CHAPTER 8

NITROGEN MOBILIZATION AND IMMOBILIZATION UNDER CONTROLLED CONDITIONS

8.1 EFFECT OF SLASH COMPONENTS ON MINERALISATION OF NITROGEN

INTRODUCTION

It is possible that some of the residues left after clear felling of the mature P.radiata trees provided enough energy material to allow a proliferation of micro-organisms in the soil and an immobilization of mineral nutrients. (Refer to Jannson, 1966, and Kuo and Bartholomew, 1966, for excellent introductions to this topic). In order to study the effects of individual components of slash on net mineralisation, an experiment was set up where each slash component was added to the soil in amounts likely to be present under field conditions.

METHOD

Soil samples each of 330 g (oven dry weight) collected from under a mature P.radiata stand (P31) were placed in

10 cm plastic pots and the treatments set out in Table 8.1.1 were added. The amount of each slash component added was estimated from data given by Will and Orman (1960) and Ovington (1954). The removal of bole material was compensated for. Wood and bark were ground before addition.

TABLE 8.1.1

Proportion of slash components added to 330 g (o.d.) of mature P.radiata forest soil for the nitrogen mineralisation investigation.

	Components	lb/2 million lb of soil
1.	Above ground bark	3,000
2.	Above ground wood	16,000
3.	Fresh needles	7,000
4.	Total above ground (bark, wood and needles)	26,000
5.	Undecomposed litter from P31 Stand	12,000
6.	Root wood	75,000
7.	Root bark	8,000
8.	Total below ground (root, wood and bark)	83,000
9.	Total organic matter	121,000
10.	Celulose (= amount of total O.M.)	121,000
11.	$\frac{1}{2}$ Glucose(= $\frac{1}{2}$ " " " ")	60,500
12.	$\frac{1}{4}$ Glucose(= $\frac{1}{4}$ " " " ")	30,250
13.	$\frac{1}{8}$ Glucose(= $\frac{1}{8}$ amount of total O.M).	15,125
14.	Cellulose (ball milled)	121,000
15.	Control	Nil

P. radiata seedlings were planted in the soil so that the actual nitrogen supply to the plant could be investigated over a period of time. This method was used in preference to sampling for mineral nitrogen levels at various intervals because these static figures would not give as good a picture of the nitrogen supplying power of the soil. The soil samples in the plastic pots were placed on top of 600-ml beakers. Excess applied water was retained in the beakers and poured back over the soil when soil moisture levels subsequently dropped. This procedure eliminated any leaching of soluble nutrients. One seedling of *P.radiata* collected from Tasman Forest, and germinated on damp filter paper, was planted in each pot.

All pots were kept in a glasshouse with temperature and humidity control and were watered every second day. Root systems were extracted from the soil by a jet of water. Shoot and root portions were oven dried at 102 $\pm$ 1° C separately and weighed after being cooled in a desiccator.

RESULTS AND DISCUSSION

The results are presented in Table 8.1.2. Only the application of fresh needles stimulated dry matter production in this experiment. Litter depressed the growth rate although one may have expected a stimulation in the growth rate when litter was added since the litter was mainly composed of partially decayed needles. It appears that the release of nutrients from fresh needles was greater than release from

Table 8.1.2 Weight (o.d.) of shoot and root of P.radiata seedlings grown in soil treated with residues remaining after clear felling.

Treatment		Shoot weight in g	Root weight in g	Shoot/ root %	Total(1+2) Dry weight	Average
Control	1	1.0876	0.4452	246.4	0.7664	0.7117
	2	0.9152	0.3986	212.4	0.6569	
Litter	1	0.6133	0.2710	226.3	0.4422	0.5559
	2	0.7090	0.6300	112.5	0.6695	
Needles	1	0.8307	0.4546	182.7	0.6427	0.7672
	2	0.9978	0.7857	127.0	0.8918	
Root wood	1	0.4210	0.1508	279.2	0.2859	0.2996
	2	0.3687	0.2577	143.1	0.3132	
Root bark	1	0.7091	0.3487	203.4	0.5289	0.4367
	2	0.4417	0.2472	178.7	0.3445	
Shoot wood	1	0.2094	0.4129	50.7	0.3112	0.4365
	2	0.6807	0.4431	153.6	0.5619	
Shoot bark	1	0.7876	0.2765	284.8	0.5321	0.4728
	2	0.5625	0.2644	212.8	0.4135	
Below ground	1	0.3038	0.1791	169.6	0.2415	0.2832
	2	0.3334	0.3164	105.4	0.3249	
Above ground	1	0.3775	0.2212	170.7	0.2994	0.2585
	2	0.3009	0.1343	224.1	0.2176	
Total O.M.	1	0.2599	0.2607	99.7	0.2603	0.2350
	2	0.1716	0.2477	69.3	0.2097	
Cellulose	1	0.1660	0.0815	203.7	0.1238	0.1109
	2	0.1196	0.0765	156.4	0.0981	
$\frac{1}{8}$ Glucose	1	0.7207	0.2870	251.0	0.5039	0.4605
	2	0.5452	0.2890	188.7	0.4171	
$\frac{1}{4}$ Glucose	1	0.2958	0.1956	151.2	0.2457	0.2186
	2	0.2590	0.1240	208.9	0.1915	
$\frac{1}{2}$ Glucose	1	0.5200	0.1963	264.9	0.3582	0.3122
	2	0.3500	0.1826	191.7	0.2663	
Glucose	1	0.0921	0.0792	116.3	0.0857	0.1153
	2	0.1714	0.1184	144.8	0.1449	

Fig.8.1.3 *P.radiata* seedlings growing on the soil taken from under mature *P.radiata* at Tasman Forest. Soil was treated with cellulose, leaf litter and the organic matter left after clear felling.

Fig.8.1.4 *P.radiata* seedlings growing on soil taken from under mature *P.radiata* at Tasman Forest. Soil was treated with cellulose, pine needles and woody shoot residue left after clear felling.

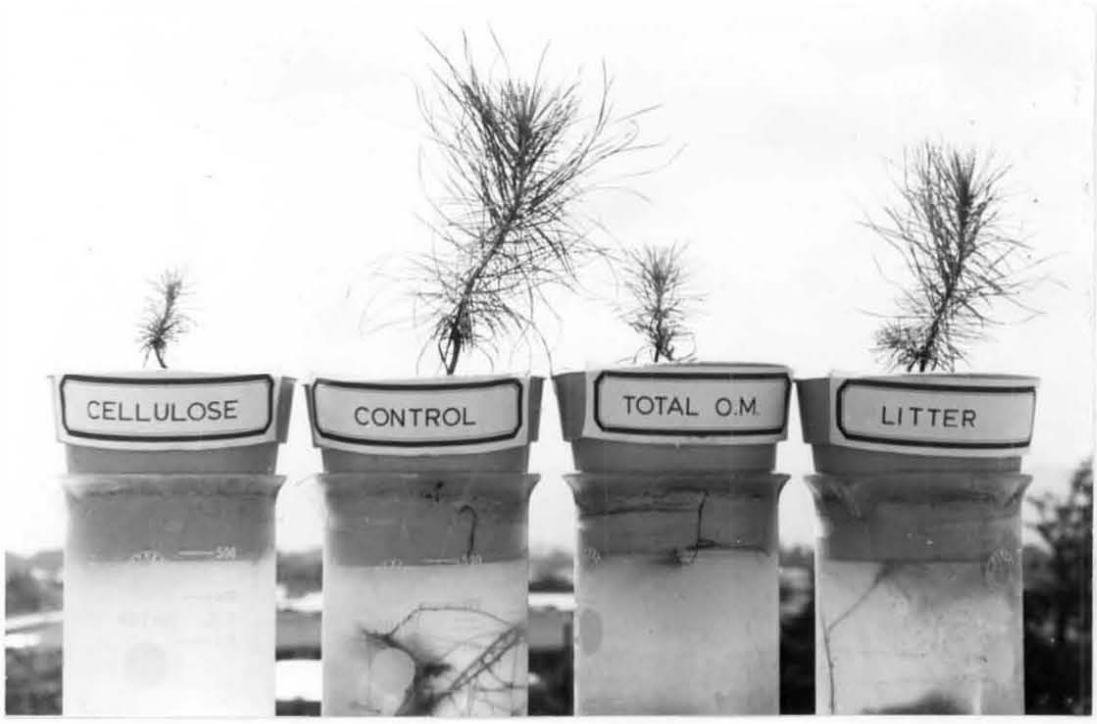


Fig.8.1.3

Fig.8.1.4

the partially decomposed litter. This was surprising in view of the greater energy to nutrient ratio probably present in the fresh needles. Perhaps the fresh needles do have a lower energy to nutrient ratio but these nutrients may be leached out rapidly on the death of the needles.

Of the woody components added root wood caused the greatest depression in growth. Root bark, shoot wood and shoot bark caused approximately equal reduction in growth. The substantial effect of bark in comparison with wood was in contrast to the slow decomposition of bark observed in the field, but it should be noted that in this experiment the bark was ground and thus would have been more available for decomposition.

The small difference between the reduction in growth caused by total above ground material and total below ground material was surprising in view of the large reduction caused by the root wood in contrast to the shoot wood. The inclusion of needles in the above ground portion might have been expected to have made the above ground retardation in seedling growth greater than that caused by the below ground components.

The inclusion of all O.M. likely to be present on the site after clear felling caused a retardation in the growth of pine seedlings to less than half of the plants growing in control soil. The same amount of pure cellulose caused a further 50% decline.

The addition of a readily available substrate such as glucose equal in weight to one-eighth of the total O.M. caused a depression in growth equal to that caused by the root bark. Glucose equal to one-quarter of the total O.M. decreased the growth rate to approximately that of the total O.M. itself. Therefore only one-quarter of the total organic matter, or less since root and shoot bark and wood were ground before addition, was as available to decomposition as glucose. Since organic matter provided mineral nutrients as decomposition occurred and glucose did not, the equivalence was probably closer to one-eighth glucose and total O.M.

The depression of growth caused by the addition of 60,500 lb/acre glucose (= $\frac{1}{2}$ glucose) appears to be anomalous, but no explanation can be found to explain the anomaly. The addition of glucose in an amount equivalent to the total O.M. had an equal depressing effect as did the same quantity of cellulose. Since glucose was more rapidly respired it may have caused greater immobilization initially but subsequently its effects disappeared. This meant that the more slowly decomposing cellulose caused an equal amount of depression of growth.

Immobilization of nutrients by slash components may therefore cause some reduction in growth of trees in Tasman Forest. However, since both growth and decomposition rates would have been higher under glasshouse conditions the effect measured in this experiment may not apply in the field. It is, however, possible that at some times during

the year when the climate is favourable this may occur.

8.2 THE EFFECTS OF THE ADDITION OF GLUCOSE AMMONIUM AND NITRATE NITROGEN ON MINERAL NITROGEN LEVELS IN SOIL AND DUFF SAMPLES

INTRODUCTION

Because several workers (Jansson, 1958; Broadbent and Tyler, 1962; Overrein, 1967a, 1967b; Simpson and Freney, 1967; Winsor and Pollard, 1956a, b, c; Nommik, 1956; and Jansson et al. 1955) have reported that ammonium is used preferentially to nitrate by soil micro-organisms, an experiment was set up to test this in Tasman Forest soil. Ammonium nitrogen was also added to the soil and duff samples to determine if any nitrification occurred within a period of two months under high levels of NH_4 - N. Glucose was added to provide an energy substrate to determine whether ammonium or nitrate would be preferentially used in the presence of high levels of easily available material. This experiment was part of one described in Section 6.13 where CO_2 evolution rates for the different treatments were measured.

METHOD

The method used has been outlined in Section 6.13. The analytical method for mineral nitrogen is described in Section 9.2. The results were not subjected to analysis of variance because the form of nitrogen may have changed throughout the course of the experiment and this complication would have made interpretation difficult. The experiment

was factorial in design, and NH_4 -N, NO_3 -N and glucose were added to the soils and duff as outlined in Section 6.13.

RESULTS AND DISCUSSION

Soil from 25 cm depth (Fig. 8.2.1)

Initially this soil contained 6 ppm NH_4 -N but no NO_3-N. After two months incubation the level of NH_4 -N increased by 11 ppm. If field conditions were the same as those in this experiment the field soil at 25 cm should show a net mineralization of 130 lb NH_4 -N/acre annually if it is assumed that 10 cm of soil weighs 2 million lb/acre. When glucose was added very little mineral N was detected after incubation, and when NH_4 -N was added mineralisation of soil nitrogen increased the final content by 7 ppm. Although no analysis of variance was undertaken the 7 ppm is probably not significantly different to the 11 ppm mineralised without added NH_4 -N. The presence of high levels of mineral -N does not appear to inhibit net mineralisation. This is useful to know if nitrogen fertilizer is to be applied.

When NO_3 -N was added some of the NO_3 -N was immobilised but the net mineralisation rate of NH_4 -N was unaffected. Since some NO_3 -N was used in the presence of NH_4 -N it is possible that the higher concentration of NO_3 -N compared with NH_4 -N meant that the NO_3 -N was immobilized to some extent.

When both glucose and NH_4 -N were supplied together 74 ppm of the resulting NH_4 -N pool was used compared with

the treatment where glucose alone was supplied in which only 16 ppm of the NH_4 -N existing in the soil and mineralised during the experiment was used.

Since the glucose main effect was the only treatment which significantly increased the CO_2 evolution rate and no interactions of NH_4 - G were significant, the added NH_4 -N may have allowed more of the carbon contained in the glucose to be synthesized into microbial tissue. In other words the production of new biomass was increased under conditions of high mineral -N in the soil.

When NO_3 -N and glucose were added together all the NO_3 -N and all except 4 ppm NH_4 -N was used. This interaction did not cause a significant effect on CO_2 evolution which was again possibly due to the increased efficiency of the micro-organisms supplied with extra nitrogen. When NH_4 -N and NO_3 -N were applied together NO_3 -N remained unused while some of the NH_4 -N was immobilised even when in the same soil without treatment net mineralisation occurred.

When NH_4 -N, NO_3 -N and glucose were added together more NH_4 -N than NO_3 -N was used. This supports the results of those who have found preferential utilization of NH_4 -N by soil micro-organisms. The discrepancy between this latter result and the result found when NO_3 -N alone was added may be due to the effects of a high concentration of a particular species of nitrogen causing the uptake of this species greater than is the case when this species is present in lower concentrations. When both nitrogen species were present in

Fig.8.2.1 Mineral nitrogen levels in soil samples with added NH_4 -N, NO_3 -N and glucose from 25 cm depth collected on five ridge and gully sites in Tasman Forest incubated for two months under laboratory conditions.

Fig.8.2.2 Mineral nitrogen levels in soil samples with added NH_4 -N, NO_3 -N and glucose from 0 - 2.5 cm depth collected on five ridge and gully sites in Tasman Forest incubated for two months under laboratory conditions.

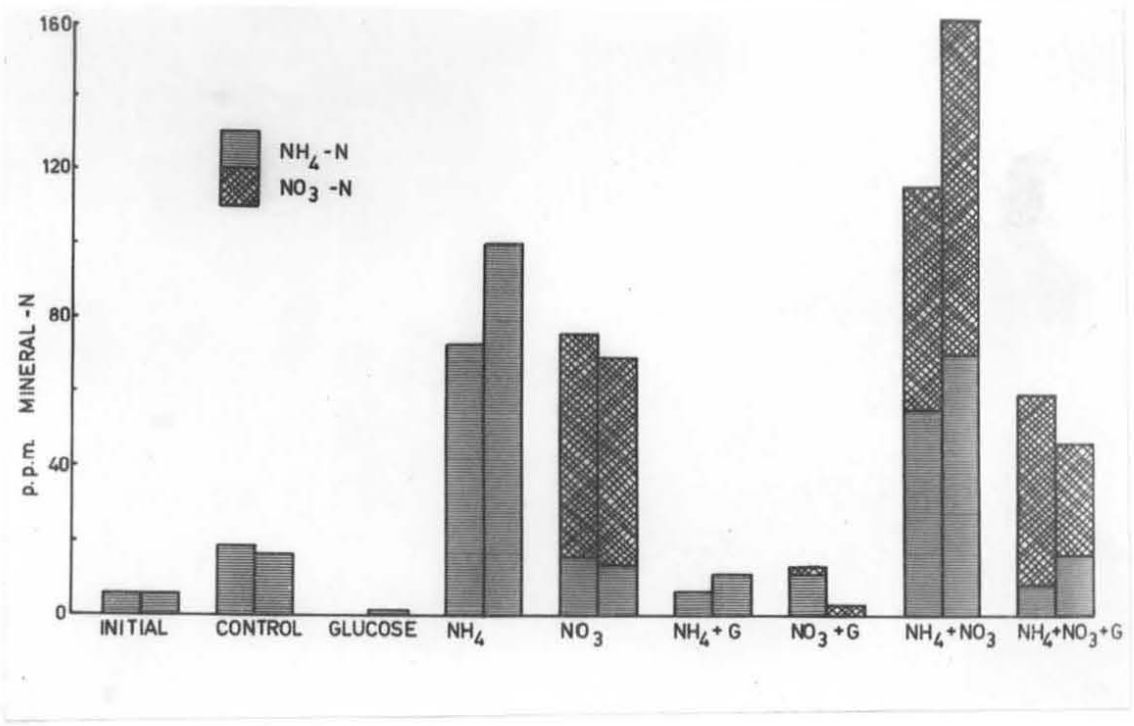


Fig.8.2.1

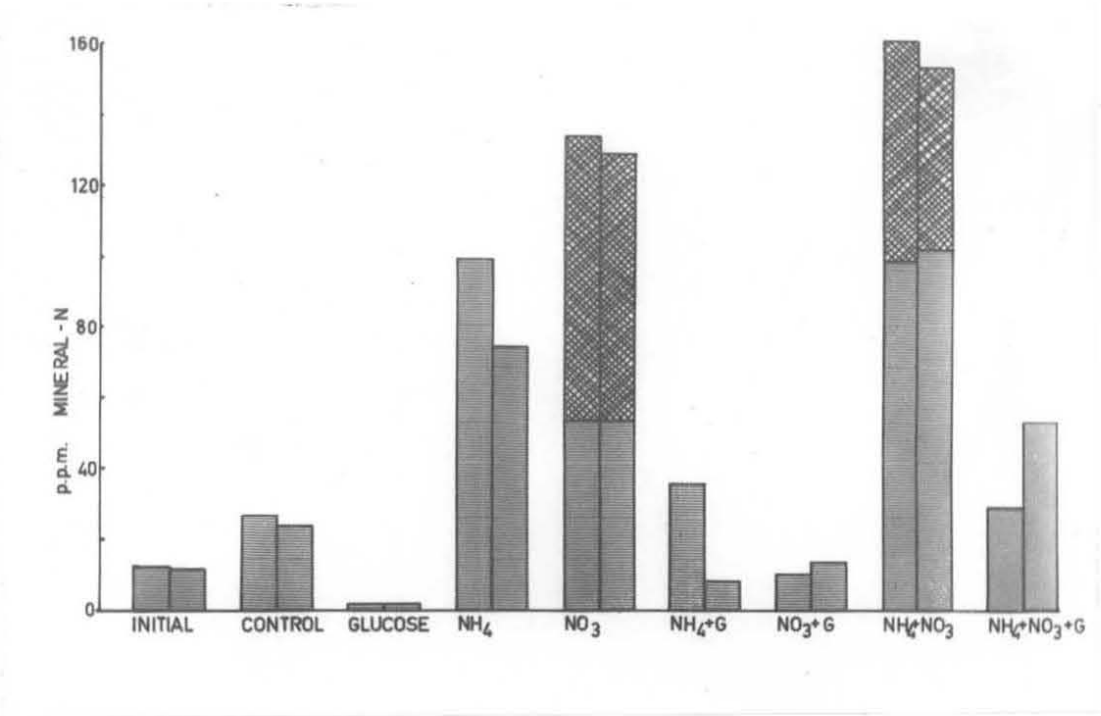


Fig.8.2.2

equal amounts NH_4 -N was used preferentially.

Soil samples from 0 - 2.5 cm depth (Fig.8.2.2)

The initial value of 11 ppm NH_4 -N in the soil sample increased to 25 ppm in the two months' incubation. This represents the equivalent of a net mineralisation in the field of 168 lb/acre/annum based on the assumptions outlined in the previous section. The addition of glucose increased the mineral nitrogen level to 2 ppm. When NH_4 -N was added 14 ppm of NH_4 -N mineralised. This result supports that obtained using soil from 25 cm depth when it was found that mineralisation was not affected by high levels of NH_4 -N.

In the presence of NO_3 -N net mineralisation was stimulated. This result was in contrast to that found with soil from 25 cm depth when NO_3 -N levels stayed constant. A change of pH with added NO_3 -N may have been responsible for the stimulation. This observation may be of some practical value should it be decided to use fertilisers in Tasman Forest.

When NH_4 -N and glucose were added together net immobilization occurred and 67 ppm of NH_4 -N was immobilized with the decomposition of glucose. When NO_3 -N and glucose were added all NO_3 -N was used. The addition of NH_4 -N and NO_3 -N brought about the immobilization of some of the NO_3 -N and the preferential use of NH_4 - was not demonstrated in this instance. Some of the NO_3 -N may have been converted into NH_4 -N because the difference between the NH_4 -N level in this treatment and the NH_4 -N level in the treatment

where NH_4 -N was applied by itself was approximately equal to the loss of 18 ppm of NO_3 -N from the 75 ppm applied to the soil at the start of the incubation.

When NH_4 -N, NO_3 -N and glucose were added together all the NO_3 -N was used, together with much of the NH_4 -N. The NH_4 -N remaining may have been re-mineralised after being immobilized because of the addition of a very available energy substrate. In the soil taken from 25 cm depth the NO_3 -N in the NO_3 -N, NH_4 -N and glucose treatment was not completely used. This perhaps indicates that nitrogen turnover was more rapid in soil from the 0 - 2.5 cm depth.

The major difference in the soil samples taken from the 0 - 2.5 cm and 25 cm depths was where the addition of NO_3 -N caused greater mineralisation of NH_4 -N in the 0 - 2.5 cm soil sample, some of the NO_3 -N was used and no stimulation of the NH_4 -N mineralisation rate occurred in the soil from 25 cm.

Duff collected from under mature P.radiata (Fig.8.2.3)

The initial NH_4 -N level increased from 138 ppm to 355 ppm; an increase of 217 ppm in two months. This is equivalent to 18 lb mineral -N/acre/year in the field. The net mineralisation rates observed in this experiment are obviously not those which occur under field conditions but they do give some idea of the potential supplying power of the soil under ideal conditions.

When glucose was added approximately 50 ppm NH_4 -N was immobilized, which was slightly less than the amount immobil-

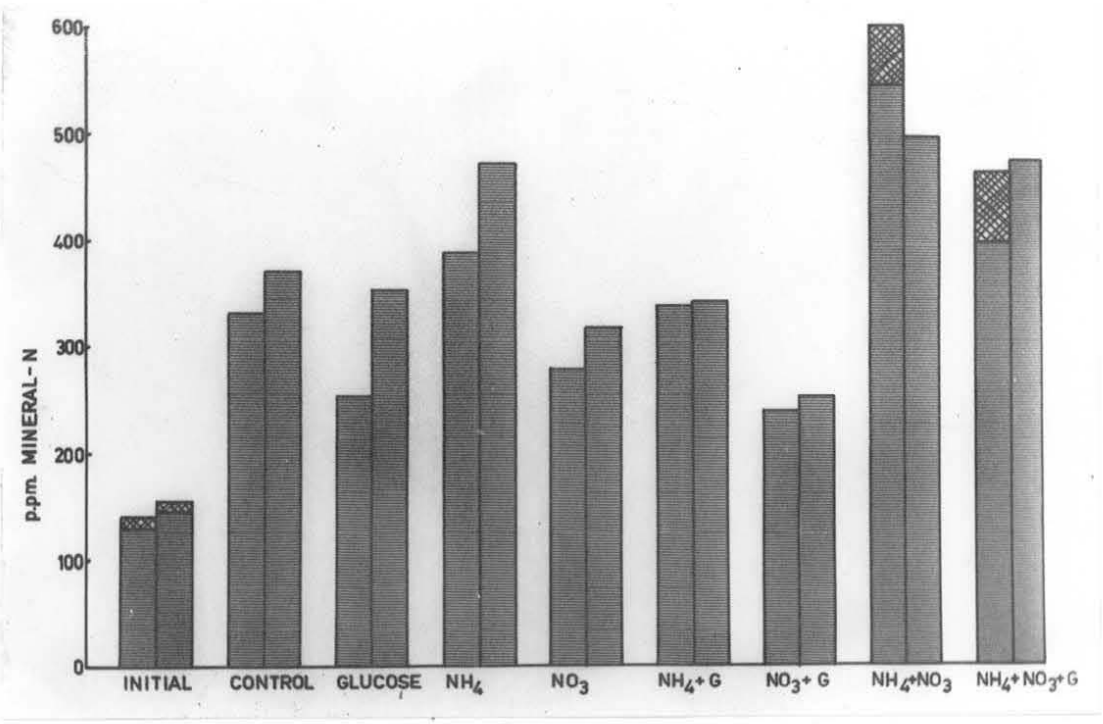


Fig.8.2.3

Mineral nitrogen levels in duff samples collected under mature P.radiata trees in Tasman Forest incubated for two months under laboratory conditions.

ized in the two soil samples. The addition of NH_4 -N did not decrease the rate of net mineralisation of NH_4. This was also observed in the soil samples tested.

No NO_3 -N was detected when analyses were carried out even where 75 ppm of NO_3 -N had been added at the start of the experiment. The level of NH_4 -N in the treatment where NO_3 -N was applied was lower than in the untreated sample, and well below that recorded for the NH_4 -N treatment when the added NH_4 -N was subtracted from the value recorded at the end of the experiment. The added NO_3 -N apparently caused some reduction in net mineralisation rate from 217 ppm to 80 ppm.

