptake on cation uptake in *Fusarium oxysporum* f. sp. *vasinfectum*. *Physiol. Plant. 23*: 294-303.

Smith, W. H. (1976). Character and significance of forest tree root exudates. *Ecology 57*: 324-331.

Sollins, P., Waring, R. H., and Cole, D. W. (1974). A systematic framework for modeling and studying the physiology of a coniferous forest ecosystem. In *Integrated Research in the Coniferous Forest Biome, Coniferous Forest Biome Bull. No. 5*, R. H. Waring and R. L. Edmonds (Eds.). University of Wasnington, Seattle, pp. 7-20.

Sollins, P., Grier, C. C., McCorison, F. M., Cromack, K., Jr., Fogel, R., and Fredriksen, R. L. (1980). The internal element cycles of an old-growth Douglas-fir ecosystem in western Oregon. *Ecol. Monogr. 50*: 261-285.

Sperber, J. I. (1957). Solution of mineral phosphates by soil bacteria. *Nature 180*: 994-995.

Stark, N. (1972). Nutrient cycling pathways and litter fungi. *BioScience 22*: 355-360.

Steinberg, R. A. (1948). Essentiality of calcium in the nutrition of fungi. *Science 107*: 423.

Tjepkema, J., and Evans, H. J. (1975). Nitrogen fixation by free-living *Rhizobium* in a defined liquid medium. *Biochem. Biophys. Res. Commun. 65*: 625-628.

Ulrich, A. (1941). Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. *Amer. J. Bot. 28*: 526-537.

Woolhouse, H. W. (1975). Membrane structure and transport problems considered in relation to phosphorus and carbohydrate movements and in regulation of endotrophic mycorrhizal associations. In *Endomycorrhizas*, F. E. Sanders, B. Mosse, and P. B. Tinker (Eds.), Academic Press, New York, pp. 209-239.

The FUNGAL COMMUNITY

ITS ORGANIZATION AND ROLE IN THE ECOSYSTEM

EDITED BY

Donald T. Wicklow
Northern Regional Research Center
Agricultural Research
Science and Education Administration
U.S. Department of Agriculture
Peoria, Illinois

George C. Carroll
Department of Biology
University of Oregon
Eugene, Oregon

MARCEL DEKKER, INC. New York and Basel

Library of Congress Cataloging in Publication Data

Main entry under title:

The Fungal community.

(Mycology series ; v. 2)
Includes indexes.
1. Fungi--Ecology. I. Wicklow, Donald T. [date].
II. Carroll, George C. [date]. III. Series.
QK604.F86 589.2'045 81-5433
ISBN 0-8247-6956-2 AACR2

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

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