SUPPRESSION OF LITTER DECOMPOSITION BY MYCORRHIZAL ROOTS OF *PINUS RADIATA*

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(Received for publication 17 September 1974)

ABSTRACT

Two experiments, designed to give more information about the suppression of litter decomposition by radiata pine (**Pinus radiata** D. Don) roots and their associated microflora, were carried out. In the first, root activity was reduced by cutting the boundaries of small areas of the forest floor in an unthinned stand. Samples collected after 3, 6, 9, and 12 months showed that less litter accumulated in areas where root activity was reduced and that the development of mycelial mats in the litter was greatest where mycorrhizal roots were present. Soil moisture content was increased where treatment had involved soil disturbance, but no significant differences attributable to treatment were observed in net amounts of major nutrients or pH. In the second experiment, litter decomposition in the presence of mycorrhizal and non-mycorrhizal roots was studied in the laboratory. Decomposition was slower when mycorrhizal roots were present. It was concluded that the presence of external mycelia of mycorrhizal fungi suppressed litter decomposition. The mechanism of suppression is not clear but moisture and pH did not play an important part in it. Competition for nutrients may have been involved. It is suggested that suppression of decomposition by mycorrhizas may be a major factor in the formation of raw humus.

INTRODUCTION

Gadgil and Gadgil (1971) described an experiment in which the roots of radiata pine (*Pinus radiata* D. Don) were partially excluded from small areas of the forest floor. This showed that the presence of living roots of radiata pine and their associated biota suppressed litter decomposition in some way. In the area where the experiment was carried out, there was an intense development of mycorrhizal roots and of fungal mycelia which were shown to be associated with them. This is typical of raw litter of temperate coniferous forests (Melin, 1925; Romell, 1935; Harley, 1940; Meyer, 1964).

The mechanism by which the living roots suppressed the activity of litter-decomposing organisms was not clear from the 1971 experiment. In a stand of this type where the upper soil layers are almost fully exploited, it can be assumed that the mycorrhizal root/fungus partnership is the dominant biotic influence in the soil of the main rooting zone. The reduced activity of the litter-decomposing organisms may therefore be the result of:

(a) The mycorrhizal roots, their external mycelia, or both being more successful competitors for nutrients or moisture;

(b) The exclusion of obligate saprophytes by direct physical or chemical antagonism by the mycorrhizal roots, their mycelia, or both.

This paper describes two experiments designed to give more information about the suppression mechanism. The first was a field trial in which the relationships between litter accumulation and certain chemical, physical, and microbiological characteristics of soil and litter samples were studied. In the second experiment, litter decomposition in the presence of mycorrhizal and non-mycorrhizal roots was measured in the laboratory.

FIELD EXPERIMENT

Experimental Layout

This study was carried out in a 22-year-old unthinned stand of radiata pine regeneration in Compartment 1039 of Kaingaroa State Forest. The soil in this compartment is classified as Taupo silty sand, upland phase (Vucetich *et al.,* 1960). The stand was pruned at age 11-12 but had received no other silvicultural treatment. There was no undergrowth in the area chosen for the study.

The experiment comprised 48 plots (1 × 1 m) sited between the trees and grouped into three blocks. Four treatments (A, B, C, and D) and four sampling times (3, 6, 9, and 12 months) were randomised among the 16 plots in each block.

Treatment A: Plot boundary cut vertically with a sharp spade to a depth of 30 cm. Litter removed on to a sheet of hessian with minimum disturbance. Plot dug and as many roots as possible removed from the soil within the plot to a depth of 30 cm. Litter carefully replaced.

Treatment B: Plot boundary cut to a depth of 30 cm. Litter removed. Soil dug over to a depth of 30 cm, but no roots removed. Litter replaced.

Treatment C: Plot boundary cut to a depth of 30 cm. No digging or root removal but litter removed and replaced.

Treatment D: Control. No cutting or digging. Litter removed and replaced.

The experiment was laid out in February 1972 and plot boundaries were recut every 2 weeks for one year.

Harvesting and Sampling

After 3, 6, 9, and 12 months, one plot of each treatment was sampled from each block. The litter layer was examined carefully and the development of fungal mycelium was visually assessed according to an arbitrary scale: 0: absent; 1: rare; 2: present but localised; 3: distributed evenly but sparsely throughout the litter layer; 4: abundant throughout the litter layer. All the litter from each plot was then carefully separated from the mineral soil and placed in polythene bags for transport to the laboratory. Cones and branches more than 0.5 cm in diameter were removed, since only fine litter was under consideration.