When glucose and NH_4 -N were added together approximately 90 ppm nitrogen was immobilized. This is almost twice as much as in the treatment with glucose alone. The difference between the NO_3 -N treatment and the NO_3 -N plus glucose treatment was only 46 ppm. Therefore, glucose caused the immobilization of more NH_4 -N (90 ppm) than NO_3 -N (46 ppm). This supports the observation that NH_4 -N was used preferentially to NO_3 -N.

With the addition of NH_4 -N and NO_3 -N some of the NO_3 -N was used even in the presence of large amounts of NH_4 -N. Net mineralisation of nitrogen from soil organic matter was partially reduced when 150 ppm of nitrogen was added. The addition of glucose, NH_4 -N and NO_3 -N gave a level approximately 80 ppm lower than that without added glucose. This result is similar to that obtained when

NH_4 -N and glucose were added. In contrast to the results presented by Robinson (1962) and Jones (1968) at no time was nitrification observed even when large amounts of NH_4 -N were present. Nitrifying bacteria were probably limited by some feature of the soil, for example, pH.

This experiment has shown that the addition of NO_3 -N and NH_4 -N to duff and soils from different depths causes different results, and therefore judgment of which was the best species of nitrogen to apply as fertiliser is more complicated than might appear at first sight.

8.3 GROWTH OF P.RADIATA SEEDLINGS ON SOIL COLLECTED FROM UNDER TREES OF DIFFERENT AGES OF REGENERATION.

METHOD

Two sets of two soil cores were extracted from each site on 25/9/68, using the method described in Section 6.15.

Cores were divided into 0 - 12 cm and 12 - 25 cm lengths and the two half cores were placed in a plastic bag, giving a total of four bags per site. Bags were tied, placed in a shallow hole and covered with 3 ± 1 cm of soil. All bags were collected on 5/5/69.

Soil collected to a depth of 20 cm from Balmoral State Forest (Compartment 31, P26, clear felled 1959) and Hanmer State Forest (Compartment 25, felled 1959) on 12/66 to a depth of 20 cm was air dried a few days after collection. Soil was also collected from Lincoln College and stored for 12 months before use.

Plastic pots 10 cm in diameter were half-filled with washed 1 cm stones and the cores were placed on top of the stones.

Soils from places other than Tasman Forest were not collected as core samples and therefore the volume of soil added was approximately equal to the volume of the Tasman Forest half cores.

Seeds of P. radiata collected at Tasman Forest on 5/5/69 were germinated on filter paper dampened with distilled water and one seedling was planted in each pot.

Pots were kept initially in a glasshouse where temperature and humidity were not controlled. Later improvements to the glasshouse allowed the humidity to be stabilised at 93 ± 2% R.H. and the temperature at 18 ± 30° C, but malfunction caused excessive dryness on one occasion which killed many of the young plants. These pots were replanted with seedlings from the original germination which were not markedly smaller than the potted plants that had survived the dryness. Pots were placed on inverted petri dish lids and sufficient distilled water was added to the soil surface to fill the petri dish whenever the dishes became dry. Weeds were removed and placed on the soil surface. Malfunction of the glasshouse control system again caused desiccation of the plants.

Insufficient time remaining before completion of the thesis meant that this experiment had to be abandoned.

CHAPTER 9

NITROGEN MOBILIZATION AND IMMOBILIZATION UNDER FIELD CONDITIONS

9.1 AVAILABLE NITROGEN

REVIEW OF LITERATURE

Many workers have attempted to assess the amount of nitrogen in the soil which may become available to plants by measuring some fraction of the nitrogen present in the soil. The choice of a particular method for determining the potentially available nitrogen status of soils has usually been based on the degrees of correlation between variously determined available nitrogen indices and nitrogen uptake by plants and, or, response to nitrogen fertilisation (Stanford and Legg, 1968). In most ingestigations some unknown portion of the total potentially available nitrogen has been measured by an arbitrary, but standardised, technique.

Most of the nitrogen which becomes available to growing crops each year is the result of mineralisation of a small part of the soil organic matter by microbial processes. The rate at which the mineralisation takes place is dependent upon

very many factors including temperature, moisture, aeration, type of organic matter in addition to the amount present that it is unlikely that determination of the amount present will provide a reliable index of the available nitrogen supply (Smith, 1966). These variables help to explain the large differences in correlation coefficients found by workers (Prasad, 1965; Smith, 1966; Hunter and Carter, 1965; Stanford, Ayres and Doi, 1965; Ozus and Hanway, 1966; Spencer, et al. 1966; Kresge and Merkle, 1957; Stanford and Legg, 1968; Keeney and Bremner, 1966; Robinson, 1967a, 1967b, 1968a, 1968b, 1968c; Jenkinson, 1968). Thus, in order to obtain significant results it is necessary to evaluate the method on the soil being investigated, and furthermore these results need to be correlated with the growth, in the field, of the particular plant species involved before any real predictive use can be made of the index. Most indices reported show some degree of correlation with plant growth measured either in the laboratory or in the field. In nearly all investigations it has been found that total nitrogen shows the lowest correlation while the quantity mineral nitrogen produced on incubation usually shows the highest correlation. Chemical indices have usually shown correlations coefficients of intermediate value.

Because it is almost impossible to assess the growth of pine trees accurately, it would have been difficult to demonstrate the correlation between the growth of mature pine trees and a chemical index of potentially available nitrogen especially with soil samples from different depths. There-

fore a chemical method involving extraction of nitrogen with boiling water, which has been shown to give consistently good correlations, was chosen, (Livens, 1959; Robinson, 1968b; Keeney and Bremner, 1966; and Jenkinson, 1968). This method has the advantage that it is less affected by the preliminary handling and storage of the soil samples and can be done more quickly than the incubation test (Robinson, 1968b) since it can be completed in a single analysis.

It was of particular interest to find out whether this method would be sensitive enough to distinguish the differences in availability of soil nitrogen between soils from an area of fairly uniform vegetation and climate. This experiment was also designed to determine whether nitrogen availability was greater in soils from gully sites than ridge sites and also if the amount of available nitrogen in the soil changed with age of the regenerating crop and the deposition of crop residues.

METHOD

Soil samples collected from ten sites (LP31, UP31; LR66, UR66; LR65, UR65; LR63, UR63; LR60, UR60) and four depths (0-2.5 cm, 5 cm, 10 cm and 25 cm) in Tasman Forest on 15/3/67, 4/7/67, 28/8/67 and 5/6/68 were mixed in equal amounts and finely ground to pass through a mesh of .016 inch diameter. A similar series of soils collected from the same sites on 25/9/68, 2/1/68 and 3/2/68 was treated in the same way. The first series of soils was mainly collected in

winter and stored for a longer period of time than the second series which was mainly collected in summer. Analyses began in May, 1969. Samples were also collected from Hanmer and Balmoral Forests and stored air dry for two years before use.

Duplicate samples, each of 5 g of soil from each series, were mixed with 30 ml of distilled water and the suspension was boiled under reflux for 60 minutes according to the method of Keeney and Bremner (1966). Twenty ml of a 10% (W/V) K_2SO_4 solution were added to the cooled soil-water mixture, which was allowed to settle before 20 ml of the clear supernatant liquid was put into a 50-ml Kjeldahl flask. Two ml of concentrated H_2SO_4 were added and the mixture heated on a hot plate. Earlier experiments showed that if a layer of brass filings, about 2 inches deep, were put on top of the hot plate and the flasks were embedded in the filings all flasks could be heated uniformly at 240° C. Heating was continued until the digest cleared. Digestion was then continued for a further 20 minutes on a similar plate but at 320° C. The flasks were allowed to cool and 10 ml distilled water were slowly added. Steam distillation and Nesslerization were carried out as described in Section 9.2.

Factors used in the analysis of variance were as follows :

1) Series:

Series (1) collected on 15/3/67, 4/7/67, 28/8/67 and 5/6/68.

(2) collected on 25/9/68, 2/1/68, and 3/2/68.

2) Depth:

Soil from (1) 0-2.5 cm from a ridge and gully position on each of five sites

(2) 5 cm from a ridge and gully position on each of five sites.

(3) 10 cm from a ridge and gully position on each of five sites.

(4) 25 cm from a ridge and gully position on each of five sites.

3) Position:

Gully - mean of five sites

Ridge - " " " "

4) Age: (1) 0 years after clear felling

(2) 1 year " " "

(3) 2 years " " "

(4) 4 " " " "

(5) 7 " " " "

RESULTS AND DISCUSSION

The results and analysis of variance are presented in Appendix 9.1A.

The variation attributable to series is not significant. This means that time of collection and effects of storage had no overall effect on the results unless storage and time of collection are interacting in such a way as to reduce the total effect. The absence of any overall effect

Fig.9.1.1 Amount of available nitrogen estimated using the method of Keeney and Bremner (1966) in soil samples collected at four depths from five ridge and gully sites under P.radiata stands in Tasman Forest.

Fig.9.1.2 Amount of available nitrogen in soil samples from four ridge and gully sites collected at four depths under P.radiata regeneration in Tasman Forest.

Fig.9.1.3 Amount of available nitrogen in soil samples collected from five age classes of P.radiata at four depths on both ridge and gully sites.

Fig.9.1.4 Amount of available nitrogen estimated by the method of Keeney and Bremner (1966) in soil samples collected at four depths on ridge and gully sites under P.radiata stands of five different ages.

Fig.9.1.5 Amount of available nitrogen in soil samples from ridge and gully sites under five age classes of P.radiata. Samples were collected at four depths.

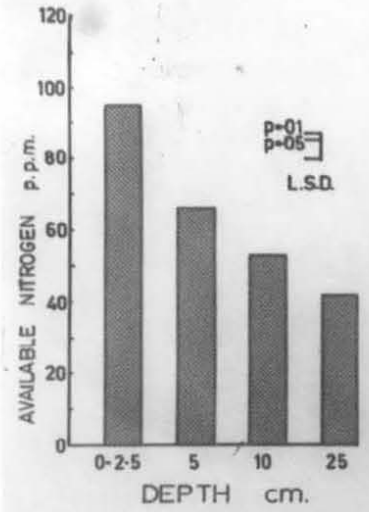


Fig.9.1.1

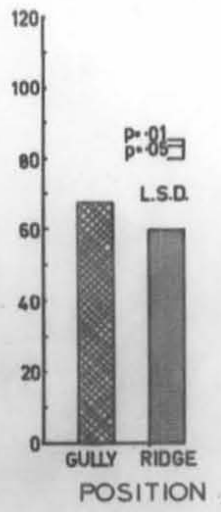


Fig.9.1.2

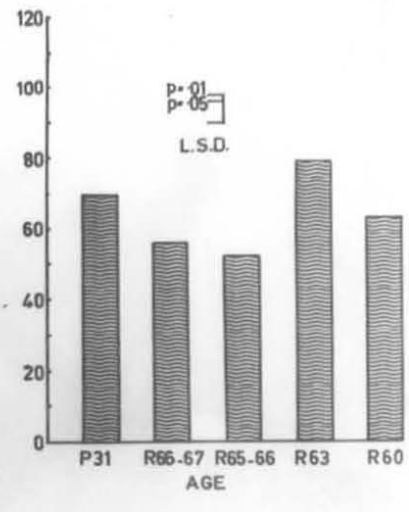


Fig.9.1.3

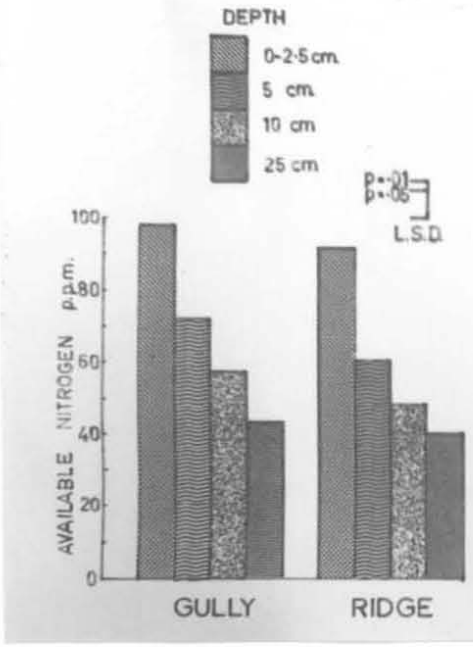


Fig.9.1.4

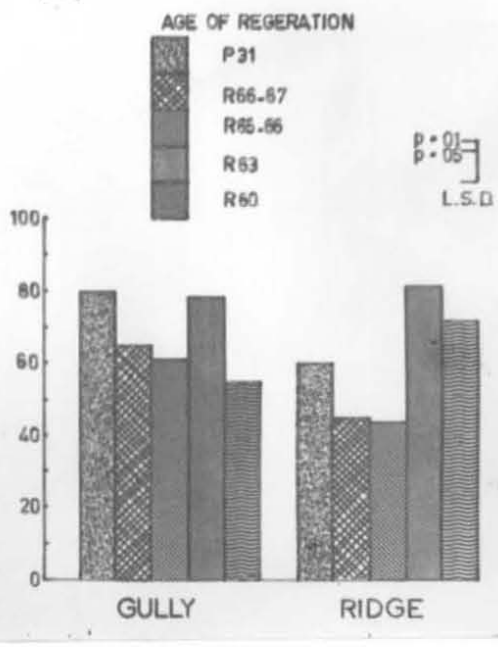


Fig.9.1.5

is in accordance with the results of Keeney and Bremner (1966) and Jenkinson (1968). On the other hand two first order interactions containing series were significant. An examination of the interaction, shown in Fig. 9.1.8 and Fig.9.1.9, demonstrates that soil samples from the ridge sites were affected more by storage and/or time of sampling than those from gully sites. Also the effect of sampling time or storage was more manifest in the R63 samples than in soil samples from other ages.

It is difficult to explain these interactions as insufficient is known about soil nitrogen on ridge sites and soil nitrogen under six-year regeneration to help explain why these should behave differently to soil nitrogen from gully or other age class sites.

Depth (Fig. 9.1.1), Position (Fig. 9.1.2) (Ridge Sites vs. Gully Sites) and Age (Fig. 9.1.3) (age of regeneration after clear felling) were all significant. The Position x Depth (Fig. 9.1.4) interaction table supports field observation that the main difference between ridge and gully sites was that the ridge sites possess a shallower top soil layer. Available nitrogen also followed this trend as shown in this experiment where the greatest difference between ridge and gully soil samples was found at the 5 cm depth followed by the 10 cm and 0-2.5 cm depths. However Kingston (1968) was unable to find a good correlation between position and depth of topsoil judged on a colour basis.

The Depth x Age (Fig.9.1.7) interaction showed the

greatest difference at the 0-2.5 cm depth and then a gradual reduction in difference until there was little noted at the 25 cm depth. The age classes with the greatest available nitrogen levels (P31, R63 and R60) were those with surface litter layers and this suggests that much of the litter nitrogen was available. The influence of undecomposed organic material deposited on the soil surface possibly extends down as far as 5 cm because at this depth the figures show the same low values for R66 and R65 whereas at 10 cm the differences have largely disappeared.

Averages for age classes also show the same trend. Availability of nitrogen dropped from 69.7 to 52.2 ppm in the three years after clear felling possibly due to immobilization and increased resistance of nitrogen to release brought about by the activities of the micro-organisms attacking the residues. It would appear that after five years the residues have been broken down and the resistant nitrogen may again be available perhaps by the increased cycling of nitrogen through the plants relative to that cycled by the micro-organisms or the more stable environmental conditions under the pine canopy. At eight years the residues from thinning operations undertaken at five years may have again increased microbial activity in relation to plant activity and the level of available nitrogen has dropped. After this stage it could be assumed that nitrogen availability would again increase and this theory is consistent with the observations of White (1966) who found an increased rate of tree growth after

Fig.9.1.7 Amount of available nitrogen estimated using the method of Keeney and Bremner (1966) in soil samples collected at four depths under five age classes of P.radiata from both ridge and gully sites in Tasman Forest.

Fig.9.1.8 Amount of available nitrogen estimated using the method of Keeney and Bremner (1966) in soil samples from ridge and gully sites in Tasman Forest collected during two periods of time.

Fig.9.1.9. Amount of available nitrogen estimated using the method of Keeney and Bremner (1966) in soil samples under five age groups of P.radiata collected from ridge and gully sites.

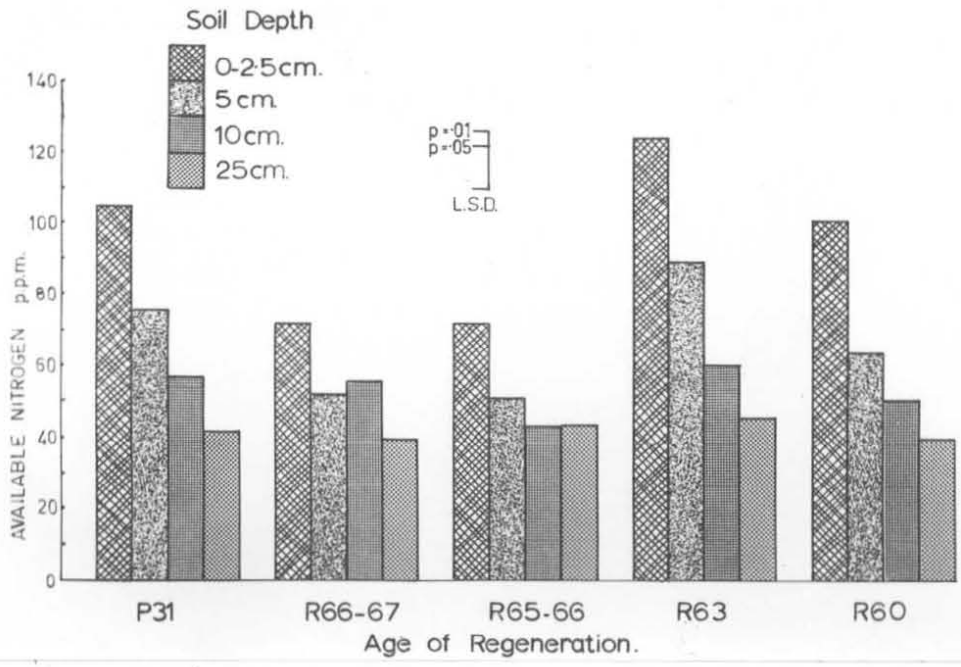


Fig.9.1.7

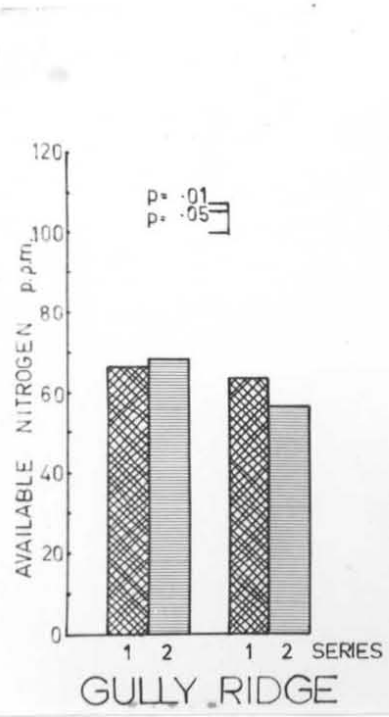


Fig.9.1.8

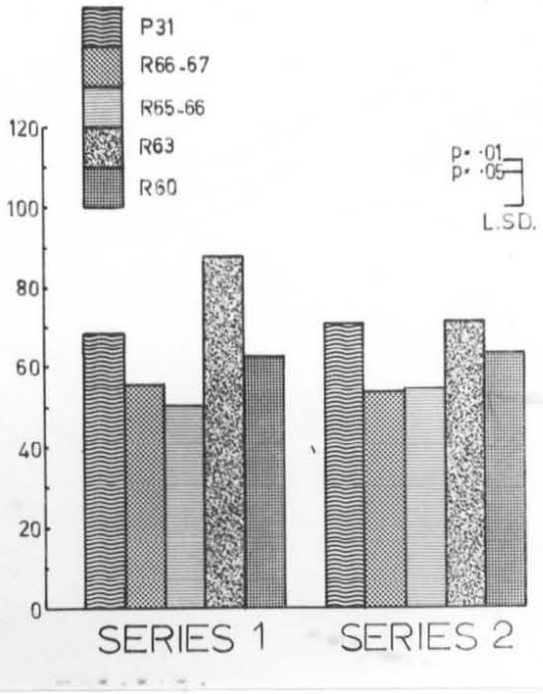


Fig.9.1.9

approximately five years.

The Age x Position (Fig. 9.1.5) interaction is more difficult to explain on the basis of current theories of nitrogen cycling. The P31, R66 and R65 age classes all show a difference of approximately 20 ppm between the gully and ridge sites. This situation is progressively reversed at R63 and R60 where more nitrogen is available on the ridge than gully sites. If this is due to an increase in mineral cycling brought about by the activities of trees compared with microbial mineral cycling then either tree growth rate should have increased on the ridges or the formation of resistant nitrogen by micro-organisms was reduced. As postulated in the introduction, if environmental conditions became such that they were less likely to cause microbial death the rate of formation of resistant nitrogen may be reduced. Canopy closure at this stage may have brought about environmental conditions conducive to the survival of micro-organisms but this should mean that ridge samples under mature pines (P31) would contain as much available nitrogen as gully samples. White (1966) has shown with a limited number of samples that growth on poorer sites (i.e. ridge sites), and presumably nutrient cycling in trees, does increase at about this age possibly due to the ability of the trees to overcome some previously limiting environmental factor.

Jenkinson (1968) has stated that the most serious objection to chemical tests as distinct from incubation tests is that no single chemical measurement is likely to give due

weight both to processes leading to mineralisation of nitrogen and those leading to immobilization. To overcome this difficulty some measure of the C:N ratio of the decomposing compounds would have to be included in the test. This would be technically very difficult. The highly significant correlations often obtained between the results of chemical tests and release of nitrogen are more likely to indicate that immobilization was not very important in the soils tested rather than that the methods used made allowance for the balance between immobilization and mobilization (Jenkinson, 1968). For this reason Jenkinson suggested spring sampling. Where there was pronounced immobilization and a high correlation of the nitrogen fraction with the plant growth, the correlation could, in fact, be with net mobilization and plant growth. A high correlation coefficient never implies that there is causation and in most cases the correlation would be between net mobilization and plant growth because immobilization rates are high (Jansson, 1958) even where net mobilization occurs.

In this instance, because correlation between available nitrogen and nitrogen available for trees was only assumed, the amount of available nitrogen measured in this study may be less related to the amount of nitrogen actually taken up by the plants unless the C:N ratios in the decomposing compounds were approximately equivalent in Tasman soils and soils reported in the literature because immobilization may reduce the amount of available nitrogen actually taken up by

the plant. The figures do, however, give an indication of the state of a certain fraction of the nitrogen in different parts of the forest.

In general, the method used produced data on "available" nitrogen which distinguished between groups of closely related soils and supported some long held theories concerning the situation in Tasman Forest. It also revealed some interesting interactions (i.e. Depth x Position, Depth x Age, and Position x Age) which could usefully be followed up.

Values obtained, using Hanmer and Balmoral Forest soil samples, are presented in Fig. 9.1.10. The result obtained show that these soils are similar to Tasman Forest soils in the amount of available nitrogen present.

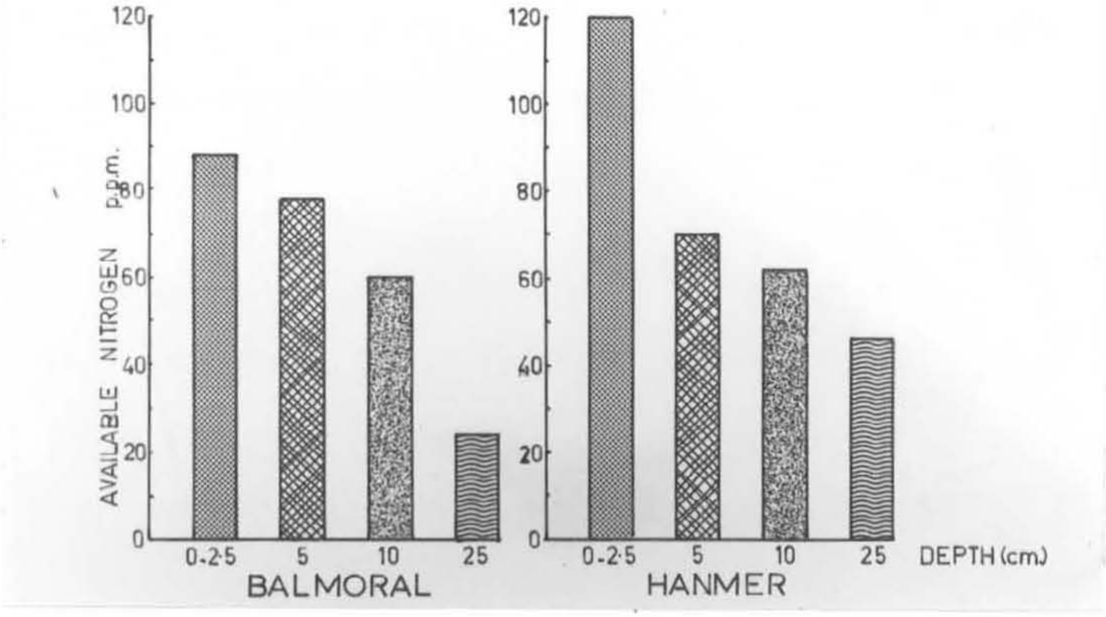


Fig.9.1.10

Amount of available Nitrogen measured, using the method of Keeney and Bremner (1966) in soil samples collected at four depths in Balmoral, and Hanmer State Forests from two stands in each forest.

