Importance and mobilization of nutrients in soft rot of wood

JAMES J. WORRALL[1] AND C. J. K. WANG

College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, U.S.A.

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Soft rot of wood by *Chaetomium globosum* and *Scytalidium lignicola* was negligible in the absence of added nutrients. Independently varying the concentrations of nutrients in double Abrams' solution (which is often used for testing soft rot of wood) showed that these concentrations are higher than necessary, and in some cases supraoptimal, for soft rot as measured by weight loss. Optimal nutrient concentrations were lower in cases of low decay capacity than in cases of high decay capacity. A suitable, reduced solution contained, per litre, 1.5 g NH_4NO_3, 2.5 g KH_2PO_4, 2.0 g K_2HPO_4, and 1 g $MgSO_4 \cdot 7H_2O$. Best results were obtained when blocks were infiltrated with the solution. Increasing osmolarity with KCl inhibited soft rot, suggesting that the solution satisfies specific nutrient requirements rather than an osmophilic requirement. P and especially N were actively mobilized into decaying blocks. As any of the nutrients were added at low levels to the external solution, decay and the influx of N increased.

Key words: wood decay, soft rot, nutrients, translocation, osmophily.

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La pourriture molle du bois associée aux activités de *Chaetomium globosum* et de *Scytalidium lignicola* est négligeable en l'absence d'apport de nutriments. Variant de façon indépendante les concentrations en nutriments de la double solution d'Abram (solution souvent utilisée dans les tests de pourriture molle du bois) a permis de démontrer que ces concentrations sont plus élevées que nécessaire et, dans certains cas, supraoptimales pour la pourriture du bois, d'après les mesures de pertes en poids. Les concentrations optimales en nutriments sont plus faibles dans les cas de faible capacité de carie que dans les cas de capacité élevée. Une solution réduite et convenable contient, par litre, 1,5 g de NH_4NO_3, 2,5 g de KH_2PO_4, 2,0 g de K_2HPO_4 et 1 g de $MHSO_4 \cdot 7H_2O$. Les meilleurs résultats ont été obtenus lorsque les blocs de bois étaient infiltrés avec la solution. Une augmentation de l'osmolarité avec de KCl a inhibé la carie, ce qui suggère que la solution satisfait les exigences spécifiques en nutriments plutôt que celle de l'osmophilie. Le P et particulièrement le N ont été activement mobilisés dans les blocs en pourrissement. Du fait que chacun des nutriments a été ajouté en faible concentration dans la solution externe, l'influence de N et le pourrissement ont été accrus.

Mots clés : carie du bois, pourriture molle, nutriments, translocation, osmophilie.

[Traduit par la rédaction]

Introduction

Soft rot is a type of wood decay caused by some deuteromycetes and ascomycetes. It is a serious problem in many parts of the world (Findlay 1984), and recent reports have suggested that soft rot may be important in utility poles in North America as well (Zabel et al. 1985, 1991). It is often most apparent under conditions stressful to basidiomycetes, such as presence of preservatives or high moisture content, and is often most severe on the surface of affected wood. Its most unique feature is the presence of diamond-shaped, more or less elongated cavities that spiral around the lumen within the S2 wall layer, but such cavities do not always occur. Damage due to soft rot has been noted with increasing frequency on wood in service. Laboratory tests of soft rot are widely used for screening preservatives and in studies of the biology and mechanisms of soft rot.

Unlike the case with white and brown rots, high nutrient levels are required for development of soft rot. This requirement has led to the use of nutrient solutions in decay studies *in vitro*. Most workers have used a nutrient solution developed for studies on biodegradation of textiles (Abrams 1948), often at twice the original concentration. A comparison of five methods showed that one using double Abrams' solution (2AS) (Duncan 1965) gave the highest weight loss (Bravery 1968). However, there are several problems with the use of 2AS. In our experience, a precipitate forms during preparation of 2AS, suggesting that at least some ingredients are present in excess. Moreover, the relative need for each nutrient has not been studied. The only nutrient that has been tested independent of the others is nitrogen, and results have not been consistent in that case. Although Kaune (1970) found that 6–9 g/L NH_4NO_3 was optimal, Kerner-Gang (1974) found that 3 g/L gave similar or higher weight losses. In addition, the nature of the salt requirement has not been addressed with respect to soft rot.