Two soil cores (12 cm deep × 4 cm diameter) were taken at random from each plot and sealed in airtight jars for determination of field moisture content. Two large samples of soil (15 × 15 cm to a depth of 30 cm) were then taken at random and mixed in a polythene bag. The presence or absence in the plots of (a) visible fungal mycelium in the soil and (b) areas of bleached soil (Will, 1968) were noted.

Laboratory Analysis

Litter samples were dried to constant weight at 70°C and then subsampled by a

quartering technique. Finely ground subsamples were analysed for total nitrogen content by a semi-micro Kjeldahl method (Bremner, 1960) and for organic carbon content by a modification of the Walkley-Black method (Metson, 1956).

The large soil samples were air-dried, mixed and quartered. The following analyses were carried out on the subsamples:

1. pH (glass electrode).
2. Total nitrogen (as for litter samples).
3. Inorganic nitrogen by extraction with KCl followed by steam distillation (Bremner, 1965).
4. Organic carbon (as for litter samples).
5. Cation exchange capacity and exchangeable Na, K, Mg, Ca by methods described in Metson (1956).
6. Total phosphorus by the sodium carbonate fusion method (Jackson, 1958).
7. Available phosphate using Bray No. 2 extractant (Bray and Kurtz, 1945).

The C/N ratio of the soil and litter samples was also calculated.

Results

Data from the measurements carried out on litter and soil samples are summarised in Tables 1 and 2. The experimental design allowed a separation of the effect of the

TABLE 1—Litter analysis data from field experiment (each figure represents the mean of subsamples from three replicate plots)

Harvested after	Treatment	Dry wt (kg)	Total N (%)	Organic C (%)	C/N	Mycelial development
3 months	A. Cut, dug, roots removed	1.89	0.98	33.3	33	2
	B. Cut, dug	2.50	0.99	33.0	33	2
	C. Cut	3.65	0.97	28.4	28	3
	D. Control	3.37	1.19	38.1	32	4
6 months	A. Cut, dug, roots removed	2.72	0.91	24.6	27	0
	B. Cut, dug	2.92	0.82	24.5	31	3
	C. Cut	3.24	1.08	34.2	32	2
	D. Control	4.02	0.96	29.7	31	3
9 months	A. Cut, dug, roots removed	2.91	0.95	24.3	25	0
	B. Cut, dug	1.66	0.99	30.1	31	0
	C. Cut	3.45	1.00	29.8	30	1
	D. Control	4.84	1.09	33.4	30	4
12 months	A. Cut, dug, roots removed	2.98	1.07	28.2	26	0
	B. Cut, dug	1.98	0.94	24.0	25	0
	C. Cut	2.10	1.25	30.4	24	2
	D. Control	4.69	1.16	30.5	26	4

TABLE 2 - Soil analysis data from field experiment (each figure represents the mean of subsamples from three replicate plots)