9.2 LEVELS OF MINERAL NITROGEN IN THE SOIL

In general, the quantity of mineral nitrogen present at any one time in a soil can be correlated with various vegetational, climatic and edaphic factors. Harmsen and Kolenbrander (1965) have reviewed the literature which tends to emphasise the following factors as being the most important :

1. The effect of moisture on mineralisation in the soil.
2. The effect of water movement on leaching.
3. The effect of nitrogen species in regard to susceptibility to leaching.
4. The effect of temperature on turnover rates.
5. The effect of inhibition by large concentrations of mineral nitrogen.
6. The effect of plant absorption.

The few reports on this topic relate, in the main, to well separated climatic zones and therefore Harmsen and Kolenbrander (1965) were able to show fairly consistent patterns for each climatic zone depending generally on the distribution of wet and dry seasons, general temperature and rate of rainfall. For example, they found in temperate, humid climatic zones, heavy rainfall in winter caused strong leaching and subsequent low values in the topsoil while increased spring temperatures caused a build up which was partially reduced by autumn rains.

Edaphic factors may influence leaching of NO_3-N. For example, Crother and Michandani (1931) and Barnes (1950)

reported levels of mineral-N of less than 4 ppm in light soils compared with 20 - 50 ppm on heavier soils (Russell, 1964). The differences were probably due to the influence of leaching although mineralisation rates may have had an influence.

In arable land mineral-N levels vary throughout the year and are greatly influenced by the growth of the crop as well as by climatic and edaphic factors. Mineral-N usually disappears rapidly as soon as the crop commences growth; the rate of disappearance depending on the rate of growth of the crop (Harmsen and Kolenbrander, 1965). After harvest the residues may either cause mineralisation or immobilization depending on the C:N ratio and the availability of the carbon in the residues. Usually measures are taken to induce net mineralisation except where leaching is of prime importance.

Soils under perennial crops, especially grasses, seem to have a lower and more constant level of mineral-N under most climates. Figures for mineral-N in grassland soils in areas with humid climates have been published by Lyon and Bizzell (1936); Bizzell (1944); Stevenson and Chase (1953); Stockli (1949), and Richardson (1938); and in arid climates by Newton (1939); Rhodes and Newell (1946); Prescott (1934); Penman (1949); Cornish (1949); Theron (1951); Theron and Haylett (1953), and Goring and Clark (1948). Work on the mineral-N content of soils has also been reported from different geographical areas; for example, Thomson and Coup

(1943) in New Zealand, Martin and Cox (1956) in Australia and Greenland (1958) in Ghana. All have shown that mineral-N under grass is consistently low and is made up largely of NH_4-N. Richardson (1938) found approximately 6 ppm NH_4-N and 1 ppm NO_3-N in an old grassland soil sampled down to a depth of 20 cm throughout the year. Eggleton (1934) showed that the NH_4-N level varied from approximately 5 to 18 ppm while NO_3-N varied from 3 - 5 ppm with spring levels of 8 ppm. Martin and Cox (1956) reported levels of approximately 1 - 2 ppm NH_4-N and 0 - 1.6 ppm under semi-arid native grassland in Southern Queensland in Australia.

The level of mineral nitrogen in forest soils and the forms in which it is present depends on the pH of the soil and on the type of humus in the forest floor (Russell, 1961). Amounts of mineral-N in forest soils tend to be higher than in grassland soils especially in the humus layers.

Bornebusch (1930) found 1284 ppm, mostly NO_3-N, in the old leaf layer of mull soils under beech and 272 ppm in mull leaf litter. The upper mull had 52 ppm while raw humus varied from 95 to 388 ppm. Under spruce the mull layer had 106 ppm and the soil immediately beneath had 7 ppm while raw humus in mor varied from 115 - 132 ppm. Cunningham (1962) studied a tropical forest soil in Ghana and found the levels of mineral-N for different depths were 36 ppm at the 0 - 5 cm depth and 12 ppm at the 5 - 15 cm depth. In another tropical forest area in Ghana, Greenland (1958) found fairly high and fluctuating nitrate levels but low and constant ammonium

levels on the floor of a natural forest. Buzlukova's (1961) analyses showed that gray forest soils of the Irkutsk Steppe under pine contained from 13 - 21 ppm NH_4-N during the growing season while the same soil under fallow did not exceed 10 ppm. Very few observations have been made on the level of mineral-N in tropical forest soils but the evidence so far points to a similarity between tropical and temperate conditions (Russell, 1961).

Sampling for mineral nitrogen was carried out on soil from Tasman Forest sites, listed in Tables 9.2.1 and 9.2.2, to assess the total effects of carbon and nitrogen mineralisation in the soil of periods after clear felling.

METHOD

Instead of bringing back soil to the laboratory for sampling and weighing, which may have resulted in major changes in mineral nitrogen content which have been shown to occur in a short time (Robinson, 1967), it was decided to take into the field bottles containing 40 ml of 2N KCl. Toluene was not added since McGarity (1958, Ph.D. Thesis, University of Sydney) found that the addition of toluene significantly increased the mineral nitrogen content.

In the field a composite soil sample of approximately 4 g from three cores from each site was placed in each bottle and transported back to the laboratory.

Although Jansson (1958) contended that the ratio of KCl to soil is important, it was considered that there would be

less error if an approximately correct soil:KCl ratio were obtained when sampling in the field than if the soil were brought back to the laboratory and carefully weighed since this would mean there would be a period of several days between collecting the sample and extraction of the ammonia with KCl solution.

Before analysis, the KCl soil mixture was shaken for 30 minutes on a wrist action mechanical shaker (Griffin and George Limited). The suspension was allowed to settle and 20 ml of the clear solution were drawn off. For analysis 10 ml of the supernatant solution were placed in a 50-ml Kjeldahl flask with a ground glass joint. After placing a suspension of 0.2 g furnace-heated MgO suspended in 2 ml distilled water into the Kjeldahl flask, the flask was attached to a steam distillation apparatus (Bremner, 1965). Fifteen ml of distillate condensed in a water jacket condenser were collected in a test tube. The rate of distillation was controlled so that the temperature did not exceed 22° C and the bottle was filled in approximately two minutes. After disconnecting the Kjeldahl flask, 0.2 g Davada alloy was added and the procedure repeated.

In preliminary analyses, difficulty was experienced in obtaining consistent results when there was mineral nitrogen in the distillate. However, when the method of Bremner (1965) using 0.001 N HCl and boric acid - bromocresol green - methyl red indicator was replaced by the method of nesslerization (Mestson, 1956) greatly improved results were obtained.

In one trial, using six sub-samples from a single sample, a deviation between the extremes of less than 2% resulted. The 30 ml of distillate was collected in a bottle containing 5 ml of distilled water. One ml of Nessler Reagent was added to this solution. The resulting solution was left for 30 minutes at 20° C. The intensity of the colour was read at 420 mu in a Beckman DB spectrophotometer using glass cuvettes with a 1 cm light path. Values of percentage transmissions obtained were compared with a standard curve made using a solution of 0, 0.4, 0.8, 1.2, 2, 3, 4 ppm N.

After analysis for mineral nitrogen had been carried out the KCl - soil suspension was weighed. This enabled the weight of KCl in the suspension to be determined, provided no water had been lost by evaporation before this stage. Water loss was avoided by sealing the bottles. The suspension was then dried at 102 ± 2° C, cooled in a desiccator, and re-weighed. Bottles were then cleaned, dried and re-weighed and the weight of the bottle subtracted. The weight of oven dry soil present was then found by subtracting the weight of KCl.

RESULTS AND DISCUSSION

The underlined values in Table 9.2.1 for the 0 - 25 cm depths are averages of those obtained for 0 - 12.5 and 12.5 - 25 cm depths. This was carried out so that the results could be expressed on a ground surface area basis.

TABLE 9.2.1

Mineral nitrogen in various sites at Tasman Forest expressed in ppm. Calculated average values from 0-12.5 cm and 12.5-25 cm samples = x and two figures in one category represent duplicates.

Soil depth in cm	3 February 1968 SAMPLING SITES									
	LP31	UP31	LR66-67		UR66-67		LG65-66		UR65-66	
0-2.5		66.4	36.9		7.1		29.0		15.3	
5	11.2		16.5	23.0	11.5	19.3	21.8	19.4	10.3	10.4
10	2.1		25.1		27.0		6.1		16.5	
25	0.0		12.0		7.5		9.0		8.8	
0-25	19.9		22.6		13.2		16.5		12.8	

	LR63		UR63		LR60		UR60	
0-2.5	11.3				29.0		52.2	
5	28.9	-	56.8	47.2	11.4	6.8	20.3	-
10	7.1		33.8		5.7		11.7	
25	6.6		5.5		2.4		17.5	
0-25	13.5		29.7		11.5		25.4	

Two samplings completed did not appear to show any clear cut trends although there was an ill-defined tendency of mineral nitrogen to decrease with increasing soil depth. In general, the values tended to be higher than those obtained by Stone and Will (1965), especially the organic matter layer (0 - 2 cm). Will and Stone sampled in winter (July) while the samples in this study were obtained in February and early June which may have affected the result but, as pointed out in the introduction, most workers have found lower contents in the wet cold season than the hotter drier season. Perhaps rain during winter leached some of the mineral nitrogen of the organic layer into the soil below or the differences were caused by differences in analytical methods, for example, mineralisation in the 2N KCl-soil solutions during transport. Robinson (1967) noted increases in mineral nitrogen in tropical soils using a neutral KCl extracting reagent at ambient temperatures. He found this was the best way of storing soil samples without alteration of nitrogen content. The only solution would be to carry out analyses on the site.

Jannson (1958) and McGarity (1958) have suggested that amounts of less than 8 ppm NH_4-N may have little biological significance and may be an artefact of extraction; if this is so, then some of the deeper Tasman Forest soil samples with NH_4-N contents of less than 8 ppm may have no free nitrogen available for uptake by plants.

9.3 MINERALISATION IN THE FIELD

REVIEW OF LITERATURE

Very few workers have studied mineralisation rates under field conditions. Most investigators who have studied this phenomenon have done so using controlled laboratory conditions and related their results to field conditions.

METHODS

Soil obtained from Tasman Forest was used to study net mineralisation rates at different sites under nearly natural conditions.

During the dry summer the soil in Tasman Forest became extremely hard and could not be penetrated by the soil sampler. For this reason pieces of soil chipped from the particular levels were sampled, placed in plastic bags and buried 2.5 cm below the soil surface. After incubation the soil sample was mixed and sampled. Analysis of mineral nitrogen in the soil sample was carried out using Bremner's method described under "Amounts of Mineral Nitrogen".

RESULTS

The results of analysis for mineral nitrogen levels before and after incubation are presented in Table 9.3.1. Duplicate samples were taken in most cases, the individual results are presented to show the variation between samples.

DISCUSSION

During the dry summer period there was a slight immobilization of mineral nitrogen. The environmental conditions of the soil inside the bag would have been similar to those in the surrounding soil as the bags were permeable to water, especially at temperatures of 25° C and higher. Temperatures inside the bags would probably have been close to that of the surrounding soil. The results suggest that during dry periods in the summer no nitrogen is available to any plants in the forest, unless the conditions inside the bag caused greater immobilization than that in the surrounding soil.

TABLE 9.3.1

Mineral-N in ppm found at two sampling dates in soil from 5 cm depth in Tasman Forest. Samples were held in plastic bags on the site where the soil was extracted.

Date of sampling	Sites															
	LR66-67		UR66-67		LR65-66		UR65-66		LR63		UR63		LR60		UR60	
3.2.68	16.5	23.0	11.5	19.3	21.8	19.0	10.4	-	28.9	-	47.0	56.0	11.4	7.0	20.3	24.7
6.5.68	14.0		0.0	9.0	15.1	11.9	7.5	-	15.0	13.0	53.0	57.0	9.0	8.0	18.8	24.7

TABLE 9.2.2

Mineral nitrogen in various sites at Tasman Forest expressed in ppm

Soil Depth in cm	5 June, 1968 SAMPLING SITES LP31	UP31	LR66-67	UR66-67	LR65-66	UR65-66	LR63	UR63	LR60	UR60
0- 2.5			44.6	16.1	32.6	6.1	39.2	72.9	7.6	10.4
5			11.2	2.2	11.5	2.6	18.5	41.8	0.0	7.5
10			7.8	1.0	-	1.1	10.3	11.8	11.5	6.0
25			1.0	14.0	6.0	2.4	24.5	11.7	25.0	4.0
0		16.8	32.3	16.9	13.5	12.3			16.8	17.3
12.5		16.5	14.1	19.2	9.7	5.2				25.0
12.5		3.1	2.0	3.1	5.4	3.0			5.2	1.4
-25		3.0	2.1	0.0	13.9	5.4			10.4	1.2
0-25	22.3 23.9	9.1	12.5	10.0	10.5	6.5	20.5		12.3	15.4 11.2

CHAPTER 10

AGENTS OF DECOMPOSITION

10.1 FUNGAL DECOMPOSITION OF PINE NEEDLES

10.11 INTRODUCTION

A thorough study of the agents responsible for the decomposition of plant remains is essential for a complete understanding of the ecosystem. A greater knowledge of the ecosystem, in turn, enables the repercussions of man-made disturbances to be predicted with greater accuracy and adjusted for with greater ease.

Although in a short-term study this section of the work cannot be completed fully, it is hoped to elucidate some of the phenomena associated with decomposition of one complex plant part, viz. P.radiata needles. It was decided, firstly, to study the species of fungal agents and their patterns of distribution in an attempt to elucidate something of their interrelationships and the effects of generalized environmental features on them. Because none of the methods so far conceived would completely fulfil this objective it was decided to use several methods and endeavour to correlate

the results. The next step yet to be undertaken involved the determination of the activities of each of these isolates both by themselves and in conjunction with other fungi. This can be accomplished in part by allowing decomposition of previously sterilized needles by selected fungi in various combinations (Section 6.32).

The total activities of these fungi under different environmental conditions can also be assessed in part by studying carbon mineralisation under these conditions, but since these aspects of fungal activity fall into the category energy dissipation they have been considered under this heading.

The final step in investigating the breakdown of needles would be a detailed examination of the activities of each of the fungi involved; for example, the role of the different species in the breakdown of individual compounds and the detailed physiology of the fungi themselves. However, this type of study is outside the scope of the present investigation.

10.12 REVIEW OF LITERATURE

(1) Methods

Methods of characterisation of micro-organisms fall into two groups. Firstly, direct observation of some part of the fungal body, either with or without the aid of microscope, stains, etc. The second method, culturing,

involves placing the material containing the fungus on a transparent substratum so that it will grow out when it can be observed more easily.

The latter method has been more widely used and subjected to many modifications but the former can also yield a great deal of information if a method of making the fungal hyphae visible against the plant tissue can be found. Often both methods have been used by one investigator. Hayes (1965) plated small pieces of P.sylvestris needles on to 2% malt agar after three different treatments: 1. plated soon after collection; 2. kept in a sterile damp chamber for a time; 3. surface sterilized with 0.1% $AgNO_3$ and then plated. Pugh (1958) incubated Carex paniculata leaves before plating but he used a greater range of media (Carex extract, potato dextrose and Czapek-Dox agars) and $HgCl_2$ as a sterilant as well as $AgNO_3$. Kendrick and Burges (1962) found that 2% malt agar was the most satisfactory medium they ried. To reduce the fungal population on the needle surface they used a wetting agent followed by numerous washings with sterile water on the undecomposed needles. On needles from the F_2 layer this method was not found to be very effective so mercuric chloride was used instead. Hudson and Webster (1958); Webster and Dix (1960); and Webster (1956) gained supplementary information using direct microscopic examination together with plating. They examined the plant surface for fruiting bodies and also plated pieces of stalk on to maize extract agar with rose bengal, penicillin and streptomycin.

Caldwell (1963) in a study of the colonization of beech litter used two methods: plating on to Czapek-Dox agar with 0.05% yeast extract added adjusted to pH 4.0 together with observations on sections made with a sledge microtome and stained in Cartwright's (1929) Safranin picro-aniline blue solution.

Witkamp (1963) cut up the leaves, mixed them with sterile water, and made dilution plates. This is a method which is usually considered to be more suitable for substrates which disperse more easily than leaves, for example, soil.

It seems to be generally agreed that no one method is entirely satisfactory and that a range of methods will supply information which is more truly representative of what occurs in nature.

(ii) Agents

Hudson (1968) has reviewed the whole topic of the ecology of fungi on plant remains above the soil but the following review deals with one section of this subject in more detail.

There is no well-defined and well understood theory to explain the variation in the agents involved in fungal decomposition of leaf litter. Perhaps the variation is a result of numerous interacting factors and will never be able to be explained by one simple theory.

The different fungal floras found on the leaves of plants of related or even the same species (sometimes referred to as substrate plants) in different geographical

areas is one of the most outstanding features of the literature in this field.

Factors which may be responsible for this variation include :

a) Nutritional factors such as the abundance of carbohydrates.

b) Microclimatic factors.

c) The length of time the substrate plant has had an influence on the fungal flora of the particular place studied.

d) The stage of development of the substrate plant and/or its organs.

e) The characteristics of the indivual fungi themselves including their antagonisms to each other.

f) The species of substrate plant involved.

g) Difference in geography with its associated macro-environmental differences.

Although it is not possible to evaluate exactly the role of all these factors some broad assumptions can be made which can be illustrated from published results. In only a few cases are there reports of work where the variables listed above were held constant.

Needle decomposition in Pinus sylvestris has been studied by several authors - Kendrick and Burges (1962) at Delamere and Hayes (1965 a and b) at Marian-y-Winllan and Bannock. Their results include examples of the variation in fungal flora in the needles of one substrate plant.

Kendrick and Burges (1962) studied the fungal flora of the L, F and H layers of the forest floor leaf little of 50-year old trees and found the seven most important species present on the living needle to be Coniosporium sp., Lophodermium pinastri, Aureobasidium pullulans, and Fusiococcum bacillare. The incidence of the latter three species increased rapidly as the needles died but six months after needle fall these fungi declined and Desmazierella acicola, Sympodiella acicola, Helicome monospora, Trichoderma virile, and Penicillium sp. became dominant for the next two years. D.acicola was the only fungus which was colonizing the internal tissues during this period. When the needles became part of the F_2 layer they were colonized by fungi which were both internal and external colonies: Trichoderma virile, Penicillium sp., a Basidiomycete and a sterile mycelium.

Hayes (1965) also studied the decomposition of P. sylvestris needles and found some similarities with the results obtained by Kendrick and Burges (1962) although differences were also apparent. Among the early colonists, Hayes found only Lophodermium pinastri and one other species to be common to both studies, but reported, in addition, a Penicillium species, Haplographium penicilloides and Aspergillus fumigatus. Of the later invaders Trichoderma virile, Verticicladium trifidum (= Desmazieriella so.) were found together with Oidiodendron fuscum, and Cylindrocarpon chrenbergi. These latter two were not reported by Kendrick and Burges (1962). Hayes (1965) found Fusiococcum bacillare

throughout his study but it was only present in abundance on the leaves in the first six months at Delamere. A needle surface fungus Mucor hiemalis was also present throughout only on F_1 and F_2 needles at Delamere. Two other fungi not recorded by Kendrick and Burges (1962) were found throughout the period of Hayes' study. Compared with the initial colonisers at Marian-y-Winllan mentioned above (Hayes, 1965a) the decomposition at Bannock (Hayes, 1965b) was started by Graphium sp. followed shortly by Geotrichum candidum and Penicillium funiculosum and continued by G. candidum, P. spinulosum and Pachybasium hamatum in addition to Mucor hiemalis and T. viride. Oak wood litter fungi were studied by Hering (1965) in England who compared his results with those of Hosca's (1961) study of an oak litter at Turin. Hering concluded from the comparison that a substantially different succession pattern was present in the two localities.

The results discussed above demonstrate that while there are similarities present, intraspecific differences are also significant. Reasons for these detailed differences may well be numerous and complex.

From the studies of Kendrick and Burges (1962), Hayes (1965a and b) and Ward (1952), Hayes (1965b) has contended that the length of time that a substrate plant has had an influence on the main fungal flora of an area is important in determining phenomena such as the number of different

species of fungi in a particular area. The 150-year history of P. sylvestris forests at Rannock has probably been mainly responsible for the 120 species found compared with 70 species from Delamere (Kendrick and Burges, 1962) and 78 species from Rufford (Ward, 1952). Gremmen (1957) also studied P. sylvestris leaf decomposition in the Netherlands but his study differed from that of Kendrick and Burges (1962) and Hayes (1965a and b) in at least three main features. Nutritionally, leaves still attached to fallen trees are higher in carbohydrates than those leaves which have fallen naturally. Secondly, the area of study was different and associated major environmental features, and thirdly, the microclimate was probably different.

Tentatively Gremmen (1957) divided decay of needles into two successive stages; each stage being characterised by certain fungi which, however, varied depending on the microclimate. Firstly, the Sclerophoma stage where the needles were grey, lying in heaps under dry conditions or still attached to a dead tree. Now and then these heaps would be wetted by rain, but they were soon dried out again by wind. The characteristic species were Cenangium acicolum and Sclerophoma pityophila. Secondly, the Desmarierella-stage where the needles were black and lay in heaps under wet or very wet conditions almost in contact with or very near the soil. Characteristic species were Dasyscyphus pulverulentus, Desmazierella acicola, Phialea acuum and sometimes Ophioectria scolecospora. Decomposition in this stage was distinct-

ly more advanced than in the former, and there is no doubt that the Desmazierella-stage was the more active of the two stages.

The first stage had no species in common with those reported by Kendrick and Burges (1962) and Hayes (1965a and b) but the second stage resembles in its microclimate and had one important species D. acicola in common with those found by English workers.

The colonization of naturally fallen needles and of needles shed after infection by a parasitic fungus, e.g. Lophodermium pinastri, which was poor in sugars, was quite distinct from the colonization of needles on felled trees which were rich in nutrients. Leaves attached to branches which were above the ground present a different microhabitat to fungi from those which were close to the soil or are incorporated into other litter on the surface. Leaves above ground probably dry out often while those on the ground, being less exposed, are probably less prone to desiccation.

In these latter two respects Gremmen's study resembles work done by Hudson and Webster (1958), Webster (1956) and Pugh (1958) on Dactylis sp., Agropyron sp. and Carex paniculata where the fungi responsible for the decomposition of inflorescence and vegetative stalks were enumerated. These studies differ from Gremmen's in that the organ was being decomposed while still attached to a living plant, whereas in Gremmen's study the tree was severed from its roots. Probably substances were being withdrawn into the active

part of the plant while decomposition was taking place.

The microclimate conditions of the inflorescences reported by Webster (1956) probably apply also to needles in Gremmen's work. Webster (1956) found that relative humidity values approaching saturation were commonly recorded within 10 cm of the ground in the grass with steep humidity gradients in the first meter. The density of grass sward would, of course, make it more likely that saturation would occur than in more loosely packed needles except for places where needles were closely packed on a fallen tree. Although Webster (1956) did not make an exhaustive study of the rate of drying the example which he quotes is revealing. After a period of light rain (0.07") between 7.15 and 9.30 a.m. on a day with fine weather and a 10 m.p.h. wind the upper two internodes came to an equilibrium value of 3-5% moisture within four hours. While at lower levels the water content was still high after 11 hours of drying conditions. Since the minimum safety levels for mould growth on grains has been found to be 12-16% (in Webster, 1956) the upper internodes would be too dry for most species of fungi to cause decay soon after rainfall had ceased.

Webster (1956), Hudson and Webster (1958) and Webster and Dix (1960) studied the production of fruiting bodies on the stalks and related the observed dissimilarities to variations in water content, nutrition, host resistance and competition. They present evidence to show that the upper sheaths had a higher concentration of nutrients than the

lower internodes. Then there was a reverse in nutrient levels because of the rapid rate of senescence in the upper internodes. These conditions induced vigorous sporulation of primary saprophytes which was greater on leaves having a rapid senescence and was prolonged on those tissues having a fluctuating water content.

This situation is probably similar to that of pine needles which fall naturally or remain attached to a fallen tree. In the needles which fall naturally nutrient reserves were lower and a better moisture microclimate was experienced because the needles were in close proximity to the ground whereas in needles attached to a fallen tree nutrient reserves were higher but the needles were subject to rapid drying after rain. This frequent drying probably tends to induce a long dominance of primary saprophytes in the attached needles.

In contrast to the work reported on Pinus sylvestris, Hudson and Webster (1958) have found that despite differences in growth habit and time of flowering in Dactylis sp. and Agropyron sp. the pattern of fungal colonization was very similar. Although most of the fungal groups recognized by Webster (1956) were present Acrothecium sp. was not found. Other fungi were more commonly recorded on Agropyron than on Dactylis.

A comparison of the work of Ward (1952, cited in Chesters, 1960) who studied Pinus nigra and P. sylvestris and Kendrick and Burges (1962) who studied P. sylvestris reveals

that there is conflicting evidence concerning the inter-specific variation of closely related species. Hayes (1965) has reported that the species of fungi found by these two workers differed completely. There may be an element of geographical variation with its associated environmental variation included in this comparison because the areas studied were some 30 miles apart.

Webster, Hudson and Dix (1956, 1957 and 1958) and Brandsberg (1967) found that different species of substrate plant may have the same fungal flora responsible for decomposition. This means that different substrate plants are not necessarily responsible for differences in fungal flora. Brandsberg (1967) investigated the fungal flora of Abies grandis, Pinus monitcola, and P. ponderosa litter in Idaho and a younger P. ponderosa stand in Washington and found few differences among fungi from various sites. He found the same 15 species were found commonly on all sites listed. (The abstract reporting his work was confused on this aspect.)

These results were confirmed by Hayes (1965a) who reported a similar fungal attack on the litter of Abies grandis, Picea sitchensis and Pinus sylvestris at Coed Marian-y-Winllan Caernarvonshire. Thus the differences in fungal attack reported by previously mentioned workers could well be due to geographical differences together with which can be assumed habitat differences. However, allowance must also be made for lack of uniformity in methods of study.

It is quite likely that some similarities or differences could be due to the techniques used which may give preference to certain species, but the importance of this variable would be hard to estimate without a controlled study.