The objectives of the present work were to (*i*) determine the optimal concentration of each nutrient present in AS independent of the other nutrients; (*ii*) distinguish between a nutrient requirement and osmophily; and (*iii*) compare the relative mobilization of nutrients from the solution into the wood.

Materials and methods

Blocks

Blocks 1 cm (tangential) × 2 cm (radial) × 0.5 cm (longitudinal) were cut from kiln-dried southern yellow pine (*Pinus taeda* L.) and yellow birch (*Betula alleghaniensis* Britton). They were dried in a forced-air oven at 103°C for 24 h, cooled in a desiccator, and weighed. They were brought to 90–100% moisture content (dry-weight basis) by soaking in distilled water, unless otherwise noted.

Nutrient solutions

2AS consisted of 6 g NH_4NO_3, 5 g KH_2PO_4, 4 g K_2HPO_4, 4 g $MgSO_4 \cdot 7H_2O$, and 2.5 g glucose, per litre. Glucose was not present in Abrams' original solution but was a modification (Duncan 1965). Kaune's (1970) solution contained 6 g NH_4NO_3, 2.56 g K_2HPO_4, 1.02 g

[1]Author to whom all correspondence should be sent to the following address: 350 Illick Hall, 1 Forestry Drive, Department of Environmental and Forest Biology, State University of New York, Syracuse, NY 13210, U.S.A.

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$MgSO_4 \cdot 7H_2O$, and 0.25 g KCl, per litre. Glucose was added to Kaune's solution (2.5 g/L) and micronutrients and vitamin B_1 were not added. All solutions were adjusted to pH 6 with HCl. In one experiment, the inorganic nutrients present in 2AS were independently varied in concentration while holding the others constant. KH_2PO_4 and K_2HPO_4 were kept in a proportion of 5:4. Each ingredient was tested at the concentration in 2AS, $\frac{1}{2}$-, $\frac{1}{4}$-, $\frac{1}{8}$-strength 2AS, and 0.0 g/L. Sufficient KCl was added as each nutrient was reduced to maintain the osmoticum at the level in 2AS (324 mosmol/L). This way, the requirement for the nutrient itself could be determined without confounding by overall salt concentration. Vermiculite chambers were used (see below).

Decay chambers

Experiments were conducted using either vermiculite or agar as a base. In the first case, 15 g vermiculite and 90 mL nutrient solution were added to each 16-oz French square jar. Four moistened blocks were placed in each jar with the long axis vertical and the upper surface even with the top of the vermiculite, and jars were autoclaved 30 min. For agar chambers, 50 and 20 mL of nutrient solution with 2% agar were added to 16- and 6-oz jars, respectively, so that, when laid horizontally, the agar would reach a level about 3 mm below the mouth of the jar. After autoclaving, jars were laid on their sides for solidification. Autoclaved supports (Whatman No. 1 filter paper, 25 × 15 mm, one per block) were placed on the solidified agar. Blocks were autoclaved for 30 min at 121°C and laid flat on the supports in each jar. Three jars containing four blocks each (12 replicates) were used for each wood–fungus combination.

Cultures and inoculation

Chaetomium globosum Kunze ex Steud. (isolate P-591) and *Scytalidium lignicola* Pesante (P-53 and P-57), isolated from utility poles, were grown for 2 weeks on 2% malt extract agar before plugs 4 mm in diameter were cut from the margins of actively growing cultures. One plug was placed in contact with each block. Chambers were incubated at 28°C.

Analyses

After 12 weeks, blocks were cleaned of external mycelium and vermiculite. One block from each treatment was stored for potential microscopic observations. The dry weights of the remaining 11 replicates were determined as above. Weight losses were corrected by subtracting weight losses of the uninoculated control blocks. Blocks were bulked within treatments and ground to pass through a screen with 0.85-mm openings. A portion was used directly for nitrogen analysis and a portion was ashed for other analyses. Total nitrogen was analyzed by Kjeldahl digestion, P by spectrophotometric measurement of the reaction with vanadate–molybdate (Wilde et al. 1972), and K, Ca, and Mg by atomic absorption photometry (Bickelhaupt and White 1982). Nutrient concentrations in blocks are expressed on the basis of the original, undecayed, dry weight of the blocks. This corrects for the increase in concentration that occurs as wood mass is lost and allows more direct comparison of net movement of nutrients into blocks.