Harvested after	Treatment	Inorganic N (µg/g)	Total N (%)	Organic C (%)	C/N	pH	Cation exchange capacity (m.e./100 g soil)	Exchangeable bases (m.e./100 g soil) Na	K	Ca	Mg	Total P (ppm)	Available P (ppm)	Moisture (%)
3 months	A. Cut, dug, roots removed	13.38	0.16	3.3	21	5.2	9.6	0.1	0.4	0.3	0.3	399.4	11.33	45
	B. Cut, dug	7.74	0.14	3.7	25	5.2	9.0	0.1	0.3	0.3	0.4	379.5	13.82	52
	C. Cut	13.22	0.13	2.8	22	5.2	8.8	0.1	0.3	0.2	0.3	334.7	13.21	40
	D. Control	7.28	0.15	3.9	26	5.1	10.3	0.2	0.4	0.3	0.4	400.5	15.26	41
6 months	A. Cut, dug, roots removed	8.14	0.15	3.3	21	5.3	9.4	0.1	0.4	0.3	0.4	358.5	15.71	51
	B. Cut, dug	14.39	0.14	3.2	23	5.2	8.3	0.2	0.5	0.3	0.3	353.0	14.63	51
	C. Cut	14.25	0.14	3.5	25	5.2	9.5	0.2	0.6	0.3	0.3	375.7	13.76	52
	D. Control	7.25	0.15	3.3	23	5.2	9.1	0.1	0.5	0.3	0.3	368.0	13.78	50
9 months	A. Cut, dug, roots removed	15.09	0.17	3.0	19	5.1	10.1	0.1	0.8	0.3	0.4	381.3	7.99	58
	B. Cut, dug	12.92	0.15	3.4	22	5.1	9.2	0.1	0.3	0.3	0.4	348.5	11.18	51
	C. Cut	13.87	0.19	3.9	21	5.1	10.6	0.1	0.5	0.4	0.5	392.0	13.23	48
	D. Control	10.93	0.15	2.9	20	5.2	9.0	0.2	0.5	0.3	0.3	362.4	10.53	33
12 months	A. Cut, dug, roots removed	14.37	0.23	4.8	21	5.2	11.9	0.1	0.4	0.4	0.5	380.5	12.63	38
	B. Cut, dug	13.48	0.20	3.8	19	5.2	9.8	0.1	0.3	0.4	0.4	325.5	14.55	33
	C. Cut	19.07	0.16	3.0	19	5.2	9.7	0.2	0.5	0.2	0.2	364.7	11.57	33
	D. Control	11.22	0.17	3.8	22	5.2	12.3	0.1	0.4	0.3	0.4	332.2	6.12	23

cutting treatment (C) from that of digging and cutting (B) in the analysis of variance, but since digging without cutting was impossible, the effect of digging alone could not be assessed. Of all the 18 soil and litter characteristics measured, only litter dry weight, mycelial development in the litter and soil moisture content were found to be influenced by experimental treatment (Table 3).

The cutting treatment (C) caused a large reduction in litter weight per plot. Where plots were dug and cut (treatment B), litter weight was reduced even more. The removal of roots from dug and cut plots (treatment A) had no additional significant effect on litter weight. Time of sampling made no significant difference to the results.

Mycelial development in the litter was found to be significantly reduced by the cutting treatment (C) and by the digging and cutting combination, whether roots were removed or not (treatments A and B). Time of sampling had no significant effect. All samples of mycelia examined possessed clamp connections and were therefore basidiomycetous.

There was significantly less moisture in the plots sampled after 12 months than at other sampling times. Soil moisture content did not appear to be affected by cutting alone (treatment C) but values for dug and cut plots, with or without root removal (treatments A and B), were significantly higher. Large variations in moisture content were observed between individual plots, especially those sampled at 9 and 12 months.

No relationship could be detected between the results of any of the measurements and the degree of soil bleaching or the degree of mycelial development in the soil.

TABLE 3—Summary of analyses of variance of the field experiment, showing degrees of freedom (df) and mean square ratios (MSR) for litter weight, mycelial development, and soil moisture percentage

Source of variation	df	Litter wt MSR	Mycelial development MSR	Soil moisture % MSR
Treatments (T)	3	10.7**	18.7**	4.9*
Treatment contrasts				
(A + B) - (C + D)	1	22.5**	35.0**	10.1**
(C) - (D)	1	8.6**	18.3**	4.2 NS
(A) - (B)	1	1.0 NS	3.1 NS	0.6 NS
Time of Harvest (H)	3	0.7 NS	2.1 NS	13.8**
Blocks (B)	2	4.4*	5.0*	0.7 NS
T × H	9	1.7 NS	1.1 NS	1.2 NS
T × B	6	0.7 NS	2.1 NS	0.3 NS
B × H	6	0.8 NS	0.4 NS	0.4 NS
T × B × H	18	—	—	—

All effects were tested against T × B × H
**P < 0.01 *P < 0.05 NS Not significant P > 0.05

LABORATORY EXPERIMENT

Material and Methods

Seed of radiata pine was surface-sterilised with 10% hydrogen peroxide for 2 hr and sown either (a) in sterile perlite to give non-mycorrhizal seedlings or (b) in a mixture of perlite and duff from a radiata pine stand to give mycorrhizal seedlings. After germination, the seedlings were given half-strength Long Ashton nutrient solution (Hewitt, 1966) twice a week. The pots were placed in a growth cabinet under the following conditions: temperature day 20°C, night 12°C; relative humidity > 90%; light intensity 80 w/m^2; photoperiod 16 hr. When the seedlings were 6 months old they were removed from the pots and their roots were washed thoroughly with sterile water. All seedlings grown in the perlite-duff mixture had profuse development of mycorrhizal roots. None of the seedlings grown in sterile perlite had mycorrhizas. The seedlings were repotted, one seedling per pot, in sterile perlite in preparation for the addition of litter.