All the causes of variation in fungal flora listed above except for variations due to different substrate plants and antagonisms among the fungi have some supporting experimental evidence obtained from the field. It is possible that investigations have not been intensive enough to bring out variations due to substrate plants. Because of the original dissimilarity of fungal floras the antagonism hypothesis has not been investigated in the field but Saito (1960) found evidence of antagonism among leaf litter fungi under experimental conditions.

The factor best supported as a cause of variation in the literature is that of macroclimate but this is so wide and incompletely known that it could be taken as an example of how little is known about the whole subject.

(iii) Pattern of decomposition

Even though the types of fungi present vary markedly two main factors impose some uniformity on the activity of the flora. Firstly, some compounds in the leaf are less readily mineralised than others and often the micro-organisms associated with the destruction of these more difficult compounds are not able to compete with those mineralising the more easily decomposed substances. Secondly, the rate of

release of minerals such as N, P, K, etc. from the substrate limits the maximum amount of decomposition which can occur at any one time because the micro-organisms need these minerals for their own growth. These restrictions usually cause a succession of dominance and a gradual alteration in the pattern of population. This gradual change has been divided arbitrarily into certain phases mainly for convenience of description.

Gremmen (1957) and Garrett (1963) concluded that in general decomposition of organic remains in nature seem to be along the following lines :

a) the pectic substances

b) the sugars

c) cellulose

d) lignin.

Smit and Wieringa (1953) added support to Gremmen's (1957) contention that pectins were decomposed first when they reported a dominance of the pectin decomposing fungus Aureobasidium pullulans on leaves ready to fall. They suggested that this fungus cleared the pectin from leaves and allowed further decomposition to proceed. While other workers, for example, Kendrick and Burges (1962) have reported the dominance of Aureobasidium sp. some have found no evidence of this genus (Hayes, 1965b).

The general pattern outlined by Gremmen (1957) and Garrett (1963) is not always found because the absence of a particular compound may cause one of the stages of decay

to be absent. In a study of the decomposition of carbohydrate deficient Pteridium sp. peticles Frankland (1966) found that the primary colonisers, species of Aureobasidium, Cladosperium, Epicoccum and members of the Sphaeropsidales which were decomposers of cellulose and/or lignin (Sui, 1951; and Domsch, 1960) were absent. The absence of sugars in this substrate reduced the phycomycete population which are considered to be responsible for their decomposition and therefore Gremmen's (b) category was bypassed. Potential lignin decomposers were frequent in the second year when an extensive breakdown of cell walls was visible. In the third and fourth years hypomycetes predominated. Sugar decomposing fungi reached a climax when by-products of cellulose decomposition were likely to be available. Frankland pointed out the exception quoted by Meredith (1960) was similar to his findings. In pine stumps lignin decomposition preceded the invasion of sugar fungi. This trend was also evident on the dead stems of grasses Agropyron sp. and Dactylis sp. on which hypocymetes and pyrenomycetes were the most numerous first invaders (Webster, 1956).

The succession in some fallen leaves in which the phycomycetes phase of very short duration (Burges, 1958) was considered by Frankland to be an intermediate type.

10.13 METHODS

Agents responsible for the decomposition of leaves remaining attached to aerial branches and those on the ground have been investigated by two methods.

Plating

The leaves attached to the branches were removed in bunches, using a pair of forceps which had been previously sterilized. Each bunch of needles was placed in a previously sterilized container; usually four containers were filled at each site. On return to the laboratory pieces of these leaves, approximately one-eighth inch in length, were plated directly on to malt agar (Difco) and incubated at 22° C for 10 - 14 days. Other leaves from the same collection were surface sterilized by soaking the leaves in a solution containing 10 parts of 'Janola' (Reckitt and Colman N.Z. Limited) to 35 parts of water for two minutes, followed by washings with two changes of sterile water. In the early part of the investigation four pieces were put on one plate but this was found to cause too much crowding and subsequently the number was reduced to two per plate. The fungi which grew from the pieces of leaf were examined microscopically (x 100). Subcultures of all commonly occurring fungi were made.

Isolations were also made from the leaf litter contained in litter bags (see Section on rate of decomposition).

Direct Examination

Leaf decomposing agents have also been studied by direct examination of cleared leaf tissue. Needles were cleared using the method of Hering and Nicholson (1964). Sodium chlorite (0.75%) was used for clearing and a graded series of ethanol - water mixtures from 10% to absolute alcohol was used for dehydration. The dehydrated leaves were made almost completely transparent by treatment with methyl salicylate, and were stained with 0.1% Safranin in methyl salicylate solution which is reputed to make the hyphae observable by differential staining. While some of the hyphae could be clearly distinguished from the surrounding leaf cells, not all were equally obvious. This method requires considerable improvement before it can be used in this instance for quantitative studies on fungi of pine needles.

Analysis

An analysis of association between species involved in the decomposition of needles was carried out, using 2 x 2 contingency tables and computed, using a programme developed by the author, on an IBM 1130 computer. Yates' correction was applied. Only analysis with larger numbers of isolations were included (Greig-Smith, 1957).

Trees were cut and the trunks removed in Tasman Forest after approximately 36 years' growth. The non-bole material left was distributed irregularly in the field and regeneration took place among the decaying residues some months after clear felling. The dense regeneration was thinned at approximately four years after clear felling and the residues from this were not removed.

In this investigation fungi involved in the decay of the leaves hanging from aerial branches of the clear felling residues and also the four-year old thinning leaves have been studied.

This experiment therefore consists of two parallel observations on the leaves of trees of two vastly different ages. In Figs. 10.1.1 to 10.1.5 year 0 is the time of clear felling, somewhere between 0 and 1 the seed germinates and at approximately year 4 the regeneration is thinned and its leaves start decomposing.

Litter bags were laid out as described in the Section on rate of decomposition of slash components and the time scale indicates the time they have been exposed in the forest.

The number of times a fungus appeared at least once on each piece of leaf tissue was recorded, averaged and expressed as a percentage of the total number of pieces plated from each site. Many of the types of fungi isolated were rare and were included in the results under the category 'others' while commonly isolated fungi were given abbreviated names in the text. Full names are set out in Figure 10.1.7.

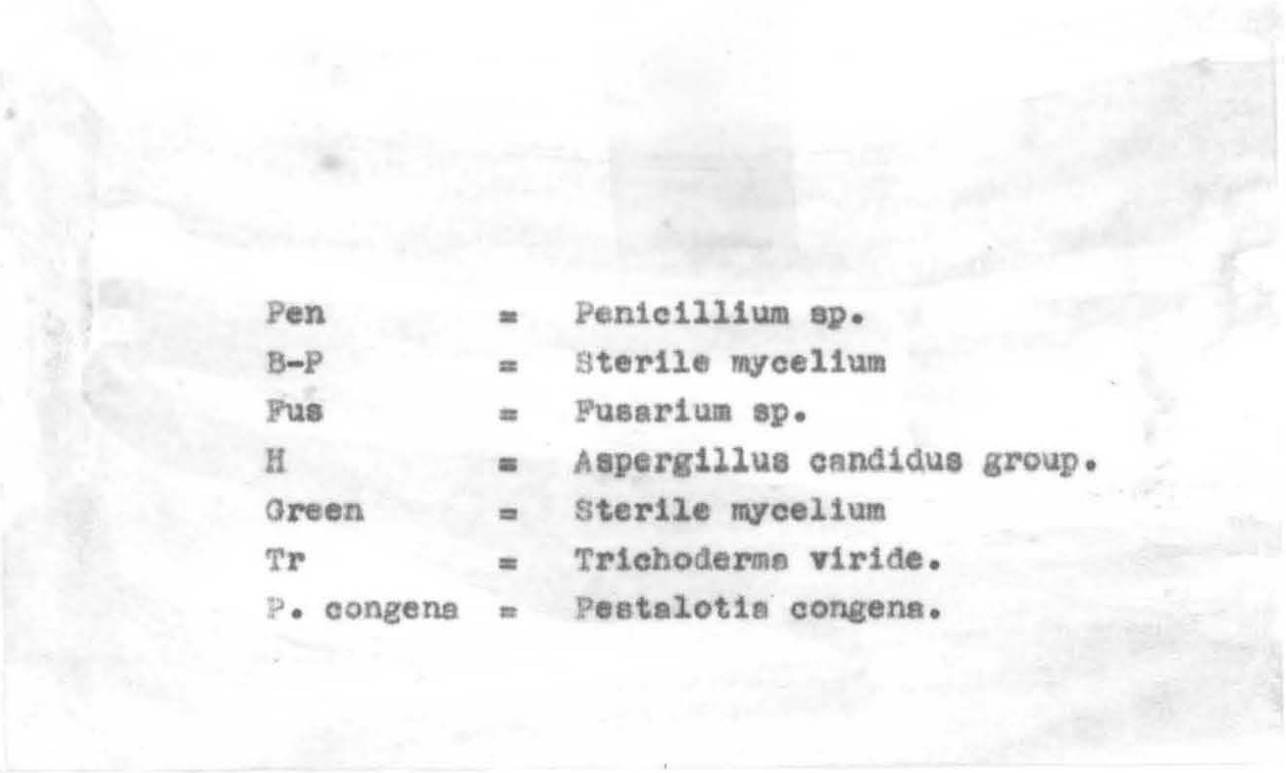


Fig.10.1.7 Fungi isolated from needles collected in Tasman Forest.

10.14 RESULTS

The results of the X^2 analysis of association between species are presented in Table 10.1.8. The % isolation of each species of fungus has been set out in Figs.10.1.1, 10.1.2, 10.1.3, 10.1.4, 10.1.5 and 10.1.6.

10.15 DISCUSSION

(i) Methods used

The selectivity of the methods used may be responsible for preferential isolation of certain fungi or groups of fungi.

Those fungi with preferences for nutrients in the malt agar would have been at an advantage. Furthermore, the method of plating out on to agar itself may allow fast growing species to dominate and perhaps even suppress the growth of slower growing fungi. Thus these fast growing fungi would appear to be the most involved in litter decomposition whereas in reality they may play a minor role. Using plating techniques, the presence or absence of individual fungi and their distribution can be studied, but their activity cannot. Thus supplementary information gained by different methods is required to complete our understanding of the process of leaf decay.

(ii) Agents in comparison with overseas work

Some of the fungi isolated in this investigation have so far failed to produce reproductive structures and there-

fore have not been identified. Of those which have been identified it has been found that, in common with other workers such as Kendrick and Burges (1962) and Hayes (1965a and b), representatives of the ubiquitous genera Penicillium and Trichoderma were frequently isolated. The other two frequently isolated ascomycete fungi Pestalotia conigena and Pirocauda sp. (Deuteromycetes) which have been identified were not commonly reported by other investigators of pine needle decomposition.

The contention that there is some variation due to geographical distances seems to be borne out in this study.

(iii) Pattern of Invasion

In common with other studies (Hayes, 1965; and Kendrick and Burges, 1962, etc.) there was a change in the fungal flora with respect to time. The surface sterilization technique used in this study has enabled partial differentiation to be made of the organisms into needle surface colonizers and those growing internally. The theory behind this is that the fungicide does not penetrate into the internal tissues and kill fungi present there. There is also the supposition that this fungicide dissipated from the surface before the internal colonisers reached the surface of the leaf. The washing with sterile water at the time when the needles were left standing would have removed most of the active ingredient from the surface.

A comparison of Figs. 10.1.1, 10.1.2 and Figs.10.1.3 and 10.1.4 for Pestalotia congena shows that the percentage

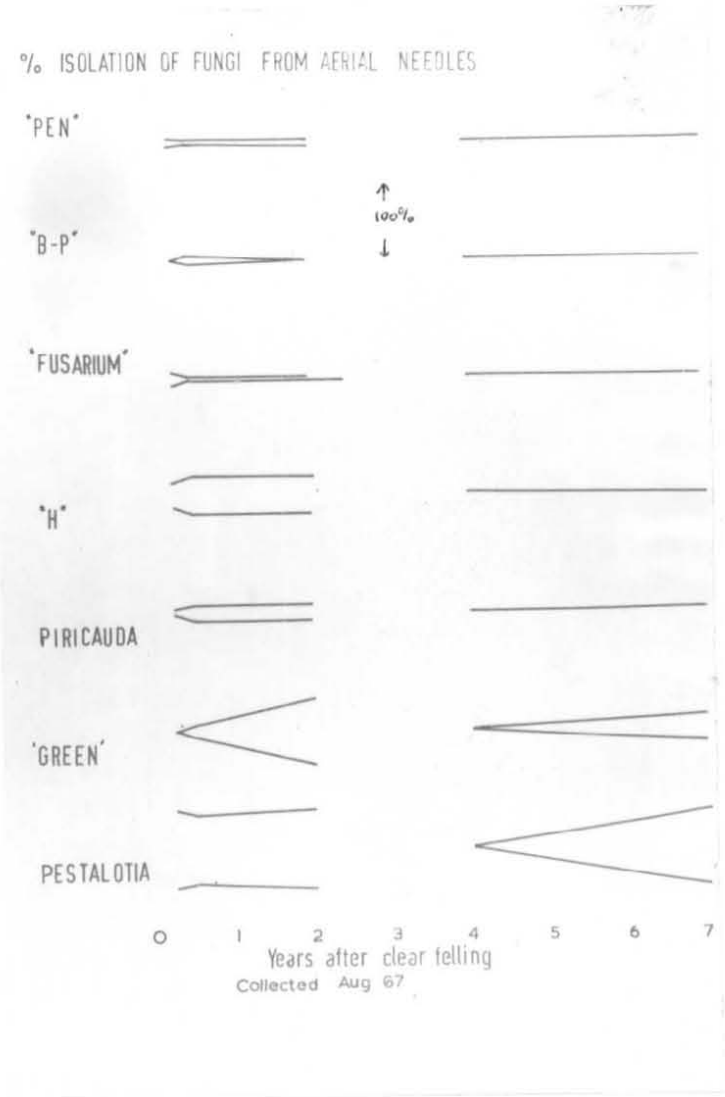


Fig.10.1.1

Percentage isolation of fungi from needles hanging from the aerial branches of P.radiata trees cut down during clear felling of mature trees and the thinning of R63 regeneration.

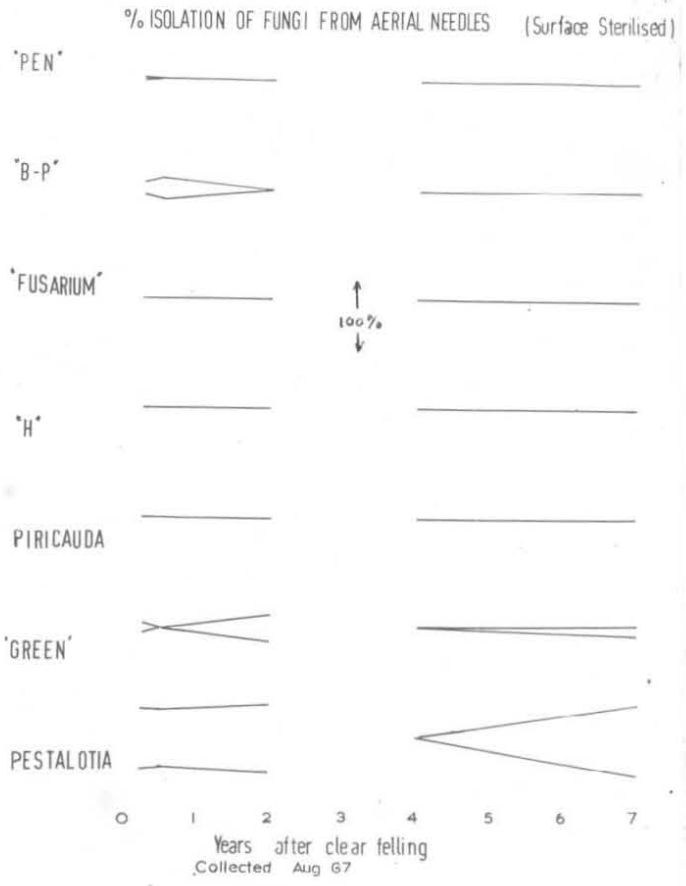


Fig.10.1.2

Percentage isolation of fungi from needles hanging from the aerial branches of P.radiata trees cut down during clear felling of mature trees and the thinning of R63 regeneration.

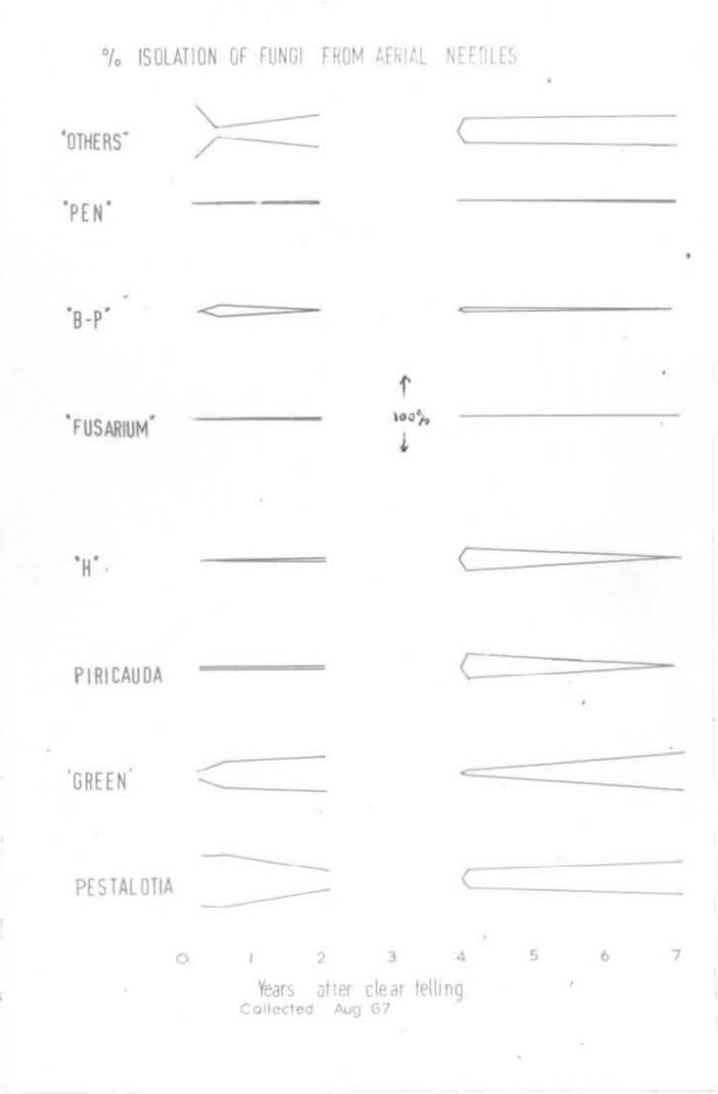


Fig. 10.1.3

Percentage isolation of fungi from needles hanging from the aerial branches of P.radiata trees cut down during clear felling of mature trees and the thinning of R63 regeneration.

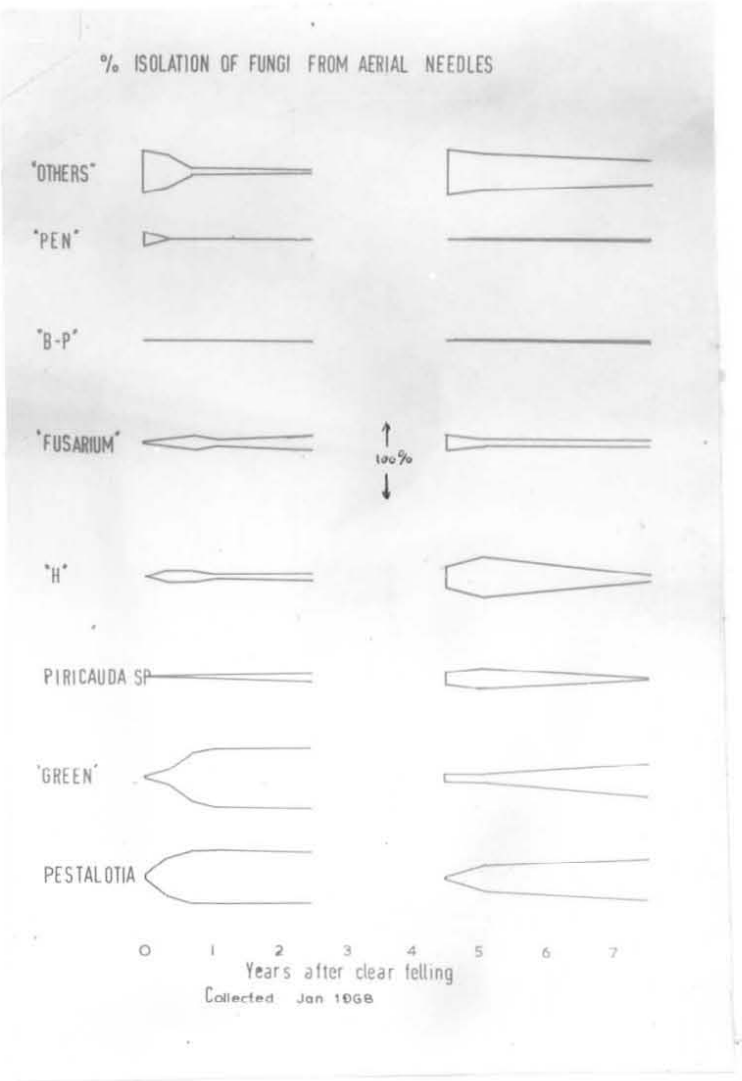


Fig. 10.1.4

Percentage isolation of fungi from needles hanging from the aerial branches of P.radiata trees cut down during clear felling of mature trees and the thinning of R63 regeneration.

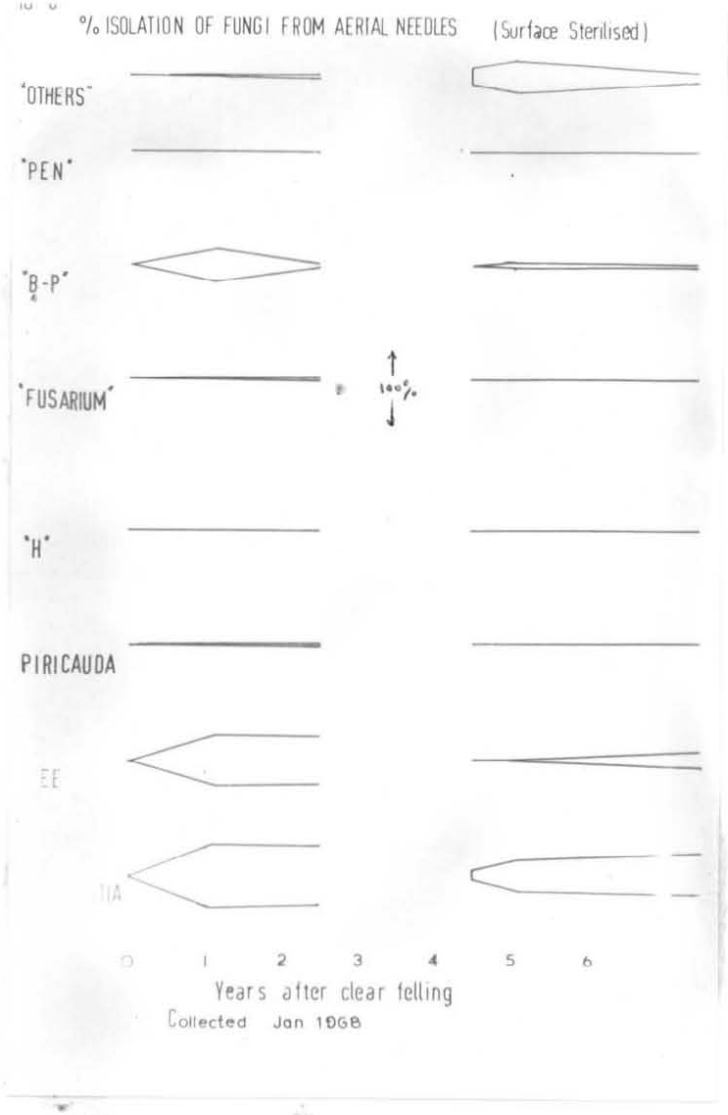


Fig. 10.1.5

Percentage isolation of fungi from needles hanging from the aerial branches of P.radiata trees cut down during clear felling of mature trees and the thinning of R63 regeneration.

isolation of this fungus was little altered by surface sterilization. From this three conclusions may be drawn. Either the fungus was present both as an internal and external colonizer, the fungus was present as an internal colonizer only but has a competitive ability which allows its invasion of the agar from the internal tissues in competition with the external colonizers, or this fungus is not affected by the fungicide treatment. P. congena was the most frequently isolated fungus and built up to its 80% of the isolates after nine months. Fig.10.1.4 and Figs. 10.1.3 and 10.1.4 show its reduced frequency of isolation from the needles of young trees thinned at about four years old on site UR63 but an earlier isolation, Fig.10.1.1, shows a build up as great as in the mature felled trees on site P31. Fig.10.1.3 shows the reduced isolation in the young trees and a slow build up in numbers, and also demonstrates a feature not present on other occasions, a decline from six months to two years. This was partly caused by a very low percentage isolation for one of the two sites (LR65-66) from which 4% isolation was obtained compared with the nearby site on the ridge (UR65-66) where 50% was found to carry the fungus.

The ascomycete "Green" shows a reduced percentage isolation with surface sterilization in all cases. This, again, may be ascribed to several causes. The fungus may be both an internal and external colonizer which is partially resistant to sterilization. Otherwise its pattern of

isolation similar to that of P.congena with approximately the same build up time in the leaves of the mature trees (P31) and a slower build up and reduced percentage isolation in the leaves of the thinned four year old trees on site R63.

The percentage isolation of Piricauda sp. and 'H' was reduced to almost zero by surface sterilization. This could mean that these fungi are particularly susceptible to the sterilising agent which has killed both their external and internal hyphae or it is only a surface colonizer which was killed by the surface sterilising.