To assess the contribution of vermiculite to the nutritional environment, a series of extractions and analyses were performed. Ninety millilitres of water, reduced nutrient solution, or 0.1 M HCl was added to 15 g vermiculite and autoclaved 30 min, just as in decay tests using vermiculite. The vermiculite was then filtered and rinsed to give a total filtrate volume of 300 mL, which was analyzed for ammonia N, P, K, and Ca, as described above. The concentrations in water alone were subtracted from the results, which were then corrected for the dilution to estimate the concentrations in the 90 mL liquid after autoclaving.

Results

In the first experiment, in which each major nutrient in 2AS was independently varied in concentration, precipitates were noted during preparation of many of the solutions, including 2AS. *Chaetomium globosum* caused much greater weight loss on birch than on pine (Figs. 1 and 2). *Scytalidium lignicola* caused less than 2% weight loss on both wood types, regardless of the treatment (Fig. 2).

Although almost no decay occurred in the absence of each nutrient, decay generally approached a maximum at nutrient concentrations far below those in 2AS (Figs. 1 and 2). In some cases, notably where less decay occurred, levels in 2AS appeared to be supraoptimal (Fig. 2).

From these results, the following nutrient levels were selected that were considered generally optimal: 1.5 g NH_4NO_3, 2.5 g KH_2PO_4, 2 g K_2HPO_4, and 1 g $MgSO_4 \cdot 7H_2O$, per L. These were used (with 2.5 g/L glucose) in an experiment designed primarily to determine the influence of osmotic concentration. Agar chambers were used. Added KCl decreased weight loss in all cases (Fig. 3).

The solution used by Kaune (1970) gave results similar to those with 2AS (Table 1). Using 2AS, we also compared using distilled water versus nutrient solution to bring the blocks to the target moisture content prior to sterilization. In most cases, more decay occurred with the nutrient solution treatment rather than with water (Table 2).

With *C. globosum* on birch, addition of low levels of the other nutrients generally increased the amounts of N and, to a lesser degree, P in the blocks (Fig. 1). With *C. globosum* on pine and with *S. lignicola*, the opposite tended to occur; at high levels of other nutrients, less N was transported into the block than at lower levels (data not shown).

In contrast, Ca and Mg concentrations in blocks were generally insensitive to the concentrations of other nutrients in the surrounding solution (Fig. 1 and other data not shown). Concentrations of Mg in blocks remained relatively constant, even in the presence of added Mg. The concentrations in the blocks of Ca, which was not added to the nutrient solution, did not vary much among the treatments, although it increased somewhat as KH_2PO_4 and K_2HPO_4 were withheld (Fig. 1).

The concentration of K in blocks generally varied with the concentration in the external solution. When KCl was added to increase osmolarity, K content of blocks increased roughly in proportion to its concentration in the solution (Fig. 3). Also, in the first experiment, as each other nutrient was progressively decreased and KCl was added to maintain the osmoticum, K content of blocks at first increased (Fig. 1). However, as the nutrient deficiencies caused a steep decline in decay, the K content of blocks began to decrease, even though K in the solution continued to rise. When KH_2PO_4 and K_2HPO_4 were reduced, the K content of blocks was also reduced, again despite the compensation with KCl.

For *C. globosum* on birch using 2AS, significantly more decay occurred on agar than on vermiculite, and the contents of N and P were higher and those of K, Ca, and Mg were lower in blocks on agar (Table 3). Nutrient contents of blocks exposed to Kaune's solution were intermediate between those of blocks exposed to 2AS in vermiculite and over agar (Table 3).