The experimental litter was made up of needles collected from near the top of a 30-m-tall radiata pine tree. They were washed in 10 changes of sterile water to remove spores present on their surfaces, dried for 2 days in a flow of sterile air in a laminar-flow cabinet and then chopped in a Waring blendor. In treatments 1, 2 and 3 (see Table 4 for details of the treatments), litter-decomposing fungi were added to the chopped needles to augment their existing microflora. These fungi had been isolated

TABLE 4—Weights of experimental litter and inoculum before and after decomposition (each figure represents the mean of six replicates)

Treatment	Wt of litter added (g) fresh litter + fresh inoculum	Wt of litter added (g) dry litter + dry inoculum	Dry wt of litter after 6 months (g) ± standard deviation
1. Mycorrhizal plant + litter + litter-decomposing fungi	20.00 + 9.8	13.57	8.23 ± 0.2
2. Non-mycorrhizal plant + litter + litter-decomposing fungi	20.00 + 9.8	13.57	7.60 ± 0.3
3. No plant. Litter + litter-decomposing fungi only	20.00 + 9.8	13.57	7.68 ± 0.2
4. Mycorrhizal plant + litter	30.00 —	17.14	9.57 ± 0.1
5. Non-mycorrhizal plant + litter	30.00 —	17.14	8.58 ± 0.03

from radiata pine litter in a previous study (Gadgil, 1970) and they were grown for 5 weeks on autoclaved radiata pine needles before being added to the experimental litter. The species of fungi and the fresh weight of the inoculum of each species added to each pot are given in Table 5. In treatments 4 and 5, litter-decomposing fungi were not added. Pure cultures of litter-decomposing fungi were used rather than natural inoculum so that the possibility of introducing mycorrhizal fungi to treatments with non-mycorrhizal plants was excluded. Samples of the experimental litter and inoculum were oven-dried to constant weight at 70°C so that the dry weight of the experimental litter added to each pot at the beginning of the study could be calculated.

There were six replicates of each treatment. The litter was spread evenly on top of the perlite and the litter surface was covered over with sterilised nylon gauze so that any needles falling from the seedlings were kept separate from the experimental litter. The seedlings were returned to the growth cabinet and they were watered with sterile water twice a week. After 6 months, the litter was removed and dried to constant weight at 70°C.

TABLE 5—Litter-decomposing fungi added to experimental litter

Species	Fresh wt of needle inoculum added to each pot (g)
Coniothyrium sp.	1.0
Mortierella sp.	1.0
*__Pestalotia algeriensis__ (Sacc. & Berl.) Guba	1.5
P. aloes Trinch	1.5
P. antennae formis Murray	1.0
P. neglecta Theum.	1.0
Stemphylium botryosum Wallr.	1.0
Strasseria carpophila Bres. & Sacc.	1.0
Zythia resinae (Ehren.) Karst.	0.8

* We have followed Guba (1961) in the classification of **Pestalotia**.

Results

An intensive development of clamp-bearing hyphae was observed in the litter of pots containing mycorrhizal plants; this was absent from the pots containing non-mycorrhizal plants and there is little doubt that the basidiomycetous hyphae were those of mycorrhizal fungi.

The dry weight of litter from the various treatments after 6 months decomposition is given in Table 4. A comparison of litter weights of those treatments where litter-decomposing fungi were added (treatments 1, 2, and 3) showed that there was significantly more ($P < 0.01$) litter where mycorrhizal roots were present (treatment 1) than where roots lacked mycorrhiza (treatment 2) or where roots were absent (treatment 3). There was no significant difference in the litter weight between treatments 2 and 3. Similar results were obtained when no litter-decomposing fungi were intentionally added to the litter. There was significantly more ($P < 0.01$) litter in treatment 4 (mycorrhizal roots present) than in treatment 5 (non-mycorrhizal roots present).