Figs. 10.1.3 and 10.1.4 show Piricauda sp. and 'H' to be much more common in the young tree needles and a decline to zero percentage isolation after six months, but Fig.10.1.1 indicates that they were more common in the mature tree leaves and remained a high percentage of the micro-organisms isolated for two years. 'H' differed from Piricauda sp. in having a greater percentage isolation.

"Pen" and "Fusarium" were never abundant at any stage but showed a tendency to be more abundant on the leaves of the mature trees probably as external colonizers although a significant percentage isolation of "Fusarium" was recorded on young tree leaves on 3 February, 1968.

The fungus "B-P" showed an increased percentage isolation in all cases with surface sterilization. This suggests that it was an internal colonizer which was able to grow on to the agar better under reduced fungal competition. In all cases the percentage isolation was highest

in the mature tree leaves (P31) and also showed a decline to zero after approximately one year.

"Others", this group, as expected, displayed variable patterns depending on the dominant species included at each stage. In general, there was a tendency for surface sterilization to reduce the number of these species, especially in the mature tree leaves. Unsterilized samples showed an initially high percentage isolation in the mature tree leaves and a sudden decline coincident with the increased percentage isolation of P. congena and "Green". This latter trend was not so pronounced in Fig. 10.1.3 where there was a slight increase in "others". Surface sterilization reduced the number of these fungi in the mature tree needles to almost zero but it had far less effect on the young tree needle. This suggests, as may be expected, that the initial colonizers of the older tree needles are external colonizers.

Isolations from litter bags (Fig.10.1.6) showed some similarities with the results recorded from needles but marked differences were apparent. The greatest difference was the large percentage isolations of "B-P". Surface sterilization has only been used in the 3 February, 1968, collection. This treatment resulted in the same increase in percentage isolation as noted for the same fungus on leaves which were attached to the fallen trees. In the case of the needles on the trees there was an early period of expansion and then decline which may perhaps have been caused

Fig.10.1.6

Percentage isolation of fungi from P.radiata needles contained in nylon net bags in Tasman Forest.

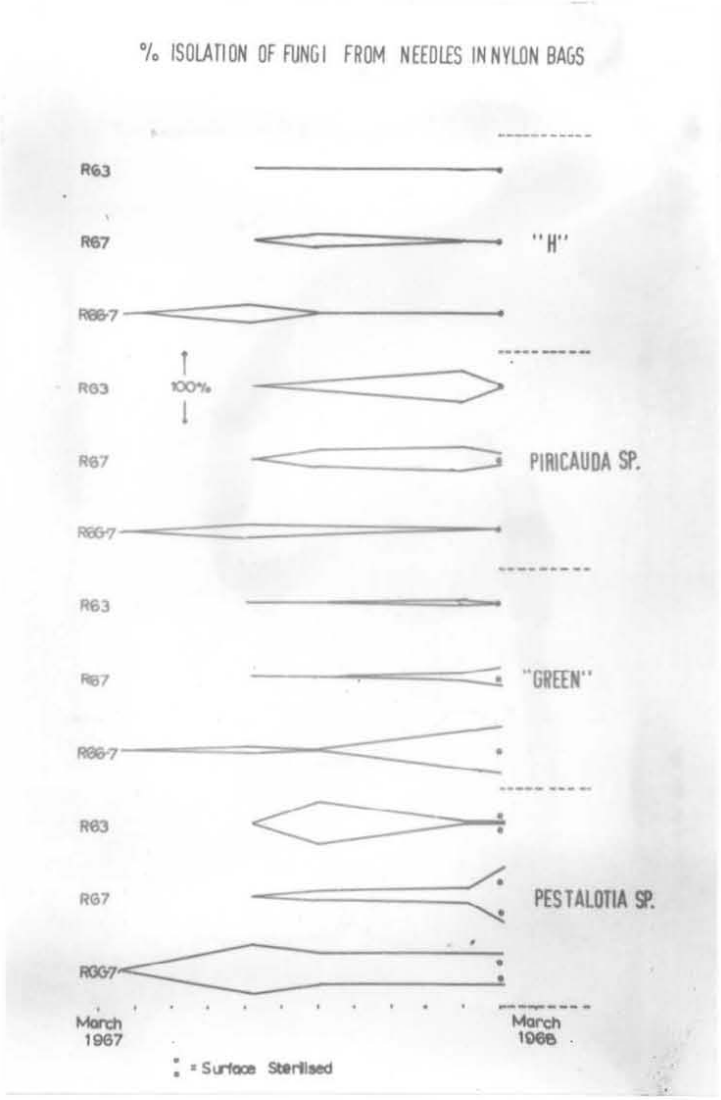


Fig.10.1.6

Percentage isolation of fungi from P.radiata needles contained in nylon net bags in Tasman Forest.

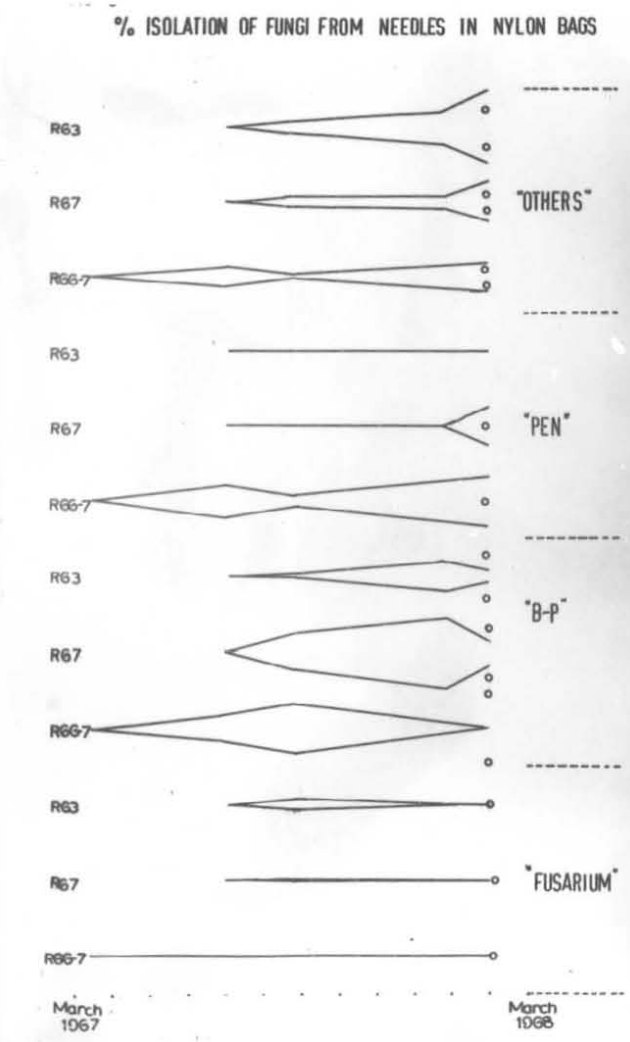
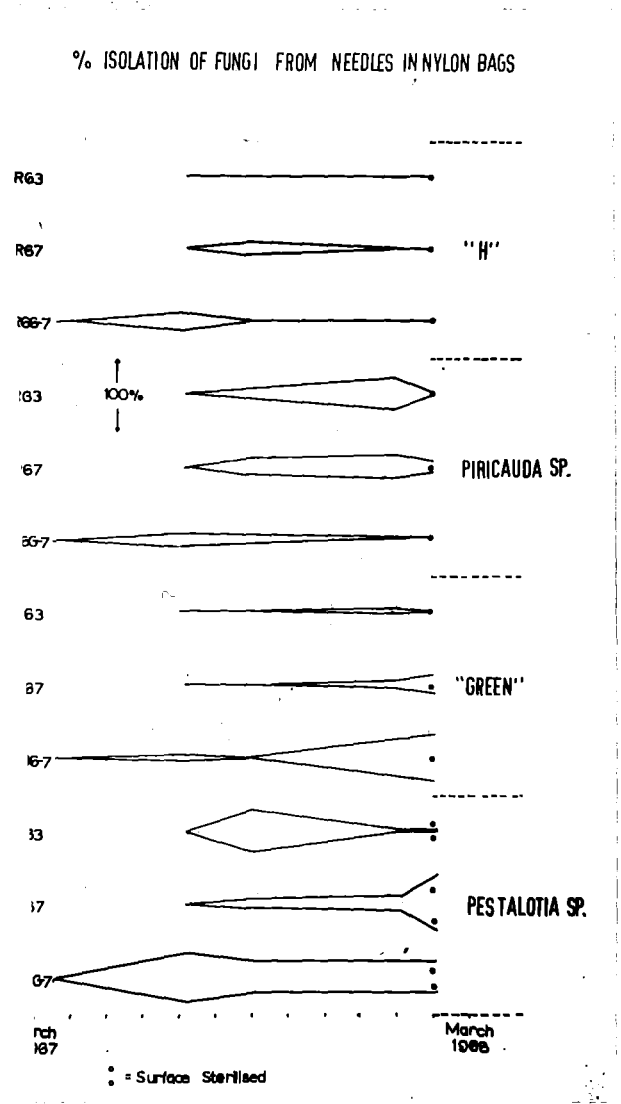


Fig.10.1.6 (continued)



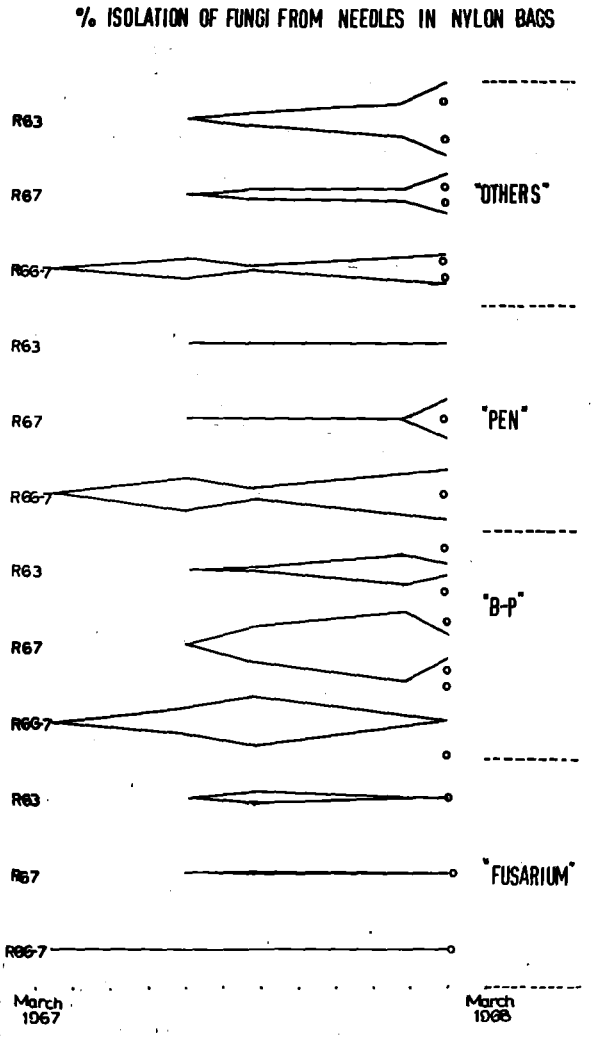


Fig.10.1.6 (continued)

by a more pronounced desiccation as the leaves aged on the trees while leaves in the litter bag with closer compaction of needles and closer proximity to the ground may have a moister microclimate which allowed greater growth of "B-P".

P.congena shows the same initial rise as "B-P" in two of the three cases but then a decline coincident with the rise in "B-P". The percentage isolation of "Green" was very reduced compared with that of the exposed needles while in the exposed needles surface sterilization reduced the number of isolations in the litter bag study it has reduced the number to zero.

Piricauda sp. occurred more frequently in the bags than in the exposed leaves attached to the tree and again was reduced to zero by surface sterilization. The low percentage isolation of Piricauda sp. on the mature tree needles in the exposed-attached needles compared with the 4-year old tree needles was not present in the litter bag. This suggests that Piricauda's absence from the mature tree needles in the first instance was due to microclimatic rather than nutritional factors.

'H' followed a reverse pattern in the bags as compared with in the exposed leaves. It was not recorded on young tree needles in the bags but was recorded in one instance in both sets of older leaf litter bags in direct contrast to the exposed leaves. Figs. 10.1.3 and 10.1.4. This, again, suggests that it was not related to the initial nutritional aspects of the leaf but to some other factor or factors which

may even modify the nutritional aspects. "Fusarium" and "Pen", in general, behave as in the exposed needles. "Fusarium" was not important at any stage but "Pen" was important, especially at one site. "Others" isolates show a gradual increase in numbers which were reduced slightly by surface sterilization but does not show the initial prominence and rapid decline, as shown in Figs. 10.1.3 and 10.1.4.

In general, there is a resemblance between the fungal invasion patterns of the two types of mature tree and young tree leaves and a resemblance between bags put out at the same time. There is also a resemblance at any one time although not so marked. This suggests, as would be expected, that the chemical properties of the leaf and the prevailing climate have an effect on the pattern of succession.

Association Analysis

Only combinations of fungal species which showed significant associations were recorded in Table 10.1.8.

The association found between species were not very consistent in needles exposed on fallen trees for different lengths of time.

The combinations P-G, BP-Tr, and Tr-Fus appear to show the most consistent associations. No associations were, however, consistent throughout.

The association of G - others and P - others cannot be easily interpreted because the group "others" included many different fungi. Other combinations which appeared significant at certain times only may have been real effects

because the association may have occurred only on needles with a particular microclimate or food supply. For example, the BP-Tr association appeared only after the needles had been exposed on fallen branches for a length of time. Also the combination P-G appeared mainly on mature (P31) needles and not on needles from young P.radiata trees.

In general, the number of associations found was disappointing and more work would be needed to confirm the associations which were found.

Fig.10.1.9 Pestalotia sp. isolated from pine needles collected from Tasman Forest, growing on malt agar.

Fig.10.1.10 Fungus "Green" isolated from pine needles collected from Tasman Forest, growing on malt agar.

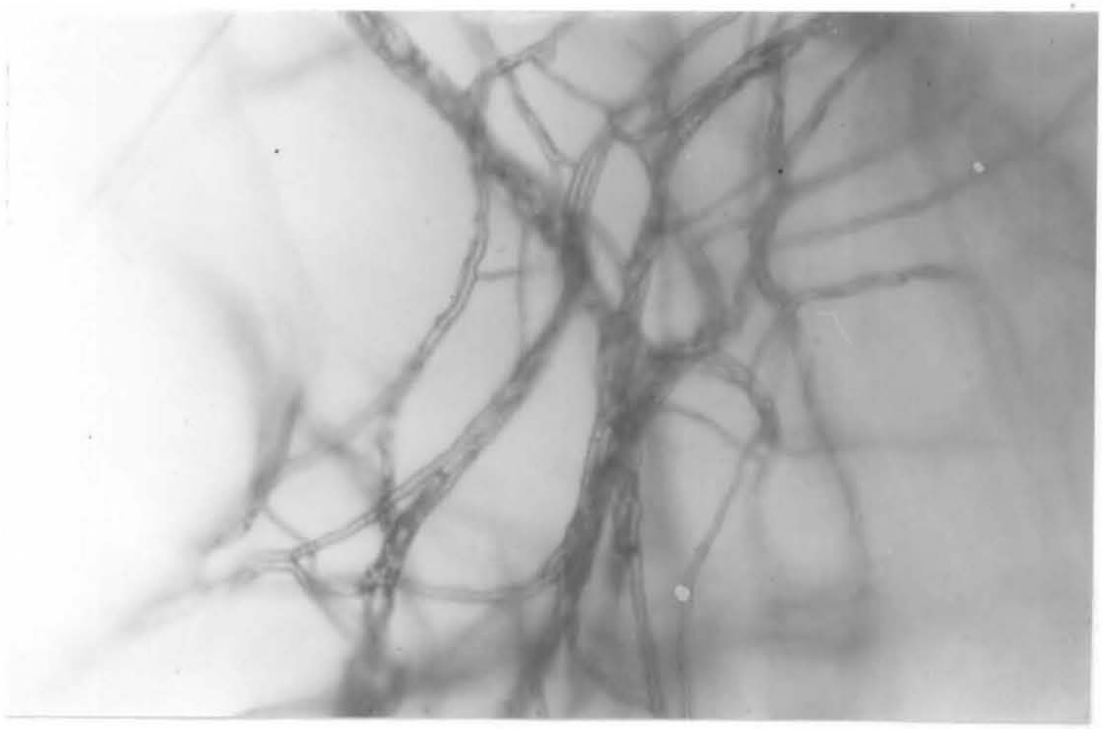


Fig.10.1.9

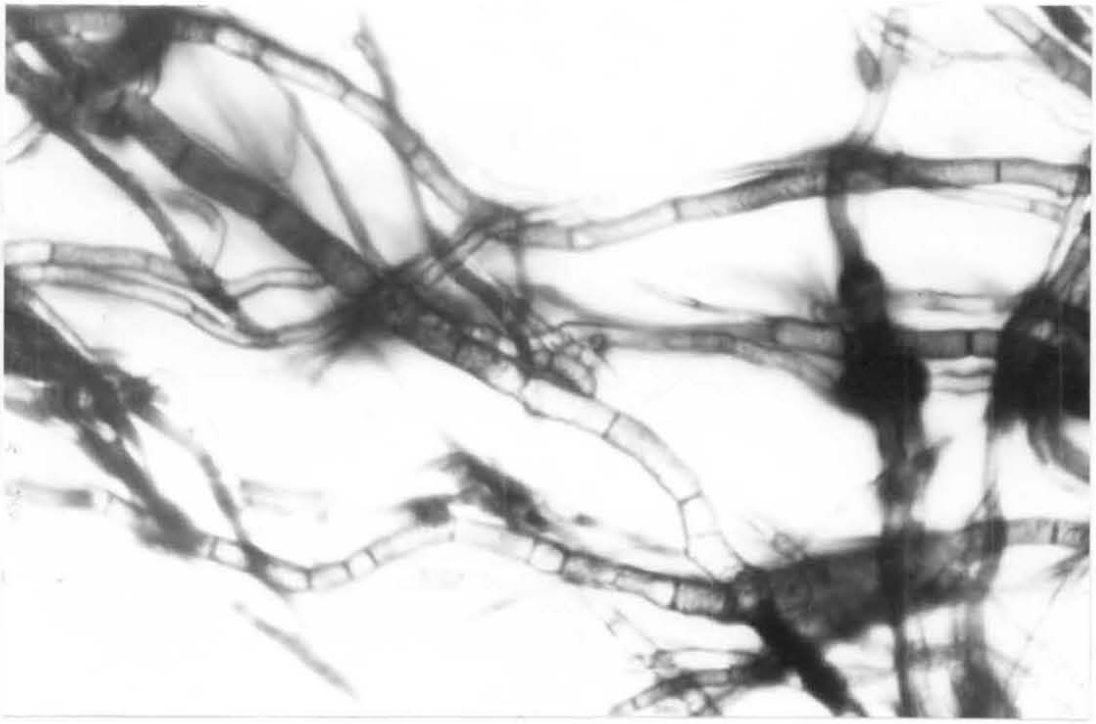


Fig.10.1.10

Table 10.1.8 Association analysis of the presence of fungi on needles collected from five sites in Tasman Forest. Significant χ^2 values only are recorded in the table. The results of 100 isolations of fungi from needles at each site were used in the analysis.

	Significant Combinations														
Sites	P-R	P-G	P-O	P-H	R-BP	R-Fus	BP-G	BP-Tr	BP-H	G-O	G-Tr	G-Fus	O-H	Tr-Fus	Fus-H
P31		**	**		**	**				**					
R66-67			**	*				**	*	**			**	**	
R65-66	*	**	*					**							*
R63							**								
R60			**					**		**	*	**		**	

10.2 MICRO-ORGANISMS IN THE SOIL

INTRODUCTION

It was considered that the number of micro-organisms at any one time would give an indication of how much nitrogen was bound up by the micro-organisms themselves. The number of micro-organisms together with studies in soil respiration could give an indication of how rapidly nitrogen turnover was occurring as a build up in numbers at any one stage might indicate an increased rate of mineral nitrogen usage by micro-organisms (Thornton and Gray, 1930); also it was hoped to relate the numbers of the various groups of micro-organisms to the breakdown of different fractions of soil organic matter under different conditions. It was also of interest to compare the soil microflora of the soils of P. radiata forests with other New Zealand soils which have already been studied and with the results found in other countries.

The numbers of micro-organisms in the soil have been studied by numerous workers, for example, Corke and Chase, 1964; Aggleton, 1934; Stenton, 1953; Badura and Badurowa, 1964; Mickovaki, 1965; Sewell, 1959; Stout, 1958, 1960 and 1961; Jensen, 1963; Latter, Cragg and Heal, 1967; and Kawada and Kawada, 1957. Results obtained with slightly different methods for the estimation of microbial numbers may vary greatly. Thus the results of different workers are not always directly comparable.

The method of Conn (1918) for the direct examination of soil was tried, using 0.015% agar as a mounting medium. However, only a few individual micro-organisms could be confidently distinguished from the soil particles. Because of these difficulties this method was abandoned and subsequently reliance has been placed on plate counts to provide a quantitative estimate.

METHOD

(1) Plate Count

Samples collected in the dry season had to be collected as pieces removed with a grubber because the soil at this period was too hard to remove complete cores which, however, could be obtained when the soil was wet when a 3.0 cm diameter tubular stainless steel soil sampler was used. Soil samples were placed in new plastic bags and brought back to the laboratory within three days where they were kept under refrigeration at approximately $6^{o} \pm 2^{o}$ C.

The samples were removed from the refrigerator and the surface of each piece was scraped with a flamed scalpel to remove surface contamination that may have occured by using a non-sterile sampling apparatus. An accurately weighed sample of approximately 10 grams was placed in a bottle containing 90 ml of sterile tap water and eight glass beads. The bottle was then vigorously shaken for two minutes by hand. A mechanical shaker was tried but was found to disperse the soil sample incompletely. No fraction of the soil was re-

moved before sampling, stones and roots being included where they occurred. Using the standard plate count dilution procedure, dilutions of 10^{-3} and 10^{-4} were prepared for soil samples obtained from each of the nine samples below 10 cm and dilutions of 10^{-4} and 10^{-5} were used for those from and above 10 cm depth. From these dilutions convenient numbers of bacteria and fungi grew on plates which were incubated at 22° C for approximately 14 days. Two media were used for routine plating: Potato dextrose agar (Baltimore Biological Laboratory) -

Potato infusion	200 g
Dextrose	20 g
Agar	15 g.

This was acidified just prior to use with 10 ml of a 10% tartaric acid solution per litre of agar. Bunt and Rovira's (1955) Soil extract agar, to which 0.1 g of Actidione (the Upjohn Co.Ltd.) per litre of agar was added, was used to enumerate the bacteria -

Agar	15.00 g
K_2HPO_4	0.40 g
$(NH_4)_2HPO_4$	0.50 g
$MgSO_4 . H_2O$	0.05 g
$MgCl_2$	0.10 g
$FeCl_3$	0.01 g
$CaCl_2$	0.10 g
Peptone	1.00 g
Yeast extract	1.00 g
Soil extract	250 ml
Tap water	750 ml.

Soil extract was prepared from Temuka silt loam soil selected from an area unlikely to have been contaminated with sprays and poisons.

(2) Analysis

Data from samplings conducted on the 4 July, 1967, 30 August, 1967, 2 January, 1968 and 3 February, 1968, were subjected to analysis of variance on IBM 360/44 and 1130 computers, using a programme developed by the author. The restricted number of sampling times used in this analysis was necessary because it was desired to primarily investigate the effect of age after clear felling on the number of micro-organisms, with a view to determining if at any stage micro-organism numbers increased to an extent likely to cause greater immobilization of the nutrients at a particular age compared with other ages. Since sampling was carried out over a period of 30 months, age classes chosen in the investigation would overlap in time and no successful analysis could be carried out.

It was also desired to determine if there were a seasonal difference in micro-organism numbers and the dates selected (mentioned above) were the most appropriate for this. All other data collected was used in a stepwise multiple regression analysis. Numbers of different groups of micro-organisms were regressed with other microbial groups as well as pH and soil water content data. Ratios of the various groups of micro-organisms were also determined and used as further independent variables. In each regression the two

microbial groups used to compute the ratios were excluded from the list of independent variables. The programme used for regression was an IBM Library programme modified to suit the data.

RESULTS AND DISCUSSION

Discussion of Method

Initially special media were used for the isolation of actinomycetes (Cooke and Chase, 1956; and Kuster and Williams, 1964) but these did not have any enhancing effect on the actinomycete count and so were replaced by Soil extract agar in routine analysis. Although Robinson (1962) found no significant increase in colonies on Bunt and Rovira's (1955) agar when compared with an unfortified soil extract agar prepared from a fertile field soil, use of a fortified medium obviated the risk of any nutrient shortage. Soil extract agar has been shown to be superior to richer artificial media by Lochhead and Taylor (1938) and Lochhead and Burton (1956), who showed that 50% of isolates taken from soil extract agar were incapable of growing on mannitol-asparagine-salts agar. On the other hand, Jensen (1962) showed that a nutritionally rich medium such as tryptone glucose extract agar could give higher counts from a beech mor than a nutritionally lean soil extract agar.

Whilst it has been argued that soil extract is not a standardised medium, Smith and Warden (1925) could find no significant differences between the numbers of colonies

developing on soil extracts made from widely different soils and James (1959) obtained similar numbers of organisms on plates of soil extract agar prepared from either a saline soil or from a clay soil. The origin of the soil extract is, of course, of less importance when values within one area are being compared but is more important when numbers are being compared with those from other localities made by other workers.