The vermiculite used in these experiments contained significant amounts of cations (Table 4). Water alone extracted small amounts of K and Mg. When the reduced nutrient solution was used, these elements actually seemed to decrease relative to the concentrations in that solution itself, probably because of uptake in the capillary spaces of the vermiculite, which were not easily rinsed. The reduced solution did extract some Ca from the vermiculite, but 0.1 M HCl extracted much higher amounts of Ca as well as K.

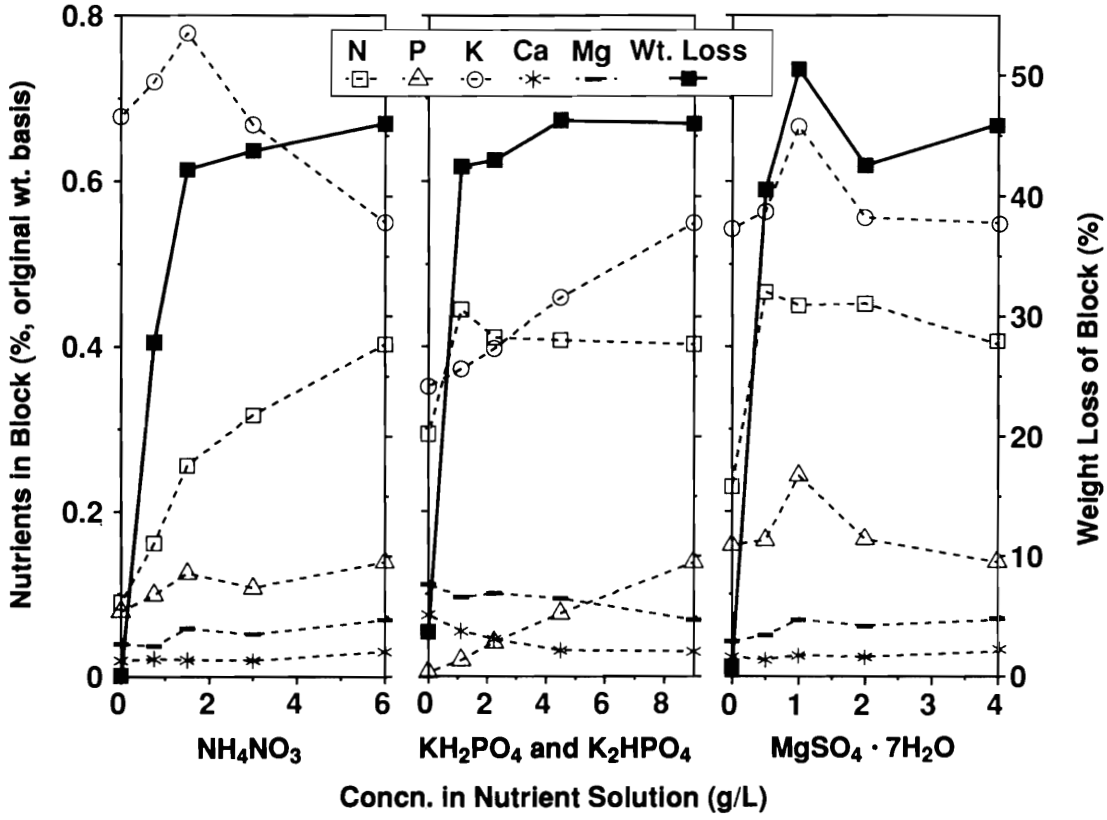

FIG. 1. Effect of independently varying solution nutrient concentrations on weight losses of and nutrient concentrations in birch blocks inoculated with *C. globosum*. Nutrients other than the one that was varied were held at the concentrations in 2AS. Weight losses are means of 11 replicates; nutrient concentrations represent single analyses of bulked replicates.