The results show clearly that litter decomposition was slower when mycorrhizal roots were present. Presence or absence of non-mycorrhizal roots made little difference to the decomposition of litter.

DISCUSSION

The field experiment showed, as before, that reduction of root activity caused by the cutting treatment led to a reduction in litter dry weight. It also confirmed that cutting caused a decrease in the amount of basidiomycetous mycelium present in the litter. Although some of the cut plots contained visible hyphae, development in the uncut plots was much greater. Hyphae seen in the cut plots may have been those of free-living saprophytic fungi or they may have been associated with roots penetrating the plots from below the 30 cm level. (Although the cutting treatment reduced root development considerably, it did not necessarily remove all root influence.) The fact that mycelial development was significantly greater in the presence of roots indicates that the majority of mycelia were associated with the roots in some way. It is possible that they were the external mycelia of mycorrhizas. This inference is supported by the laboratory experiment where basidiomycetous mycelial wefts were found only in the pots containing seedlings with mycorrhizal roots. The laboratory experiment also established that litter decomposition was suppressed by mycorrhizal roots rather than by roots alone.

Suppression of microorganisms by mycorrhizal fungi growing in association with roots has been shown by Krywolap *et al.* (1964) and by Marx and Davey (1969). In both studies, secretion of antibiotics by the mycorrhizas was demonstrated. The suppression we observed may therefore have been due to antibiotic substances. A number of other explanations are possible. For instance, uptake of water by the mycorrhizal roots could reduce the amount of moisture present to a point where litter decomposition is limited. In fact, soil moisture was not shown to be affected by root cutting without digging. It is of course possible that undetected critical differences in moisture content occurred between harvests. In the laboratory experiment moisture supply could not have been the limiting factor as all pots were watered adequately. Again, there was little difference in soil pH between various treatments of the field experiment and it would seem unlikely that either moisture or pH played a major part in limiting litter decomposition.

Uptake of major nutrients by the trees from the uncut plots did not significantly affect the levels of nutrients measured in soil and litter samples. However, the possibility that suppression of decomposition by the mycorrhizal roots was the result of competition for nutrients (Romell, 1935) cannot be disregarded. In the uncut plots, significant quantities of nutrients in short supply could have been present as cell constituents of the mycorrhizal mycelia (Stark, 1972). These would not be available to the saprophytic organisms while the hyphae were alive and, unless massive death of the mycorrhizal mycelia occurred (for instance, as a result of the cutting treatments), the activity of the saprophytes might be limited by shortage of nutrients. Death and decomposition of the mycorrhizal mycelia would release nutrients which would be used by the saprophytic organisms without any net loss or gain to the system.

A further possible explanation for suppression is that the hyphae of mycorrhizal fungi, not being dependent on the litter for an energy source, were capable of extensive colonisation of the litter and physically excluded the free-living organisms from microsites within the litter.

Whatever the mechanism of the suppression, these experiments have shown that mycorrhizal fungi play an important part in determining the course of litter decomposition. Once the mycorrhizal hyphae have permeated the litter, their influence on the free-living litter-decomposers results in the low microbial activity which is typical of raw humus (Meyer, 1959). Our results support the suggestions of Romell (1935) and Mikola (1963) that mycorrhizal fungi may make a considerable contribution to raw humus formation. Romell (1938) noted increased litter decomposition in trenched plots in a spruce stand. He attributed this to the combined effect of green manuring (from severed roots) and reduced competition from mycorrhizal fungi. Our experiments indicate that reduction of the activity of the mycorrhizal fungi would have been the more important factor.

Mycorrhizal control of litter decomposition may be a mechanism whereby the nutrient pool in stands where the upper soil layers are almost fully exploited by trees is conserved against such processes as leaching or volatilisation. It may even contribute to the stability of the climax community in temperate regions.

ACKNOWLEDGMENTS

We would like to thank the many people who assisted with maintenance, sampling and analytical work, in particular Mrs J. Muir and Miss L. Nathan. Dr W. Armstrong gave valuable advice on statistical procedure. We are most grateful to a number of colleagues who suggested improvements to the manuscript, especially Dr C. Bassett, Dr J. Butcher, Mr I. Hood, and Dr D. Rook.

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