Plate counts of successive dilutions often do not present a linear series, and it has been suggested by Meiklejohn (1957) that the phenomenon is due to either antagonism between colonies on the plate or to antibiotics in the soil which become diluted out at the higher dilutions. Whatever the cause of the increase in bacterial numbers with higher dilutions it appears to be necessary to accept for comparative counts between soils, plates which have been subject to the same number of dilutions and have similar numbers of colonies.

Unfortunately, it is not always easy to estimate accurately the number of colonies likely to be present in a sample. Consequently the problem of which dilution to count arises. In this work it has been decided to use the average of the two dilutions. It has been found that even thorough homogenizing will give variation between duplicates. For example, with white colonies on soil extract agar, one soil gave 0% variation between extremes. Another gave 100% variation and a third 10%. On the other hand, error from outside contamination in the plating stage of the procedure

was negligible as plates of high dilution occasionally contained no micro-organisms.

Results show the numbers of micro-organisms of approximately 10^6 organisms/g o.d. soil. It is now generally recognised that the plating method for the enumeration of micro-organisms leaves much to be desired, especially in the case of actinomycetes and fungi (Jensen, 1963). Skinner (1951) demonstrated that most of the actinomycete population found on agar plates exist in the soil as conidia. It is also generally recognised that the abundant colonies of Penicillium sp. and Aspergillus sp. result mainly from spores rather than pieces of mycelium. Even if spores and conidia were not present as a complicating factor the nature of the fungus and actinomycete mycelium make it difficult to count them in this manner. But, as has been pointed out on numerous occasions, no existing methods give much better results.

General Discussion

Heal, Bailey and Latter (1967) found in a study of Antartic (Signy Island) soils and English soils that the number of micro-organisms are approximately the same in similar soils but vary greatly with different soil and vegetation types. This suggested that the soil and vegetation type have greater influence on numbers than does climate. Climate caused some difference in the flora in the two areas because cold-intolerant genera were not found in the colder area.

In general, the effects of the environment are so varied that only the broadest of trends can be suggested on today's knowledge. Micro-organisms are poikilothermic and their activity within limits is proportional to temperature. If temperature decreases homiothermic animals still have to maintain a constant activity rate, usually against a dwindling energy supply, which often reduces population numbers. On the other hand, poikilothermic organisms, especially saprophytic organisms in the soil, usually do not face a declining energy supply over a short period. Thus the numbers of these organisms could increase under cold conditions because the reduced activity of individual organisms would leave more substrate available but the rate of reproduction would be slowed because of the lowered temperature and metabolic activity. Numbers of soil micro-organisms should therefore remain relatively constant with temperature changes unless the temperatures experienced are lethal. Lochhead (1926) found, in fact, that the numbers of bacteria when estimated on non-selective media were at least as high in frozen as in unfrozen soils.

Since there are many kinds of micro-organisms in soil, each having maximum metabolic efficiency over a certain temperature range, a change in the nature of the flora would be expected with temperature change. This phenomenon has been reported by Jensen (1934) and Allen and Brock (1968) who reported a characteristic flora which depended on temperature. Lochhead's (1926) observation or those of Allen and

Brock (1968) and Jensen (1934) are not mutually exclusive if it is agreed that few, if any, organisms are adapted to high activity at approximately 0^{o} C, and so under those conditions there is therefore no selective pressure to change the nature of the flora. Eggleton's (1934) observation that numbers were lower during winter is hard to explain. Either the flora was killed by the low temperatures or some other limiting factor was operating in this instance. In general, there does not appear to be any relationship between microbial numbers estimated by the plate count method and soil temperature (Jensen, 1936; Eggleton, 1934; and James and Sutherland, 1940).

It must also be realised that the effects of temperature are not always simple since other variables, such as the solubility of gases in soil solution, are affected by temperature. Moisture probably acts in a similar way. A limiting amount of moisture may mean a general decline in activity but not necessarily numbers. Some organisms are known to be more adapted to certain moisture levels than others, and this may change the nature of the flora also. Jensen (1935) and James and Sutherland (1940) have reported a relationship between the moisture content and numbers of viable bacteria because, once moisture becomes non-limiting, bacterial numbers can increase and are limited only by their own metabolic activity and the availability of energy substrates.

Fig.10.2.1 Multiple correlation coefficients obtained using a Stepwise Multiple Regression Programme on an IBM 1130. Regression was carried out on data from each soil depth.

	Soil Depth			
	0 - 2.5 cm	5 cm	10 cm	25 cm
B	W +0.359	Y +0.421, W +0.540	Y +0.380	Y +0.429, A +0.612, Ph -0.688
F	Y +0.350	Y +0.524	W -0.463, B +0.621	-
A	-	-	Ph +0.335	B +0.426, W -0.516
Y	F +0.350, Ph +0.463	F +0.524	F +0.397	B +0.429, Ph +0.531
W	B +0.359	B +0.381	F -0.463, B +0.591	Ph +0.366
Ph	-	-	A +0.335	W +0.366
A/Y	-	-	-	W -0.466
B/F	W +0.361	W +0.354	W +0.426	-
F/Y	-	-	-	-
F/A	W +0.366	-	-	B -0.381, Y +0.486
B/Y	-	-	-	-
B/A	-	-	-	W +0.382

Each analysis is the mean of 40 observations at deach depth.

W = Water content
Ph = Acidity
B = Bacterial numbers
F = Fungal numbers
A = Actinomycete numbers
Y = Yeast numbers

Sign of coefficient applies only to simple correlation coefficient not given in this table.

The number of organisms is probably usually more influenced by the energy material available in the soil than the climatic conditions outside the soil. Thus, the climatic factors were found to be less important by Heal, Bailey and Latter (1967) as mentioned previously.

From this discussion it is possible to partially explain the results of counts made on soil from the Nelson area. The results showed that in summer, when the organisms are able to become metabolically active, moisture may be a limiting factor, while in winter, when temperatures are lower and micro-organisms show reduced activity, there may be no moisture shortage but they are probably not able to divide because of energy shortage. These factors may help to keep the numbers as constant as they appear in the results. This uniformity is in contrast to the results reported by Goodfellow, Hill and Gray (1968) who found 8×10^6 bacteria/g in November while, in December, they found 1.5×10^8 in the A horizon of a pine forest in England.

Correlation of bacterial numbers with other factors

Soil water content was significantly correlated with bacterial numbers ($p = 0.05$) at the 0 - 2.5 cm and 5 cm depths although correlation coefficients were low (Table 10.2.1). Jones (1968) found a correlation of $r = 0.452^{**}$ between soil moisture and bacterial numbers which is little better than the correlation found in this study. The analysis of variance (Appendix 10.2B), on the other hand, showed no significant seasonal effect suggesting that several

factors may be operating in such manner as to cancel each other out.

The correlation with water content in the top soil layers may have been due to greater water content fluctuations in these layers compared with the 10 and 25 cm depths. Bacterial numbers were correlated with yeast numbers at the 5, 10 and 25 cm depths. Correlations, in this instance, although significant were low (0.420, 0.380, 0.429). Either the numbers of bacteria and yeasts were controlled by similar environmental factors which do not control the other groups of micro-organisms or one was dependent on some metabolite produced by the other. Without further experiments these two possibilities cannot be assessed.

The correlation of actinomycetes with bacteria at the 25 cm depth may be an artifact resulting from the use of the same soil dilution agar plates for the estimation of both groups. The correlation may, therefore, have been between plating techniques although if it were one would expect all depths to show the same correlation.

The pH of the soil at different depths (see Section 5.2) did not show greater variability at a particular soil depth. Therefore, little is known which would explain why a correlation between bacterial numbers and pH should occur only at the 25 cm depth. Perhaps at this depth other factors were not limiting, or, since there is a weak correlation between pH and water content at this depth, water con- may be the major influence. Corbet (1934) found a

Fig.10.2.2 Numbers of bacteria in soil samples collected from four ridge and gully sites at four depths under P.radiata in Tasman Forest. Numbers were estimated using plate count method. Incubation was carried out at 22° C for 10 - 14 days.

Fig.10.2.10 Numbers of actinomycetes in soil samples collected on two occasions in summer and winter from four ridge and gully sites at four depths under P.radiata in Tasman Forest. Numbers were estimated using plate count method. Incubation was carried out at 22° C for 10 - 14 days.

Fig.10.2.11 Numbers of actinomycetes in soil samples collected under P.radiata at different age classes at four depths on ridge and gully sites. Numbers were estimated using plate count method. Incubation was carried out at 22° C for 10 - 14 days.

Fig.10.2.12 Numbers of actinomycetes in soil samples collected at four depths on four ridge and gully sites under P.radiata in Tasman Forest. Numbers were estimated using the plate count method. Incubation was carried out at 22° C for 10 - 14 days.

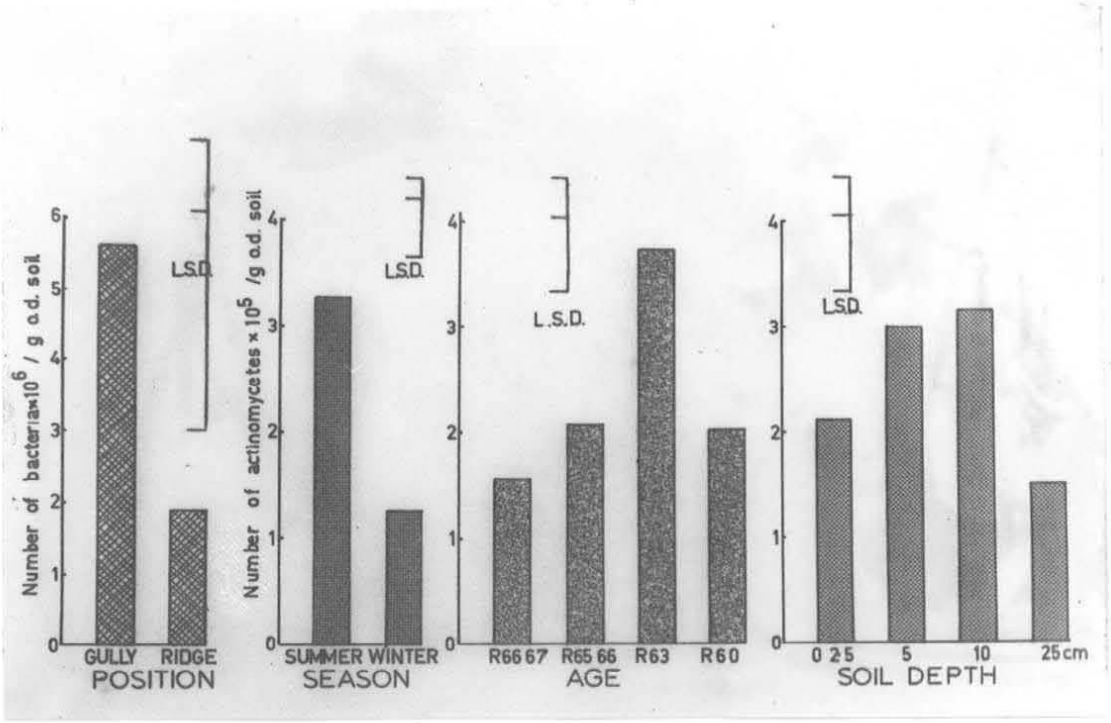


Fig.10.2.2 Fig.2.10 Fig.10.2.11 Fig.2.12

correlation between water content of the soil in a tropical region whereas Thornton and Gray (1930) did not find such a correlation in temperate soils. It is possible that some other factor was limiting microbial numbers in the soil studied by Thornton and Gray (1930) which masked the effect of moisture. Alexander (1961), on the other hand, has made the generalization "The bacterial population of various soils is closely correlated with their moisture contents". In this study the numbers were not "closely" correlated and must therefore be controlled by other factors as well as moisture, in contrast to the work reported by Jensen (1934) and Seifert (1940 and 1951).

Bacterial numbers per g soil showed greater similarity to those obtained by Corbet (1934) (5×10^5/g) than Thornton and Gray (1930) (2×10^7/g). This could be related to the nutritional status of the soils because the soil studied by Corbet (1934) was a forest soil whereas Thornton and Gray studied an agricultural soil. Goodfellow, Hill and Gray (1968) found under Pinus nigra that numbers varied from 2×10^6 to 2×10^8/g in the space of one month, while Eastwood, Frazer and Webley (1950) found population of 1×10^6-1×10^7/g in soils under 20-year old trees. Thus it would appear from the contradictory results found in the literature that there are no simple overriding factors at work but that the numbers of micro-organisms found in soils are dependent on many factors, the number of which has yet to be defined.

Correlation of fungal propagule numbers with other factors

Fungal propagule numbers were correlated with yeast numbers at the 0 - 2.5 and 5 cm depths (Table 10.2.1). This correlation may be an artifact since both fungi and yeasts were estimated using the same plates, but, as explained previously, this is unlikely. The significant correlation between fungal propagule numbers and water content at the 10 cm depth is difficult to explain because the correlation was significant only at this depth. If fungal propagule numbers were dependent on soil moisture it would be logical to assume that the water content of the soil surface layers would show the greatest correlation since these layers would tend to vary more in moisture content. Fungal numbers are also correlated with bacterial numbers only at this depth. The 10 cm depth appears to be a critical depth in terms of soil properties in Tasman Forest. In general, ridge sites soils seem to lack gray A horizon soil at this depth whereas in gully sites the A horizon usually continues down to this depth. At this depth factors limiting bacterial numbers may be common to both bacteria and fungi and hence explain the correlation. The same may hold for the correlation with water content ($r = 0.463$) because the water-holding capacity and hence the water saturation value and aeration factor may be the critical factor at this depth.

The absence of a correlation with pH is unusual (Boswell, 1955) since Jones (1968) found a significant correlation between fungi and the age of the plantation from

Fig.10.2.3 Numbers of fungal propagules in soil samples collected under P.radiata of different age classes at four depths on ridge and gully sites. Numbers were estimated using plate count method. Incubation was carried out at 22° C for 10 - 14 days.

Fig.10.2.4 Numbers of fungal propagules in soil samples collected on two occasions in summer and winter from ridge and gully sites at four depths under P.radiata in Tasman Forest.

Fig.10.2.5 Numbers of fungal propagules in soil samples collected from four depths on four ridge and gully sites at Tasman Forest.

Fig.10.2.6 Numbers of fungal propagules in soil samples collected at four depths in P.radiata stands of four ages. Soil was collected from both ridge and gully sites. Numbers were estimated using the plate count method. Incubation was carried out at 22° C for 10 - 14 days.

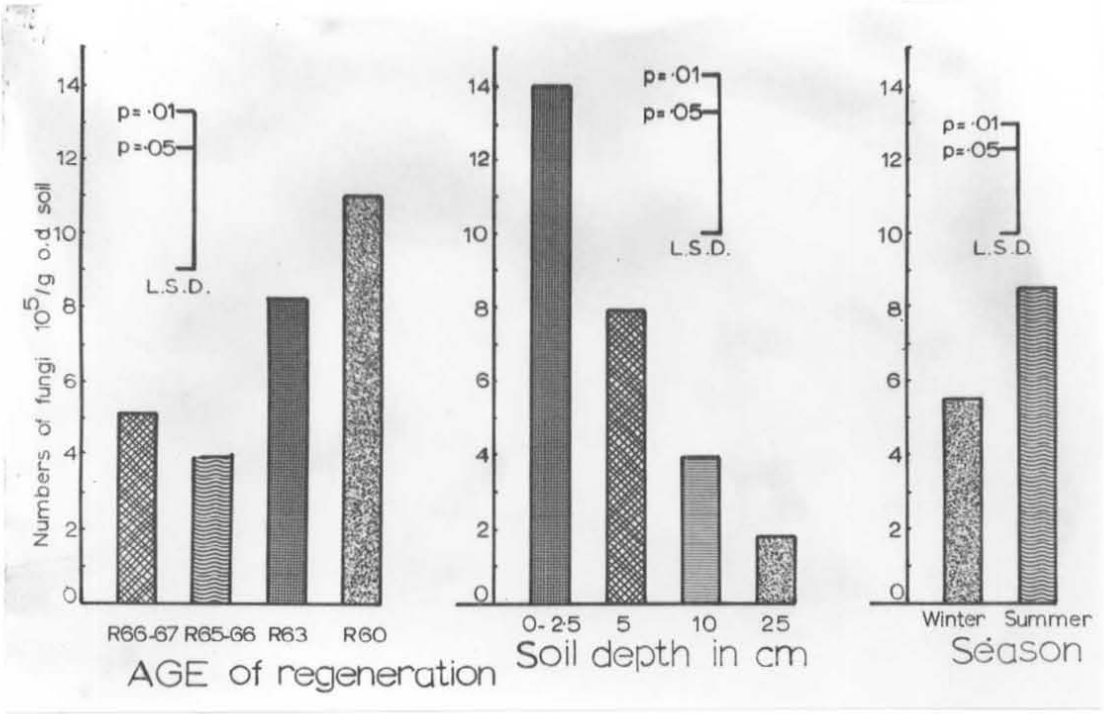


Fig.10.2.3 Fig.10.2.5 Fig.10.2.4

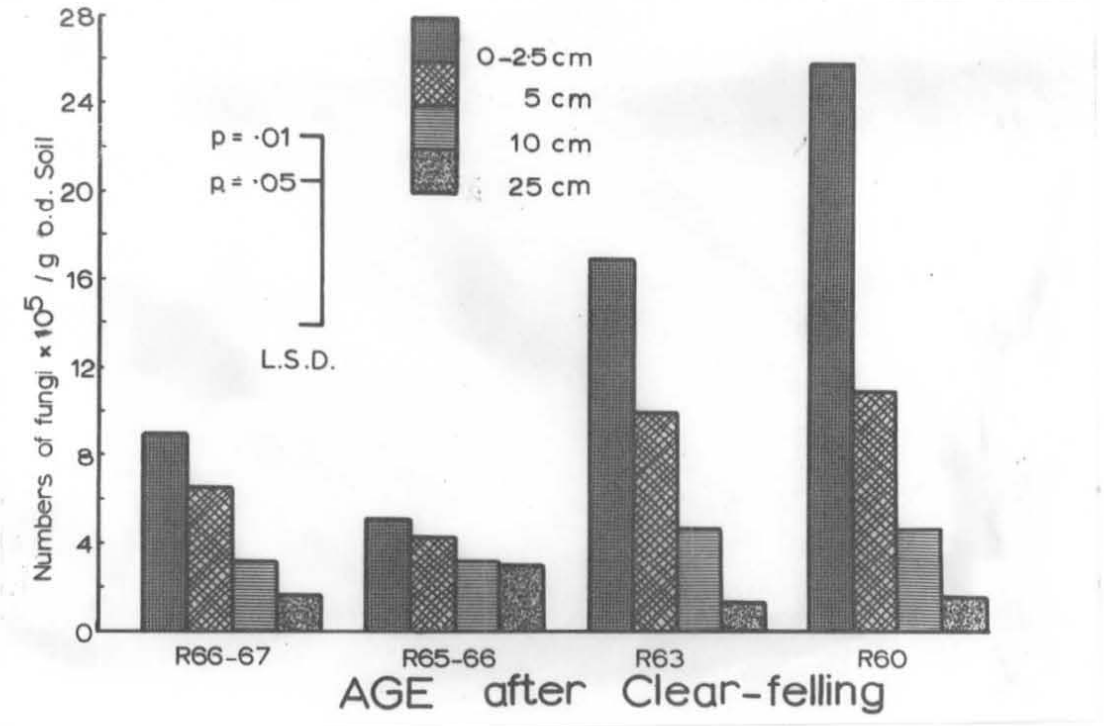


Fig.10.2.6

which the soil sample was taken and also a negative correlation ($r = -0.818^{**}$) between the level of acidity and the age of the plantation. In this study all soils sampled had had one previous crop of P. radiata and therefore the soil pH was relatively uniform not, as in Jones' case, where the plantation was the first crop. The analysis of variance table for the four sampling periods (Appendix 10.2C) shows the significant effect of age after clear felling although the Age x Depth interaction makes the main effect less pronounced. PH was probably not the cause of the age effect because its lack of correlation with fungal numbers. Fig. 10.2.3 shows a decline in numbers at R65-66 and then a significant build up after the trees start to close canopy. Perhaps the changed environment caused a greater spores production in the stands of older regeneration.

Season (Fig. 10.2.4) caused a significant effect in this study. This contrasts with the work of Williams and Parkinson (1964) who found no seasonality in conifer forest soils. It is not known which components of season were responsible for the effect. Water content of the soil seems to be unlikely in view of its low correlation with fungal numbers. Temperature may have been of importance since the numbers for summer samples were higher than for winter samples. Jones (1968) found a significant seasonal effect but an increase in numbers during spring was the main cause of the difference rather than a difference between summer and winter, although Christensen (1969), Tresner, Backus and

Curtis (1924) and England and Rice (1957) found loest numbers in summer. Jones (1968), on the other hand, found little correlation between fungal numbers and temperature while Sewell (1959) found a marked seasonal pattern of fungal activity in heathland soils in Britain which was related to temperature. The relation with temperature found by Sewell (1959) was directly opposite to that found in this study. The results of this study agree with Thornton's (1950) work where the greatest fungal activity occurred in summer and was attributed to warmer and drier soils in this season. As moisture was shown to be an insignificant factor in this study temperature therefore seems to be the most likely factor to explain the differences in fungal numbers. This agrees with Witkamp (1963) who found that temperature was more important than moisture under a humid climate.

Unfortunately, comparisons of this nature with other regions of the world are of limited value because much of the variation may be due to a limiting factor not studied in the investigation. This limiting factor may vary geographically making comparisons difficult.

Numbers decline with depth (Fig. 10.2.5) in a very regular manner although numbers at 10 cm do not differ significantly from those at 25 cm depth. The interaction age x depth (Fig. 10.2.6) shows that the increase in numbers in the R63 and R60 stands occurs mainly in the top 2.5 cm of soil. The development of a thin litter layer together

with the development of a more even microclimate under the closing canopy were probably responsible for the increases.

Correlation of yeast numbers with other factors

Yeast numbers were found to be correlated significantly with fungal propagule numbers at the top three depths, 0 - 2.5, 5 and 10 cm, but correlations were low (Fig. 10.2.1). It could be that some of the correlation was caused by the use of the same soil dilution agar plates for counting numbers although if this were an important effect there would probably have been a correlation at all depths. A more likely explanation is that the yeasts, since they are fungi, were affected by the same factors which control fungal propagule numbers although one would have imagined a correlation rather with conditions suitable for mycelial production in the fungi rather than spore production which is largely measured by the plate count method.

The effects of pH explain some significant part of the variation at the 0 - 2.5 and 25 cm depths. At both depths the relationship was positive, suggesting that pH was limiting yeast numbers. The presence of the correlation of pH with yeasts and the absence of a correlation of pH with fungi can be explained if pH is related to mycelial development and not to spore production which was largely measured by the dilution plate method.

The analysis of variance (Appendix 10.2D) shows age of regeneration and depth to be highly significant. The interactions age x position and age x depth were also significant.

Fig.10.2.7 Number of yeasts in Tasman Forest soil collected from four depths under P.radiata regeneration. Soil samples were collected on four occasions from both ridge and gully sites. Incubation of the agar dilution plates was carried out at 22° C for 10 - 14 days.

Fig.10.2.8 Number of yeasts estimated by the plate count method in soil samples from four ages of P.radiata. Soil samples were collected from ridge and gully sites at four depths on four occasions.

Fig.10.2.8a Number of yeasts estimated by the plate count method in soil samples from four depths. Samples were obtained from ridge and gully sites under P.radiata in Tasman Forest.

Fig.10.2.9 Number of yeasts in soil samples collected from ridge and gully sites under four ages of P.radiata regeneration in Tasman Forest. Numbers were estimated using the plate count method. Incubation was carried out at 22° C. for 10 - 14 days.