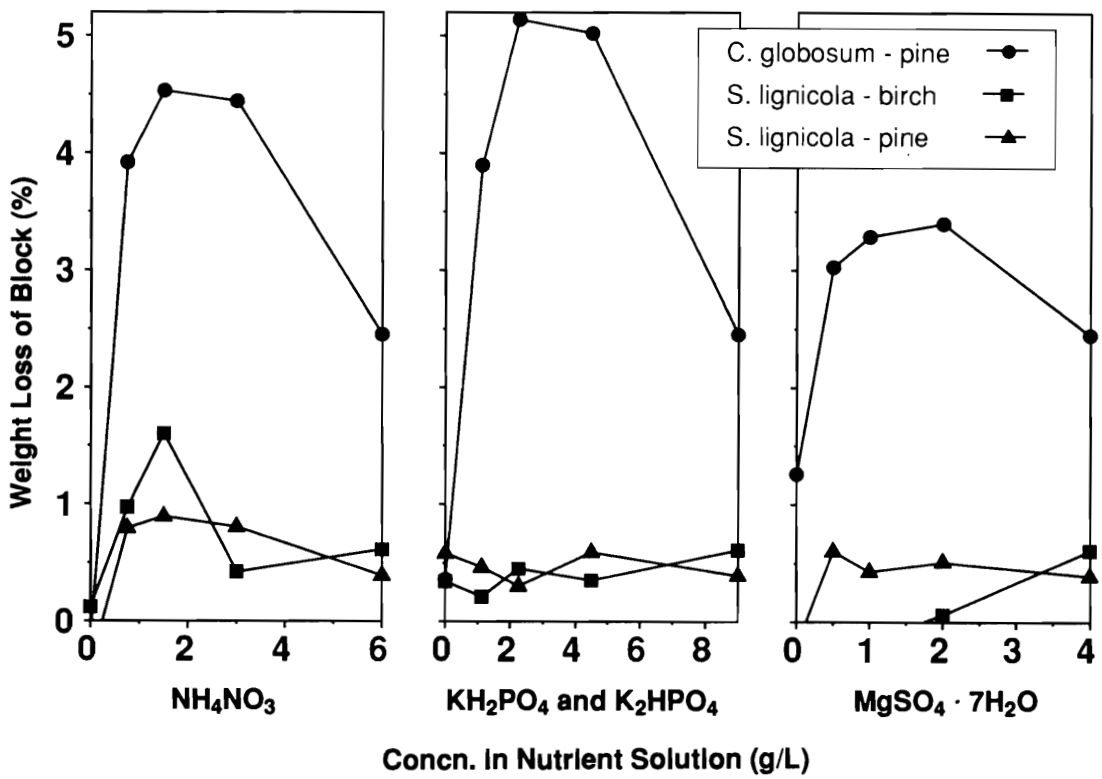

FIG. 2. Effect of independently varying nutrient concentrations on weight losses of pine blocks inoculated with *C. globosum* and of pine and birch blocks inoculated with *S. lignicola* (P-53). Nutrients other than the one that was varied were held at the concentrations in 2AS. Each point is the mean of 11 replicates.