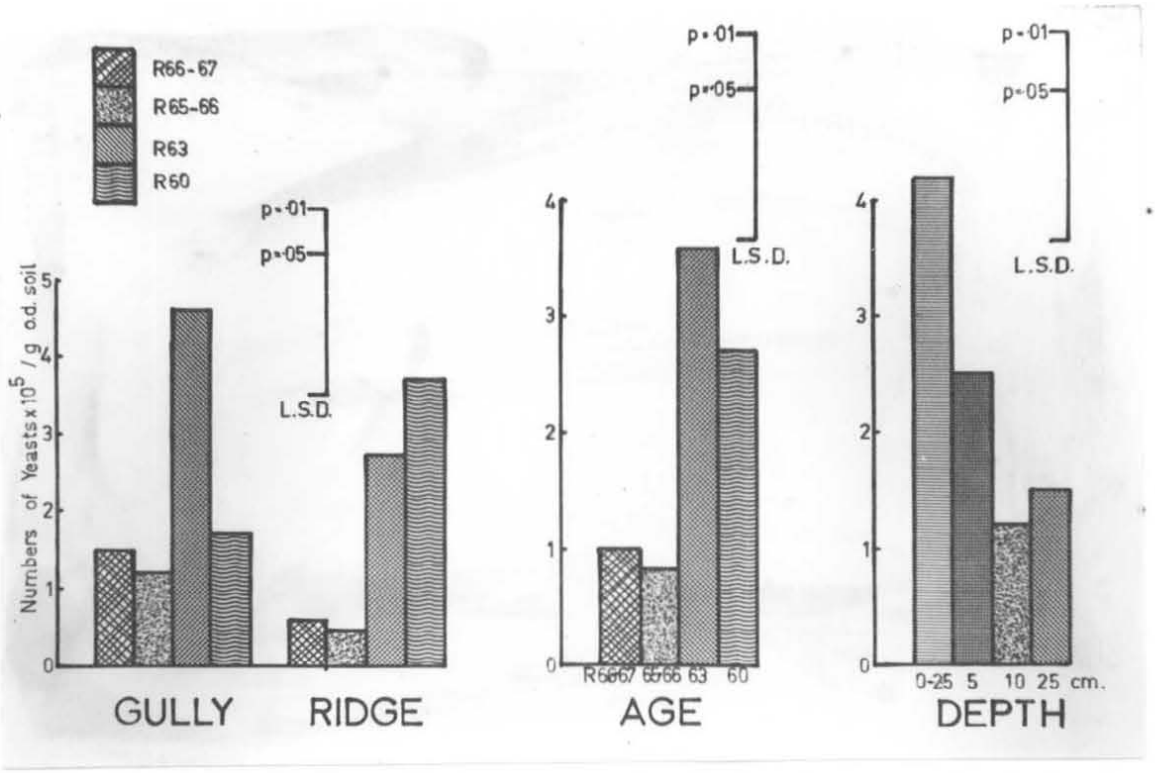


Fig.10.2.9 Fig.10.2.8 Fig.10.2.8a

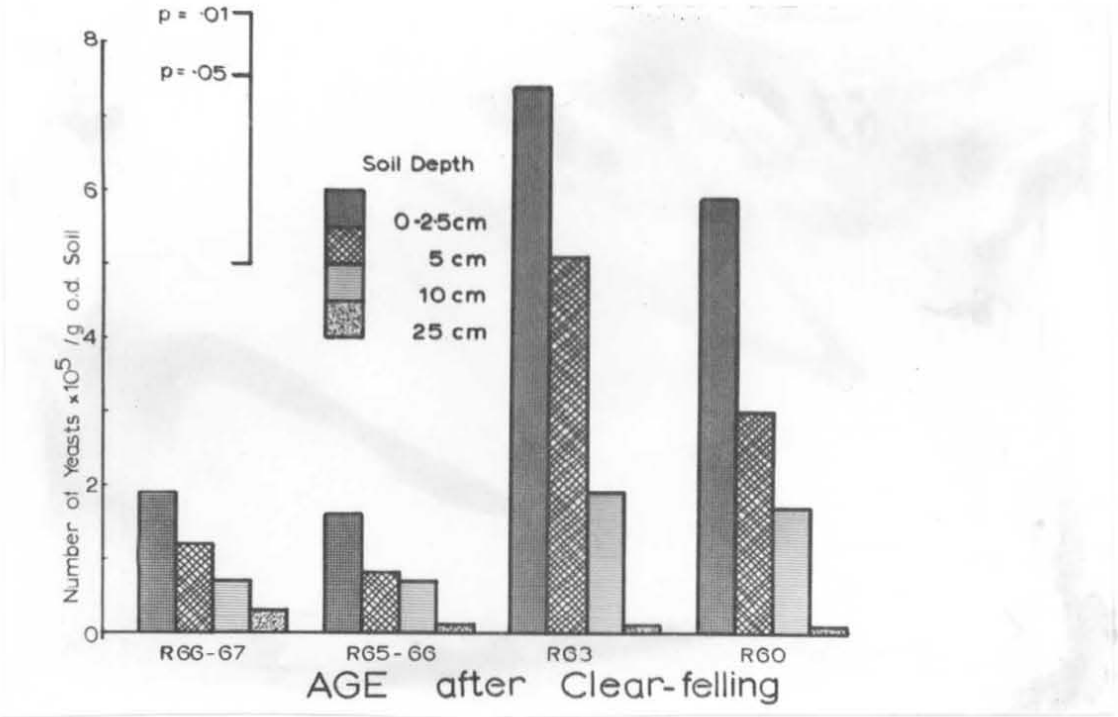


Fig.10.2.7

Yeast numbers increased markedly (Fig. 10.2.7) in the R63 regeneration stands, especially in the surface layers. At 25 cm depth there was no increase. Soil under R60 regeneration followed the same trend, but in a reduced fashion. The population increase occurred at canopy closure and may therefore have been brought about by the existence of more even environmental conditions or the development of a litter layer, although the drop in R60 stands compared with R63 stands does not support this theory. The positive correlation with pH in the top layers suggests that the acid nature of the litter layer reduces the numbers and some unknown factor, such as available food material, increases the numbers. Yeast numbers decrease with depth down to 10 cm. Numbers at 10 cm and 25 cm depth were not significantly different. It therefore appears that yeasts were most common on the readily available substrates present near the soil surface.

Age (Fig. 10.2.8)

Greatest numbers occurred in soil under R63 and R60 regeneration. Numbers in these stands were three times those at R66-67 and R65-66.

The position x age interaction (Fig. 10.2.9) showed that the number of yeasts in the early stages after clear felling (R66-67 and R65-66) in the gullies were double the numbers on the ridge sites although the two were not significantly different. In soil samples collected under R60 regeneration numbers of yeasts suddenly increased to

5 x 10^5/g o.d. soil in the gully and 2.7 x 10^5/g in ridge sites. At R60 stage numbers in the gullies declined significantly to almost the original value while numbers in the ridge sites further increased. It aould appear that the factor causing the increase was exhausted more rapidly in the gully than on ridge sites. It is, however, difficult to imagine a compound of this type since litter would be provided in increasing amounts in the R60 regeneration on gully sites. A more likely explanation is a combination of two factors. For example, the growth of pine trees has made some material available or cause a change to the optimum value of some environmental factor. Increased growth in the gully sites then produced some other conditions which have overridden the effects of the first factor. Reduced growth on the ridge sites has meant that this detrimental condition has not yet developed on these sites. A toxin produced in the accumulation of litter at the LR60 site may also have been responsible for the decline in numbers.

The number of yeasts isolated from Tasman Forest soil appears high compared to the figure 10^3/g soil quoted by Alexander (1961). Counts of 200 - 2,000 or more were not uncommon (Alexander, 1961). In this study the numbers of yeasts as high as 10^6/g soil were found in the upper soil layers emphasising the difference between this habitat and the habitats quoted by Alexander (1961).

The correlation of actinomycete numbers with other factors

The numbers of actinomycetes were not correlated with any of the factors used in the regression (Table 10.2.1) in the top layers of soil. At the 10 cm depth numbers of actinomycetes showed a low positive correlation (r= + 0.335) with the pH of the soil and at 25 cm actinomycete numbers were correlated positively with bacterial numbers and negatively with the water content of the soil. Dry conditions may have increased spore production by the actinomycetes and increased the numbers as estimated by the plate count method. There is doubt concerning the effects of dry conditions on spore or mycelial production as Cholodny (in Waksman, 1959) reported that low moisture content favoured vegetative growth whereas von Plotho (in Waksman, 1959) also observed that a dry atmosphere stimulated spore production. Meiklejohn (1957) reported an increased occurrence of actinomycetes on dilution plates from soil drying out due to drought. Jones (1968) found a significant seasonal effect and a small negative correlation (r= - 0.244) with soil moisture.

The analysis of variance of the four samplings showed season, age of soil after clear felling of first crop and the depth of soil from which the sample was taken to be significant factors (Appendix 10.2E). Numbers found in summer were almost double those found in soil samples collected in winter (Fig. 10.2.10). This may be at least partially ex-

plained by the correlation between numbers and water content of the soil as Tasman Forest soil was drier in summer. Temperature may also have had an effect. Jones (1968) found a higher correlation (r= 0.451) with temperature which agrees with the results of this study. An increase in numbers occurred in the age classes R66-67, R65-66 and R63. There was then a decline at R60 to a value similar to that of R65-66. The factors causing the decrease of numbers in gully sites at R60 may also have been responsible for the decline of actinomycete numbers.

Soil samples collected from the surface layers showed lower actinomycete numbers than samples from 5 and 10 cm depths while numbers at 25 cm were half those found in the top layers.

The water content relationships were in direct contrast to those of bacteria which showed a positive correlation at two depths.

Alexander (1961) considered that, as a group, actinomycetes were not tolerant of low pH and their population size was inversely related to the hydrogen ion concentration. The positive correlation with pH at 10 cm in this study supports this view. Corke and Chase (1964) found higher number/g soil than Waksman (1959) suggests. Gillespie, Waksman and Joffe (in Waksman, 1959) found that the critical pH for growth of the majority of actinomycetes was approximately pH 4.8 - 5.0.

Alexander (1961) adds that most strains of Streptomyces and related forms fail to proliferate or have negligible activity below pH 5.0 and the actinomycetes in highly acid environments frequently make up less than 1% of the total viable count. Jensen (1934-1936) found that although actinomycetes tolderated acid conditions (pH 3.4 - 4.1) they grew better in more alkaline conditions. Numbers found in this study, 2 x 10^5/g, are similar to those obtained by Corke and Chase (1964) in an A_2 mineral soil of pH 5.3. The soils at Tasman Forest were even lower, which suggests that Tasman Forest actinomycetes were even better adapted to the acid conditions.

The correlation of ratio of numbers of paired groups of micro-organisms with other factors

Table 10.2.1 shows that the ratio between paired groups of bacteria, actinomycetes, fungi and yeasts were correlated in the main with the water content of the soil. Many of the ratios examined showed no correlation with any of the factors tested.

The ratio bacteria: fungi showed positive correlations with soil moisture at the 0 - 2.5, 5 and 10 cm depths (r= 0.361, r= 0.354 and r= 0.426 respectively) while the bacteria:actinomycete ratios were only significant at the 25 cm depth (r= 0.382). With increasing moisture content of the soil the ratio numbers of bacteria to fungi and actinomycetes increased. These results support the findings of Jensen (1943) and Jones (1968) who found a negative correla-

tion (r= 0.498) between the actinomycete:bacteria ratio and soil moisture. Jones (1968), however, found a higher correlation with temperature (r= 0.683).

The ratio fungi:actinomycetes showed a positive correlation (r= 0.366) only in the surface layer whereas the ratio actinomycetes:yeasts showed the only negative correlation with water content of the soil at 25 cm depth. As water content increases either the number of yeasts increases or the number of actinomycetes decreases. The latter is more likely as shown by the significant correlation between actinomycetes and water content at 25 cm depth.

In the case of bacteria:actinomycetes the reduction in actinomycete numbers with increasing water content was probably responsible for the significant correlation of ratio with moisture at 25 cm. In the case of the bacteria:fungi ratio the positive bacterial response at the 0 - 2.5 and 5 cm depths and negative fungal response at 10 cm depth were probably responsible for the effects.

Total numbers of micro-organisms in Tasman, Balmoral and Hanmer Forests soils

The result of plate counts on Balmoral and Hanmer and mountain soil (Robinson, et al. 1964) samples showed that both soils had total numbers of micro-organisms of the same order as Tasman Forest soils, and thus these results suggest that the amount of microbial tissue was similar in all soils. The total number of micro-organisms in Tasman Forest soil did not change with length of time after clear felling which

suggests that at no time was there a significant amount of immobilization of nitrogen in the field due to microbial activity after clear felling, although the method used to estimate the numbers was probably insufficiently accurate to allow a detection of small percentage change in the numbers of micro-organisms and therefore the nitrogen status of the soil.

There was an almost significant position effect (Appendix 10.2F) since the ridge soils contained fewer micro-organisms than gully samples. Depth was a significant factor and, as expected, numbers were reduced with increasing depth.

Even though total numbers of micro-organisms remained fairly constant in summer and winter their activity probably varied depending on different environmental conditions.

CHAPTER 11

CONCLUSION

The major aim of this study was to investigate factors which could be associated with a lessening in the productivity of the second generation *P.radiata* crop in the Nelson District of New Zealand. A secondary aim was to examine ways of controlling the dynamics of the forest so that undesirable aspects could be reduced. Investigations were made along five main lines, as set out below.

A. The effects of different ages of *P.radiata* regeneration on the soil properties were studied. The object of these experiments was to determine if immobilization of nutrients during the growth of the young pine trees led to a reduction in the growth rate of the trees.

B. The factors which may have made the ridge sites different from gully sites in terms of tree growth were examined. Preliminary observations suggested that differences of this type rather than general productivity decline may have been responsible for the apparent reduced growth of trees.

C. The influence of different vegetation growing on the same soil type. Soil under L.scoparium, the original vegetation, and the same soil under P.radiata were compared to see if any major change occurred in the ecosystem.

D. Experiments were set up to ascertain the effects of soil types under different climatic conditions on the growth of P.radiata, especially the ability of the soils to support a vigorous second crop of P.radiata. This approach was used to see if there were differences between the soil types which would account for the reported slow growth of the second generation P.radiata on the Moutere Gravels.

E. Other variables, not normally found under natural conditions, on the properties of the soil, including the addition of mineral nutrients and energy substances, were studied. The effects of these additions, together with changes in the environment helped to show up variation between soil types. A study of the organisms in the P.radiata ecosystem was also started as an introduction to an investigation of the stability of ecosystems, especially those which are maintained in a monotypic condition. The aim of these studies was to determine if changes could be made to the ecosystem which would bring about increased growth of the trees.

A. It was found that many soil properties did vary with the age of P.radiata regeneration but none changed in a way that was correlated with the change in growth rate after five years as reported by Whyte (1966). The numbers of micro-organisms did not increase significantly at any stage in the growth of pine regeneration although some groups of micro-organisms varied in numbers at different stages. This suggests that the amount of nutrients bound up in microbial tissue did not increase markedly at any stage. The simple enumeration of numbers of micro-organisms is, of course, subject to large errors and differences in nitrogen levels which could affect plant growth may not have been detected with this technique.

Actinomycete numbers increased with the age of the regeneration up to R63 stands and then declined in the R60 regeneration. The numbers of fungi and yeasts also increased with increasing age of regeneration but most of the increase occurred in the top litter and soil layers. Available nitrogen also followed this trend but also showed a peak in the soil under the mature trees. This suggests that the litter layers contain the highest concentrations of available nitrogen. Yeast numbers at R63 were higher in the gully sites than on the ridge sites but at R60 the trend was reversed. Soil moisture also followed this trend. This is the only evidence found for Whyte's (1966) proposal that growth rates on the ridges increase at about this age.

The pH of the soil was highest in the R66-67 and R63 stands but no group of micro-organisms followed this same trend. This is to be expected as low correlation coefficients were found between micro-organism numbers and pH. The decomposition of root wood cubes was faster under older regeneration suggesting that conditions under these trees were more suitable for wood decomposing fungi or that more nutrients were available at these later stages.

B. Marked differences were found between ridge and gully sites in the ability of soil to decompose root wood. The rate of decomposition in gully sites was only half that on ridge sites. Either some physical factor allowed faster decomposition on the ridges with consequent immobilization of nutrients and reduced tree growth or the reduced tree growth allowed more nutrients to be used by the micro-organisms attacking the wood. The former explanation seems more reasonable because the addition of nutrients did not increase root wood decomposition under laboratory conditions, although it is possible that both processes were in operation. With increased age of regeneration the decomposition rate on the ridge sites became greater relative to that in gully sites. If the increased rate was the result of greater net mobilization of nutrients from ridge soil then tree growth could also be expected to increase on ridge sites of this age.

Bacterial numbers showed a significant difference

between ridge and gully sites. Analyses for available nitrogen revealed the same trend. Also less available nitrogen was found at the 5 cm depth in ridge sites compared with gully sites. These results support theories that soil erosion has reduced top horizon soil from the ridge sites since the major differences occurred at the 5 cm depth. PH was higher in all gully sites than ridge sites except at R65-66. No explanation could be found for this result but it did not seem to affect other soil properties since no other factors were correlated with pH.

C and D. The differences between soils from Lincoln College, Hanmer, Balmoral and Tasman Forests, used in the experiments, were in some cases very marked. Generally, Lincoln soil showed greatest activity but there were differences even within the same soil type. Soils varied both in the amount of O.M. and the energy content of the O.M. Lincoln soil contained the greatest quantity of O.M., Hanmer and Balmoral soil contained an intermediate amount while Tasman Forest soils had the least. It was somewhat surprising to find that the energy content of Tasman Forest soil was the highest and this may be a reflection of the extensive degree of decomposition of O.M. in this soil. The rate of decomposition of root wood cubes was more rapid in Hanmer than in Lincoln soil although the rate of decomposition of cellulose was highest in Lincoln soils, which suggests that the non-forest Lincoln soils may not possess a flora capable of

decomposing the more complex substances rapidly.

Hanmer soil had slightly more available nitrogen than Balmoral or Tasman soil and therefore would probably be able to support a high rate of decomposition of carbonaceous material than the other forest soils. Oxygen uptake decreased in the order Balmoral, Hanmer, Tasman under P.radiata and Tasman under L.scoparium. The O_2 uptake with changing temperature revealed a difference between Tasman Forest soils and the others. The greatest increase in O_2 uptake occurred in Tasman Forest soils between 25° C and 27° C but in the other soils it occurred between 13° C and 23° C. It is thought that this may be related to a difference in mean temperature in the respective areas. Carbon dioxide evolution rate decreased with a temperature change of 24° C to 31° C in Tasman Forest soils while it rose in the other soils. Moisture content affected O_2 evolution when the water content was raised to 40%. There was a decline in CO_2 production in Tasman and Balmoral soils because of waterlogging, which did not occur in Hanmer soil. In general, differences have been found between the soil types but it remains to be determined how much these differences are capable of affecting tree growth.

E. In these experiments the effect of different additives were tried. Both energy sources and mineral nutrients were added to the soils. No measurable response was found to the application of nitrogen except for a slight positive

response to nitrogen by the organisms responsible for decomposing root wood when the nitrogen availability was limited by the application of sawdust. It is also interesting to note that the application of nitrogen did not affect the rate of soil respiration. The addition of woody substrates in amounts equivalent to those which would be found in the field after clear felling reduced the growth rate of seedlings, demonstrating that immobilization could be induced and measured under laboratory conditions. Increased respiration in Tasman Forest soil on the application of glucose further demonstrated that nutrients were available to support increased microbial activity. The slight build up in nitrogen levels in soil samples incubated in the field in the absence of plant roots showed that a small amount of net mineralisation of nitrogen occurred in the field. That this was primarily the result of low gross mineralisation and not high immobilization was, to some extent, demonstrated by the slow decay of root wood blocks in Nelson soils compared with those in Hanmer Forest soils, although added glucose decomposed almost equally well in both soils.

The mineral nitrogen content of Tasman Forest soils, to which glucose was added, fell to zero but analyses for mineral nitrogen in field soils have shown that there was always some mineral nitrogen available, indicating that microorganisms decomposing carbonaceous clear felling residues were not immobilizing all the nitrogen in field soil.

Net immobilization of nitrogen in Tasman Forest soils under optimum laboratory conditions was great enough to supply vigorous pine growth but this rapid mineralisation does not occur in the soil under field conditions. Therefore, environmental factors rather than the resistance of nitrogen compounds to decomposition were controlling nitrogen release.

The addition of minor elements reduced the rate of decomposition of root wood cubes and since the decomposition of this material did not supply a great deal of nitrogen it may be useful to control the amount of decomposition of root wood by the application of these elements as long as tree growth is not affected.

A recommendation as to the best form of nitrogen to apply as fertiliser to promote increased tree growth presents a difficult problem. The preferential use of applied NH_4 -N over NO_3 -N by micro-organisms was demonstrated, in general, in this investigation. It would, therefore, seem that NO_3 -N would be the best fertiliser to apply for maximum uptake by trees except that under high concentrations of NO_3 -N there was a tendency for the micro-organisms to use NO_3 -N. The application of mineral N did not affect mineralisation rates except where NO_3 -N was applied to duff samples. Before the application of fertiliser to control either the rate of breakdown of carbonaceous material or as nutrients designed for maximum plant use more trials would be needed to assess the overall effects

more closely.

The major fungi responsible for decomposing needles were fewer in number than those found by other workers, and the smaller number of species may be causing some instability as postulated by Florence (1967). Investigation into this type of problem is only in its infancy although people have grown crops in monoculture for thousands of years.

Endeavours to control numbers of micro-organisms and species by changing the environment is also a field which has only recently started to be investigated, and much more work is required to elucidate the factors concerned; thus in this investigation 20% of the variation in micro-organism numbers was explained. Control of the whole ecosystem is an ideal not yet able to be achieved but studies such as those undertaken in this work and in more importantly co-ordinated investigations, for example, the International Biological Programme, should be able to make much more information available within the next few years.

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APPENDIX 5.1A

Source of variation	S.S.	df	M.S.	F
Reps	$.2687 \times 10^2$	1	$.2687 \times 10^2$	0.8
Season	$.4654 \times 10^4$	1	4654×10^4	134.8 ***
Age	$.2661 \times 10^3$	3	$.8870 \times 10^2$	2.6 ≃ *
Position	$.1445 \times 10^1$	1	$.1445 \times 10^1$	0.0
Depth	$.1832 \times 10^4$	3	$.6107 \times 10^3$	17.7 **
Reps x Season	$.8633 \times 10^3$	1	$.8633 \times 10^3$	25.0 **
Reps x Age	$.8903 \times 10^2$	3	$.2968 \times 10^2$	0.9
Reps x Position	$.3117 \times 10^1$	1	$.3117 \times 10^1$	0.1
Reps x Depth	$.1684 \times 10^3$	3	$.5612 \times 10^2$	1.6
Season x Age	$.1569 \times 10^3$	3	$.5231 \times 10^2$	1.5
Season x Position	$.1336 \times 10^1$	1	$.1336 \times 10^1$	0.0
Season x Depth	$.1557 \times 10^4$	3	$.5191 \times 10^3$	15.0 **
Age x Position	$.6515 \times 10^3$	3	$.2172 \times 10^3$	6.3 **
Age x Depth	$.2049 \times 10^3$	9	$.2277 \times 10^2$	0.7
Position x Depth	$.1261 \times 10^3$	3	$.4203 \times 10^2$	1.2
Error		88	$.3452 \times 10^2$	
Total	$.1364 \times 10^5$	127	$.1074 \times 10^3$	

Analysis of variance to show factors causing differences in water content of soil from Tasman Forest.

APPENDIX 5.2A

Source of variation	S.S.	df	M.S.	F.
Replications	2.33	1	2.33	37.0 **
Season	0.39	1	0.39	6.2 *
Age	3.28	3	1.10	17.3 **
Position	0.07	1	0.07	1.2
Depth	0.26	3	0.09	1.4
Reps x Season	0.03	1	0.03	0.5
Reps x Age	0.11	3	0.04	0.5
Reps x Position	0.01	1	0.01	0.1
Reps x Depth	0.23	3	0.08	1.2
Season x Age	0.12	3	0.04	0.6
Season x Position	0.35	1	0.35	5.5 *
Season x Depth	0.41	3	0.14	2.1
Age x Position	0.88	3	0.29	4.6 **
Age x Depth	1.20	9	0.13	2.1 *
Position x Depth	0.03	3	0.01	0.2
Error	5.55	99	0.06	
Total	15.28	127	0.12	

Mean = 4.8

CF = 2953.0

Analysis of variance to show factors causing differences in acidity of soil from Tasman Forest.

APPENDIX 6.1A

Source of variation	S.S.	df	M.S.	F	
Reps	0.113	2	0.057	1.39	
Time	2.792	1	2.792	68.8	**
Moisture	0.558	3	0.186	4.58	**
Temperature	3.809	3	1.269	31.30	**
Soil Type	1.554	2	0.777	19.15	**
Reps x Time	0.141	2	0.071	1.74	
Reps x Moisture	0.136	6	0.028	0.56	
Reps x Temperature	0.299	6	0.050	1.23	
Reps x Soil	0.175	4	0.044	1.08	
Time x Moisture	0.067	3	0.022	0.55	
Time x Temperature	0.425	3	0.142	3.49	*
Time x Soil	0.320	2	0.159	3.94	*
Moist. x Temp.	0.564	9	0.063	1.55	
Moist. x Soil	0.604	6	0.101	2.48	*
Temp. x Soil	1.631	6	0.272	6.71	**
Error		230			
Total	22.48	288	0.078		

Analysis of variance to show effect of environmental factors on CO_2 production in soils from Hanmer, Balmoral and Nelson Forests.

APPENDIX 6.1B

Time x temperature interaction from analysis of variance in Appendix 6.1A

		Temperature			
		8	15	24	31
Time	1	0.05	0.16	0.22	0.27
	2	0.19	0.27	0.48	0.48

APPENDIX 6.1C

Time x soil interaction from analysis of variance in Appendix 6.1A

		Soil type		
		Hanmer	Balmoral	Nelson
Time	1	0.23	0.12	0.19
	2	0.46	0.27	0.33

APPENDIX 6.1D

Source of variation	S.S.	df	M.S.	F	
Reps	0.032	2	0.016	1.66	
Time	1.248	1	1.248	127.7	***
Moisture	0.709	7	0.101	10.37	**
Temperature	2.202	3	0.734	75.14	**
Reps x Time	0.017	2	0.009	0.88	
Reps x Moisture	0.160	14	0.012	1.17	
Reps x Temperature	0.043	6	0.007	0.73	
Time x Moisture	0.074	7	0.010	1.08	
Time x Temperature	0.337	3	0.126	12.86	**
Moisture x Temp.	0.750	21	0.036	3.65	**
Error		125			
Total		191	0.036		

Analysis of variance to show effect of environmental factors on CO_2 production of soil from Tasman Forest.