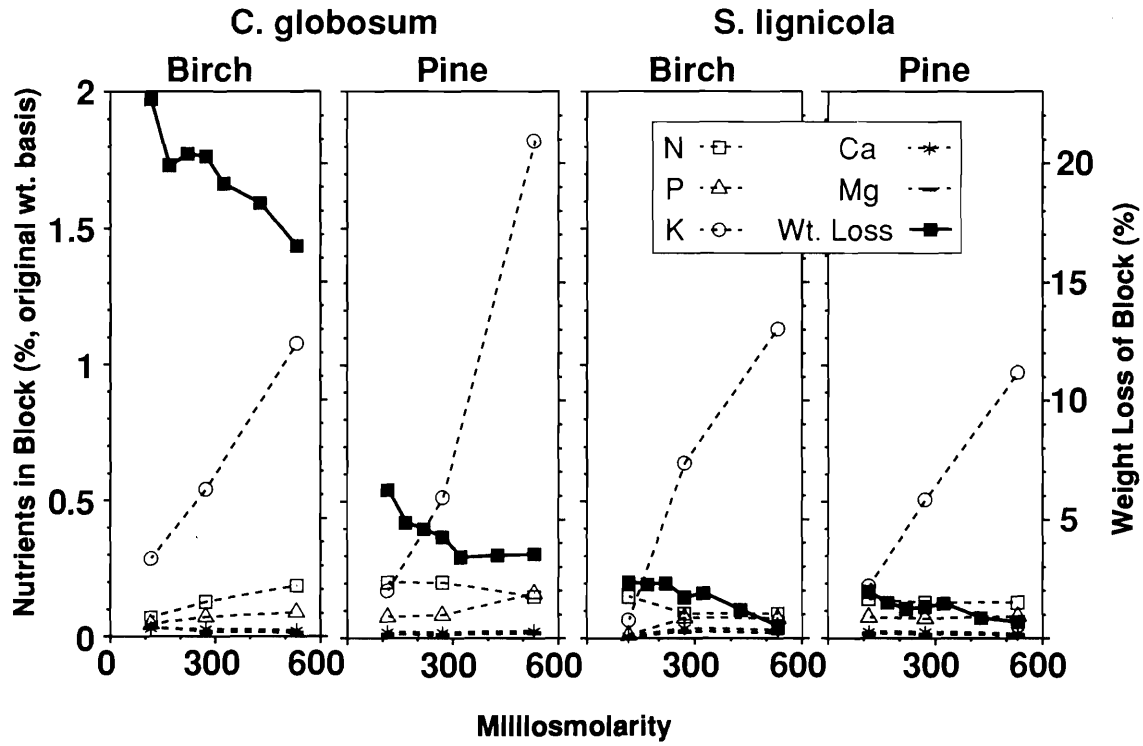

FIG. 3. Effect of osmolarity, regulated by addition of KCl to the reduced nutrient solution, on weight loss and nutrient concentration of birch and pine blocks decayed by *C. globosum* and *S. lignicola* (P-57). The osmolarity of 2AS is 324 mosM. Weight losses are means of 11 replicates; nutrient concentrations represent single analyses of bulked replicates.

TABLE 1. Weight loss (%) associated with two nutrient solutions

	C. globosum		*S. lignicola* (P-53)	
Solution	Birch	Pine	Birch	Pine
2AS	46	2.5	0.61	0.40
Kaune's	42*	4.2*	0.26*	0.66

NOTE: The experiment was conducted in vermiculite chambers. Each value is the mean of 11 replicates.

*Significantly different from the corresponding weight loss for 2AS as determined by an *F*-test; $P = 0.05$.

TABLE 2. Effect of infiltrating water versus 2AS nutrient solution on weight loss

	Weight loss (%)			
	C. globosum		*S. lignicola* (P-57)	
Block infiltration[a]	Birch	Pine	Birch	Pine
Water	38.2	1.3	3.8	1.2
2AS	54.7**	2.0	4.7*	0.6**

[a]Liquid used to bring blocks to approximately 95% moisture content. All blocks were supported on filter paper over 2AS agar.

*,**Significantly different from the corresponding water treatment at $P = 0.05$ and $P = 0.01$, respectively, as determined by an *F*-test. Each value is the mean of 11 replicates.

Discussion

The concentrations of nutrients in 2AS were too high for generally optimal soft rot in the types of tests conducted here. The presence of a precipitate also suggests that some salts are too concentrated in 2AS. By independently varying the concentration of each nutrient, we have selected concentrations that give good results: 1.5–2 g NH_4NO_3; a total of 4.5 g (in a 5:4 ratio) KH_2PO_4 and K_2HPO_4; and 1 g $MgSO_4 \cdot 7H_2O$, per litre.

Results of several experiments suggest the general conclusion that, for *C. globosum* on birch, high nutrient levels may be optimal, but that for other wood–fungus combinations, such high nutrient concentrations are supraoptimal and may be markedly inhibitory. In the experiment in which nutrients were varied, *C. globosum* continued high decay rates of birch at the highest nutrient levels and the rates even tended to increase slightly. Similarly, *C. globosum* caused significantly more decay of birch on 2AS than on Kaune's solution, which has lower nutrient levels (except for N) than 2AS. Also, in the comparison of infiltration with water versus nutrient solution, the latter led to significantly greater decay of birch by *C. globosum* than did water.

For other wood–fungus combinations, where less decay occurred, decay tended to decrease at the higher levels of N, P, and $MgSO_4$. Similarly, Kaune's solution, which has lower overall nutrient concentrations than 2AS, led to decay greater than or not significantly different from that associated with 2AS. Finally, infiltrating with nutrient solution versus water led to increases less significant than with *C. globosum* on birch (and in one case a significant decrease). Because these distinctions were observed for one isolate between two wood types as well as between fungi, they suggest that higher optimal nutrient levels are associated with high decay capacity, but that such nutrient levels may be inhibitory when decay capacity is lower.

Because KCl added to the reduced nutrient solution reduced

TABLE 3. Effect of treatment on nutrient concentrations in birch blocks exposed to *C. globosum*

Physical support	Solution	Inoculated	Weight loss (%)[a]	% original block weight				
				N	P	K	Ca	Mg
Vermiculite	2AS	No	0[a]	0.22	0.09	0.42	0.023	0.050
Vermiculite	2AS	Yes	46	0.40	0.14	0.55	0.030	0.068
Agar	2AS	Yes	63	0.45	0.15	0.37	0.023	0.044

[a]Weight losses are the means of 11 replicates. Nutrient concentrations represent single analyses of bulked replicates.

TABLE 4. Nutrients (mg/L) extracted from vermiculite

Solution	K	Ca	Mg	Ammonia N
H_2O + vermiculite[a]	19[b]	0	2	0
"Reduced" solution[c]	1823	0	118	208
"Reduced" + vermiculite[a]	1151	15	73	138
HCl + vermiculite[a]	245	156	2	1

[a]Fifteen grams of vermiculite was autoclaved with 90 mL solution, filtered, and rinsed. Concentrations are expressed on the basis of the original 90 mL.
[b]Values represent single analyses.
[c]Solution alone without vermiculite.

decay, the fungi were not osmophilic in terms of decay. Thus, the high nutrient requirement for soft rot reflects specific requirements rather than a general salt effect.

Vermiculite has a high cation exchange capacity that changes the concentrations of nutrients in solution. Although the total amount of cations extracted with HCl is less than that in the nutrient solution, the vermiculite was not pulverized, and it seems likely that the extracted amounts are a small portion of those present on the exchange surfaces. Fungal hyphae may be able to grow between the thin plates of vermiculite and further solubilize the cations.

Ca is not added in the nutrient solutions, but it was extracted in fairly large amounts from the vermiculite. The occasional superior performance of vermiculite over agar observed with some fungi (J. J. Worrall and C. J. K. Wang, unpublished) could be due to a Ca requirement that is satisfied by vermiculite. However, birch blocks contain Ca in excess of any likely requirement for this micronutrient (Table 3).

The added nutrients were essential for obtaining significant soft rot. With respect to potassium phosphates, P appears to be the element controlling decay rate in these experiments, because ample K was always present. Although S was not analyzed, it appears to be the limiting nutrient in $MgSO_4$. Some Mg is present in vermiculite, and the concentration of Mg in blocks did not vary greatly (unlike weight loss) in response to added $MgSO_4$.

King and Waite (1979) demonstrated the translocation of N to wood by fungi, including *C. globosum*, and King et al. (1989) showed that nitrogen content strongly influences the levels of CCA (chromated copper arsenate, a wood preservative) necessary to inhibit soft rot. In the latter study, N concentrations were apparently based on postdecay dry weights, so some of the increase in N concentration was likely due to mass loss during decay rather than mobilization. Mobilization of other nutrients has apparently not been studied previously with respect to wood decay.

In our study, nitrogen was the most actively mobilized of all the nutrients. Increases in other nutrients in the solution led to increased concentration of N in the blocks. The greatest difference between concentrations in uninoculated and inoculated blocks was observed with N. Phosphorus showed a similar, although lesser, tendency, but K, Ca, and Mg showed little evidence of mobilization into the blocks by the fungi. When decay of birch by *C. globosum* was increased by using agar rather than vermiculite as a support, N and P concentrations in blocks were higher, but K, Ca, and Mg concentrations were lower. The higher weight losses when blocks were infiltrated with nutrients rather than water also suggest a limited ability of the fungi to translocate at least some nutrients into the blocks.

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