Temp/Temp x Time	=	$\frac{75.14}{12.86}$	=	5.84	**
Temp/Moist x Temp	=	$\frac{75.14}{3.65}$	=	20.58	**
Moist/Moist x Temp	=	$\frac{10.37}{3.65}$	=	2.84	**

Analysis of Interactions

APPENDIX 6.1D (continued)

Time x temperature interaction from the analysis of variance table presented in Appendix 6.1D

		Temperature °C			
		8	18	24	31
Time	1	0.06	0.17	0.27	0.20
	2	0.20	0.29	0.57	0.35

APPENDIX 6.1E

Source of variation	S.S.	df	M.S.	F
Temperature	$.7449 \times 10^{-3}$	2	$.3725 \times 10^{-3}$	7.3 **
Moisture	$.5642 \times 10^{-2}$	2	$.2821 \times 10^{-2}$	55.4 **
Reps	$.1566 \times 10^{-3}$	1	$.1566 \times 10^{-3}$	3.1
Time Reps	$.1706 \times 10^{-2}$	2	$.8530 \times 10^{-3}$	16.7 **
(Kind) D vs L	$.6366 \times 10^{-2}$	1	$.6366 \times 10^{-2}$	124.9 **
Temp. x Moist.	$.1312 \times 10^{-2}$	4	$.3281 \times 10^{-3}$	6.4 **
Temp. x Reps	$.8026 \times 10^{-4}$	2	$.4013 \times 10^{-4}$	0.7
Temp. x Time Reps	$.1187 \times 10^{-2}$	4	$.2968 \times 10^{-3}$	5.8 **
Temp. x Kind	$.5253 \times 10^{-6}$	2	$.2626 \times 10^{-6}$	0.005
Moist. x Reps	$.5210 \times 10^{-3}$	2	$.2605 \times 10^{-3}$	5.1 **
Moist. x Time Reps	$.1437 \times 10^{-2}$	4	$.3591 \times 10^{-3}$	7.1 **
Moist. x Kind	$.1831 \times 10^{-3}$	2	$.9153 \times 10^{-4}$	1.8
Reps x Time Reps	$.1133 \times 10^{-3}$	2	$.5667 \times 10^{-4}$	1.1
Reps x Kind	$.5606 \times 10^{-4}$	1	$.5606 \times 10^{-4}$	1.1
Time Reps x Kind	$.3989 \times 10^{-4}$	2	$.1994 \times 10^{-4}$	0.4
Error		75		
Total	$.2332 \times 10^{-1}$	108	$.2179 \times 10^{-3}$	

$$CF = 0.6177 \times 10^{-1}$$

Analysis of variance to show effect of environmental factors on CO_2 production in litter and duff from Tasman Forest.

APPENDIX 6.1F

Time x temperature interaction from the analysis of variance table presented in Appendix 6.1E.

		Temperature		
		8	18	25
Time	1	0.196	0.174	0.200
	2	0.192	0.205	0.322
	3	0.341	0.237	0.285

Moisture x replication interaction from the analysis of variance table presented in Appendix 6.1E.

		Moisture %		
		20	60	100
Replications	1	0.180	0.289	0.284
	2	0.095	0.304	0.283

APPENDIX 6.1G

Source of variation	F	F	F	F	F
Treatment	0.38	0.82	2.79	3.58	7.32 **
NH_4	0.14	0.05	0.51	2.51	4.20
NO_3	0.06	0.46	0.24	0.24	9.25 *
G	2.00	2.90	5.47 *	0.41	27.66 **
NH_4 + NO_3	0.07	1.65	0.36	7.98 *	2.10
NH_4 + G	0.07	0.41	11.38 **	2.60	0.63
NO_3 + G	0.30	0.04	0.14	11.07 *	5.11
NH_4 + NO_3 + G	0.13	0.23	1.40	0.27	2.29
		Main Effects			
NH_4	0.1769	0.1375	0.0387	0.2675	0.2850
NO_3	0.1150	-0.4039	-0.0262	0.0825	0.4230
G	0.6444	1.0120	-0.1267	-0.1074	0.7315
NH_4 + NO_3	-0.1205	-0.7624	0.0322	0.4775	0.2019
NH_4 + G	0.1229	-0.3789	0.1827	-0.2725	0.1104
NO_3 + G	-0.2450	-0.1125	0.0202	0.5625	-0.3145
NH_4 + NO_3 + G	0.1634	-0.2879	-0.0642	-0.0875	-0.2105
	First	Second	Third	Fourth	Fifth
		Sampling			

Analysis of variance to show effect of addition of glucose, NO_3-N and NH_4-N to litter collected under mature P.radiata at Tasman Forest. Incubation was at 24 ± 1° C. (F value only shown).

APPENDIX 6.1H

Source of variation	F	F	F	F	F
Treatment	0.94	4.55 *	0.14	0.91	2.41
NH_4	2.39	3.61	0.34	0.16	2.03
NO_3	2.53	0.39	0.29	0.13	4.49 *
G	0.82	9.99 *	0.01	0.05	0.87
NH_4 + NO_3	0.18	2.49	0.29	0.13	6.46 *
NH_4 + G	0.03	8.08 *	0.01	0.03	2.79
NO_3 + G	0.36	0.92	0.02	5.81 *	0.21
NH_4 + NO_3 + G	0.27	6.37 *	0.02	0.02	0.00
		Main Effects			
NH_4	0.2412	0.0715	0.0290	0.0710	0.1867
NO_3	0.2482	-0.0235	-0.0270	-0.0650	-0.2777
G	0.1412	0.1190	-0.0050	-0.0410	0.1222
NH_4 + NO_3	-0.0667	-0.0595	0.0270	0.0650	-0.3327
NH_4 + G	0.0283	0.1070	0.0050	0.0295	-0.2187
NO_3 + G	-0.0943	0.0360	-0.0070	0.4279	0.0597
NH_4 + NO_3 + G	0.0808	-0.0950	0.0070	0.0245	0.0078
	First	Second	Third	Fourth	Fifth
		Sampling			

Analysis of variance to show effect of addition of glucose, NO_3-N, and NH_4-N to duff collected under mature P.radiata at Tasman Forest. Incubation was at 24 ± 1° C.

APPENDIX 6.1I

Source of variation	F	F	F	F
Treatment	4.40 *	2.09	0.103	14.19 **
NH_4	1.54	0.46	0.709	0.21
NO_3	0.07	0.53	0.891	0.26
G	27.4 **	12.69 **	4.7000 *	85.25 ***
$NH_4 + NO_3$	0.13	0.61	0.030	0.02
NH_4 + G	0.50	0.03	0.630	0.96
NO_3 + G	1.11	0.25	0.150	7.29 *
$NH_4 + NO_3$ + G	0.03	0.06	0.110	5.41 *
	Main Effects			
NH_4	4.114	1.007	1.447	0.383
NO_3	0.886	1.075	1.622	0.420
G	17.340	5.243	3.727	7.641
$NH_4 + NO_3$	1.198	1.146	0.306	-0.111
NH_4 + G	-2.340	-0.243	1.359	0.814
NO_3 + G	3.489	0.728	0.658	2.234
$NH_4 - NO_3$ + G	-0.572	0.382	0.570	-1.925
	First	Second	Third	Fourth
	Sampling			

Analysis of variance to show the effect of addition of glucose, NO_3-N and NH_4-N, to soil collected from the surface 0-2.5 cm from eight sites under regenerating P.radiata at Tasman Forest. Incubation was at 24 $\pm$ 1° C.

APPENDIX 6.1J

Source of variation	F	F	F	F
Treatment	6.67 **	3.51 *	16.04 **	1.36
NH_4	0.38	0.90	7.28 *	0.24
NO_3	0.98	0.13	3.52	0.04
G	43.49 **	19.79	84.10 ***	9.48 *
NH_4 + NO_3	0.30	0.90	4.05	0.28
NH_4 + G	0.91	1.92	6.21 *	0.05
NO_3 + G	0.52	0.64	2.79	0.08
NH_4 + NO_3 + G	0.12	0.26	4.33	0.38
		Main Effects		
NH_4	3.750	0.9037	1.734	0.7084
NO_3	6.041	0.3467	-1.206	0.2769
G	40.200	4.2360	5.896	4.2270
NH_4 + NO_3	-3.333	-0.9027	1.293	0.7720
NH_4 + G	5.833	1.3190	-1.602	-0.3384
NO_3 + G	4.374	0.7627	-1.074	0.3999
NH_4 + NO_3 + G	-2.083	-0.4867	1.337	0.8960
	First	Second	Third	Fourth
		Sampling		

Analysis of variance to show effect of addition of glucose, NO_3-N and NH_4-N to soil collected at a depth of 25 cm from eight sites under regenerating P.radiata at Tasman Forest. Incubation was at 24 $\pm$ 1° C.

APPENDIX 6.1K

Source of variation	S.S.	df	M.S.	F
Reps	1.12	2	0.56	0.09
Time	138.90	2	69.46	11.13 **
Rate	146.40	2	73.22	11.74 **
Soil	732.80	5	146.50	23.50 **
Reps x Time	36.06	4	9.01	1.45
Reps x Rate	21.25	4	5.31	0.85
Reps x Soil	105.60	10	10.56	1.69
Time x Rate	21.74	4	5.44	0.87
Time x Soil	176.70	10	17.67	2.83 **
Rate x Soil	271.50	10	27.15	4.35 **
Error	673.40	108	6.24	
Total	2325.00	161	14.4	

Effect of added cellulose on respiration of soil samples from Tasman Forest ridge sites, Hanmer Forest and Lincoln College.

APPENDIX 6.1L

Source of variation	S.S.	df	M.S.	F.
Position	0.60	1	0.60	0.24
Reps	7.44	2	3.72	1.48
Time	25.96	2	12.98	5.15 **
Rate	24.08	2	12.04	4.78 **
Age	10.90	2	5.45	2.16
Position x Reps	11.38	2	5.69	2.26
Position x Time	13.03	2	6.51	2.59
Position x Rate	2.21	2	1.11	0.44
Position x Age	15.04	2	7.52	2.98
Reps x Time	5.62	4	1.40	0.56
Reps x Rate	12.58	4	3.14	1.25
Reps x Age	14.95	4	3.74	1.49
Time x Rate	17.69	4	4.42	1.76
Time x Age	7.33	4	1.83	0.73
Rate x Age	1.51	4	0.38	0.15
Error	302.40	120	2.52	
Total	472.8	161	2.94	

Carbon dioxide production of soil samples collected from three sites at Tasman Forest incubated with added cellulose.

APPENDIX 6.1M

Source of variation	S.S.	df	M.S.	F
Reps	23.32	2	11.66	0.48
Rate	22.53	2	11.26	0.47
Soil	485.4	5	97.09	4.03
Reps x Rate	53.82	4	13.45	0.56
Reps x Soil	291.20	10	29.12	1.21
Error	481.70	20	24.08	
Total	1571.00	53	29.64	

Effect of added glucose on soil respiration in soil samples previously amended with cellulose at different rates.

APPENDIX 6.1N

Source of variation	S.S	df	M.S.	F	
Position	2.49	1	2.40	0.11	
Reps	79.02	2	39.51	1.77	
Rate	101.50	2	50.79	2.28	
Age	185.90	2	92.97	4.17	*
Position x Reps	112.10	2	56.06	2.51	
Position x rate	9.09	2	4.55	0.20	
Position x Age	116.50	2	58.27	2.61	
Reps x Rate	96.33	4	24.08	1.08	
Reps x Age	54.96	4	13.74	0.62	
Rate x Age	35.33	4	8.83	0.40	
Error	624.40	28	22.30		
Total	1417.00	53			

Effect of glucose on Tasman Forest soil samples collected from ridge and gully sites and amended previously with cellulose at different rates.

APPENDIX 6.2B

Equations are of the form Log rate (O_2 uptake uL/g soil/hr) = c + b Log time (days)

Day one included in the analysis		c.c.
Lincoln	log R = 1.5846 - 0.4368 log T	r= 0.789
Hanmer	log R = 0.3883 - 0.1622 log T	r= 0.512
Balmoral	log R = 0.9723 - 0.3859 log T	r= 0.737
Tasman Manuka	log R = 0.7920 - 0.6521 log T	r= 0.925
Tasman P31	log R = 1.1385 - 0.5423 log T	r= 0.931
Tasman R60	log R = 0.9170 - 0.5277 log T	r= 0.917
Mean of above soils	log R = 1.0358 - 0.4338 log T	r= 0.862

Day one excluded from the analysis		c.c.
Lincoln	log R = 2.1831 - 0.6961 log T	r= 0.992
Hanmer	log R = 1.1630 - 0.4978 log T	r= 0.940
Balmoral	log R = 1.4681 - 0.6007 log T	r= 0.905
Tasman Manuka	log R = 0.7953 - 0.6536 log T	r= 0.888
Tasman P31	log R = 1.4979 - 0.6980 log T	r= 0.991
Tasman R60	log R = 1.2280 - 0.6845 log T	r= 0.979
Mean of above soils	log R = 1.4622 - 0.6185 log T	r= 0.982

APPENDIX 6.2B

Source of Variation	S.S.	df	M.S.	F	
Repsday	2.3600	5	0.472	19.08	**
Reps	0.3076	1	0.3076	12.43	**
Soil	27.84	5	5.568	225.00	**
Temperature	0.046	1	0.0456	1.84	
Repsday/Reps	0.172	5	0.034	1.39	
Repsday/Soil	1.492	25	0.0597	2.41	**
Repsday/Temp.	3.576	5	0.7152	28.90	**
Reps/Soil	2.634	5	0.527	21.29	**
Reps/Temp.	0.00477	1	0.0047	0.19	
Soil/Temp.	0.0714	5	0.0143	0.58	
Error		85	.0248		
Total	40.60	143	0.2839		

CF = 94.00

Mean = 0.8079

The effect of temperatures 23.4° and 25.4^{4} C on oxygen uptake in soil samples from Tasman, Hanmer and Balmoral Forests and Lincoln College, using a Warburg apparatus.

APPENDIX 6.2D

Source of variation	S.S.	df	M.S.	F	
Repsday	6.137	2	3.068	60.29	**
Reps	2.813	1	2.813	55.28	**
Soil	94.970	5	18.990	373.10	***
Day	153.000	5	30.600	601.30	***
Repsday/Reps	0.378	2	0.189	3.71	*
Repsday/Soil	2.805	10	0.281	5.51	**
Repsday/Day	10.590	10	1.059	20.81	**
Reps/Soil	1.002	5	0.200	3.94	**
Reps/Day	5.921	5	1.184	23.26	**
Soil/Day	56.090	25	2.243	44.08	**
Error		745	.05088		
Total	341.1	215	1.586		

CF = 614.2

Mean = 1.686

Oxygen absorption in soil samples from Tasman, Hanmer and Balmoral Forests and Lincoln College using a Warburg apparatus incubated at 23.4° C for a period of 25 days.

APPENDIX 6.2E

Source of variation	S.S.	df	M.S.	F	
Reps/day	0.0001335	1	0.001335	0.01	
Reps	0.005733	1	0.005733	0.50	
Soil	1.030	5	0.2061	19.60	**
Day	0.2614	3	0.08715	8.29	**
Repsday/Reps	0.01652	1	0.01652	1.57	
Repsday/Soil	0.1035	5	0.02070	1.96	
Repsday/Day	0.1953	3	0.06512	6.19	**
Reps/Soil	0.09854	5	0.01970	1.87	
Reps/Day	0.002089	3	0.000696	0.07	
Soil/Day	0.2765	15	0.01843	1.75	
Error		53	0.0105		
Total	2.548	95	0.02682		

CF = 8.015

Mean = 0.2889

Oxygen uptake by soil samples from Tasman, Hanmer and Balmoral Forests and Lincoln College measured with a Warburg apparatus, incubated at 13.6° C after preincubation at 23.4° C.

APPENDIX 6.2F

Source of variation	S.S.	df	M.S.	F	
Repsday	0.8271	2	0.4135	13.91	**
Reps	0.2853	1	0.2853	9.60	**
Soil	3.670	4	0.9176	30.88	**
Day	1.188	3	0.3960	13.33	**
Repsday/Reps	0.0309	2	0.0155	0.52	
Repsday/Soil	0.2568	8	0.0321	1.08	
Repsday/Day	1.814	6	0.3024	10.18	**
Reps/Soil	0.4455	4	0.1113	3.75	**
Reps/Day	0.1508	3	0.0503	1.69	
Soil/Day	0.3314	12	0.0276	0.93	
Error		74	0.0297		
Total		119			

CF = 41.50

Mean = 0.5880

Rate of oxygen absorption in soil samples from Tasman, Balmoral and Hanmer Forests after seven days' preincubation. Incubation carried out, using a Warburg apparatus, at four periods up to 25 days after initial wetting of samples.

APPENDIX 6.2G

Soil x replication interaction from the analysis of variance presented in Appendix 6.2F.

		Replication	
		1	2
Soil	Balmoral	.64	.83
	Hanmer	.69	.92
	P31	.34	.28
	R60	.55	.68
	Manuka	.50	.47

APPENDIX 6.2H

Repsday x day interaction from the analysis of variance presented in Appendix 6.2F.

		Repsday		
		1	2	3
Day	1	.63	.80	.85
	2	.21	.80	.56
	3	.46	.68	.43
	4	.60	.42	.61

APPENDIX 6.2I

Source of variation	S.S.	df	M.S.	F.	
Repsday	1.609	5	0.3218	4.72	**
Reps	0.1077	1	0.1077	1.58	
Soil	16.390	5	3.278	48.09	**
Temperature	5.021	1	5.021	73.67	**
Repsday/Reps	0.164	5	0.033	0.48	
Repsday/Soil	1.615	25	0.0646	0.95	
Repsday/Temp.	0.7820	5	0.1564	2.29	
Reps/Soil	1.090	5	0.2181	3.20	*
Reps/Temp.	0.0768	1	0.0768	1.13	
Soil/Temp.	0.3142	5	0.0628	0.92	
Error		85	.06830		
Total		143			

CF = 102.1

Mean = 0.8424

Effect of temperatures 23.4° and 27.4° C on oxygen uptakes in soil samples from Tasman, Hanmer and Balmoral Forests and Lincoln College, using a Warburg apparatus.

APPENDIX 6.2L

Source of variation	S.S.	df	M.S.	F
Repsday	0.8829	1	0.883	12.410 **
Reps	0.8153	2	0.408	5.730 **
Treatment	1.6500	3	0.550	7.740 **
Day	118.5000	4	29.62	416.500 **
Repsday/Reps	0.3890	2	0.195	2.734
Repsday/Treatment	0.9912	3	0.330	4.645 **
Repsday/Day	0.5125	4	0.128	1.801
Reps/Treatment	1.321	6	0.220	3.095 **
Reps/Day	0.5148	8	0.064	0.905
Treatment/Day	3.491	12	0.291	4.090 **
Error		74	0.071	
Total	134.3	119	1.128	

CF = 369.5

Means = 17.5 ul O_2/5 g soil/hr.

Analysis of variance of results obtained when glucose and nitrogen were applied to Tasman Forest soil samples. Oxygen uptake was measured, using a Warburg apparatus.

APPENDIX 6.3A

Source of variation	df	F value
Fungus G	1	2.316
P	1	5.060 *
BP x BPF	1	3.074
BP x P	1	1.751
BPF x R	1	1.931
BPF x G	1	3.191
BPF x Fus	1	7.722 **
R x P	1	2.024
R x Tr	1	11.386 **
G x P	1	2.216
G x Fus	1	3.556
P x Tr	1	2.119
P x Fus	1	4.344 *
Error	27	
Total	63	

Analysis of variance of results of decomposition of pine needles by various fungi in pure culture. Only significant or nearly significant F values are presented.

APPENDIX 7.2A

Source of variation	S.S.	df	M.S.	F	
Position	8205.0	1	8205.0	311.30	***
Replications	67.3	1	67.3	2.55	
Depth	3154.0	3	1051.0	39.90	**
Age	539.3	2	269.6	10.23	**
Position x Reps	333.8	1	333.8	12.66	**
Position x Depth	1796.0	3	598.8	22.72	**
Position x Age	1361.0	2	680.7	25.83	**
Reps x Depth	1167.0	3	389.1	14.76	**
Reps x Age	173.9	2	87.0	3.30	
Depth x Age	4447.0	6	741.2	28.12	**
Error	606.1	23	26.35		
Total		47	464.9		

Mean = 32.10% loss of weight

CF = 49,470

Analysis of variance of results of decomposition of root wood cubes buried in the soil at four depths in Tasman Forest for 22 months.

APPENDIX 9.1A

Source of variation	S.S.	df	M.S.	F
Reps	240	1	240	1.57
Series	384	1	384	2.51
Depth	59000	3	19660	128.40 **
Position	2592	1	2592	16.92 **
Age	16200	4	4050	26.44 **
Reps x Series	207	1	207	1.35
Reps x Depth	637	3	212	1.39
Reps x Position	148	1	148	0.97
Reps x Age	222	4	55	0.36
Series x Depth	468	3	156	1.02
Series x Position	1177	1	1177	7.69 **
Series x Age	2073	4	518	3.38 *
Depth x Position	2991	3	997	6.51 **
Depth x Age	18020	12	1501	9.81 **
Position x Age	7946	4	1986	12.96 **
Error	17295	113	153	
Total	129600	159	815	

CF = 647,400

Mean = 63.61

APPENDIX 10.2B

Source of variation	S.S.	df	M.S.	F
Reps	$.9336 \times 10^{13}$	1	$.9336 \times 10^{12}$	0.115
Season	$.1280 \times 10^{15}$	1	$.1280 \times 10^{15}$	1.573
Age	$.3273 \times 10^{15}$	3	$.1091 \times 10^{15}$	1.340
Position	$.4437 \times 10^{15}$	1	$.4437 \times 10^{15}$	5.449 *
Depth	$.4271 \times 10^{15}$	3	$.1424 \times 10^{15}$	1.749
Reps x Season	$.5093 \times 10^{14}$	1	$.5093 \times 10^{14}$	0.626
Reps x Age	$.2423 \times 10^{15}$	3	$.8077 \times 10^{14}$	0.992
Reps x Position	$.3264 \times 10^{14}$	1	$.3264 \times 10^{14}$	0.401
Reps x Depth	$.1621 \times 10^{14}$	3	$.5403 \times 10^{13}$	0.066
Season x Age	$.2881 \times 10^{15}$	3	$.9603 \times 10^{14}$	1.179
Seasons x Position	$.1235 \times 10^{15}$	1	$.1235 \times 10^{15}$	1.517
Seasons x Depth	$.1029 \times 10^{15}$	3	$.3430 \times 10^{14}$	0.421
Age x Position	$.1925 \times 10^{15}$	3	$.6418 \times 10^{14}$	0.788
Age x Depth	$.5808 \times 10^{15}$	9	$.6454 \times 10^{14}$	0.793
Position x Depth	$.3332 \times 10^{15}$	3	$.1111 \times 10^{15}$	1.364
Error		88		
Total	$.1046 \times 10^{17}$	127	$.8239 \times 10^{14}$	

C.F. = $.1803 \times 10^{16}$

Analysis of variance to show factors causing differences in numbers of bacteria found in soil from Tasman Forest.

APPENDIX 10.2C

Source of variation	S.S.	df	Variance	F	
Reps	$.3429 \times 10^{11}$	1	$.3429 \times 10^{11}$	0.077	
Season	$.2788 \times 10^{13}$	1	$.2788 \times 10^{13}$	6.306	*
Age	$.9522 \times 10^{13}$	3	$.3174 \times 10^{13}$	7.178	**
Position	$.4268 \times 10^{11}$	1	$.4268 \times 10^{11}$	0.096	
Depth	$.2803 \times 10^{14}$	3	$.9344 \times 10^{13}$	21.130	**
Reps x Season	$.6519 \times 10^{12}$	1	$.6519 \times 10^{12}$	1.474	
Reps x Age	$.3069 \times 10^{13}$	3	$.1023 \times 10^{13}$	2.314	
Reps x Position	$.2856 \times 10^{13}$	1	$.2856 \times 10^{13}$	6.459	*
Reps x Depth	$.3479 \times 10^{13}$	3	$.1160 \times 10^{13}$	2.623	
Season x Age	$.4953 \times 10^{12}$	3	$.1651 \times 10^{12}$	0.373	
Season x Position	$.5022 \times 10^{12}$	1	$.5022 \times 10^{12}$	1.136	
Season x Depth	$.1081 \times 10^{13}$	3	$.3603 \times 10^{12}$	0.815	
Age x Position	$.4558 \times 10^{12}$	3	$.1519 \times 10^{12}$	0.343	
Age x Depth	$.1310 \times 10^{14}$	9	$.1455 \times 10^{13}$	3.291	**
Position x Depth	$.4018 \times 10^{12}$	3	$.1339 \times 10^{12}$	0.303	
Error			$.4421 \times 10^{12}$		
Total	$.1054 \times 10^{15}$	127	$.8301 \times 10^{12}$		

C.F. = $.6242 \times 10^{14}$

Analysis of variance to show factors causing differences in numbers of fungal propagules found in soil from Tasman Forest.

APPENDIX 10.2D

Source of variation	S.S.	df	M.S.	F	
Reps	$.7244 \times 10^{11}$	1	$.7244 \times 10^{11}$	1.037	
Season	$.2193 \times 10^{12}$	1	$.2193 \times 10^{12}$	3.140	
Age	$.1792 \times 10^{13}$	3	$.5973 \times 10^{12}$	8.553	**
Position	$.4469 \times 10^{11}$	1	$.4469 \times 10^{11}$	0.640	
Depth	$.2905 \times 10^{13}$	3	$.9683 \times 10^{12}$	13.870	**
Reps x Season	$.1607 \times 10^{12}$	1	$.1607 \times 10^{12}$	2.301	
Reps x Age	$.1356 \times 10^{12}$	3	$.4520 \times 10^{11}$	0.647	
Reps x Position	$.1406 \times 10^{12}$	1	$.1406 \times 10^{12}$	2.013	
Reps x Depth	$.4845 \times 10^{12}$	3	$.1615 \times 10^{12}$	2.313	
Season x Age	$.2097 \times 10^{12}$	3	$.6991 \times 10^{11}$	1.001	
Season x Position	$.2263 \times 10^{12}$	1	$.2263 \times 10^{12}$	3.241	
Season x Depth	$.2278 \times 10^{12}$	3	$.7595 \times 10^{11}$	1.088	
Age x Position	$.6981 \times 10^{12}$	3	$.2327 \times 10^{12}$	3.333	**
Age x Depth	$.1298 \times 10^{13}$	9	$.1442 \times 10^{12}$	2.066	**
Position x Depth	$.7363 \times 10^{11}$	3	$.2454 \times 10^{11}$	0.352	
Error		88	$.6980 \times 10^{11}$		
Total	$.1483 \times 10^{14}$	127	$.1168 \times 10^{12}$		

C.F. = $.5438 \times 10^{13}$

Analysis of variance to show factors causing differences in numbers of yeasts found in soil from Tasman Forest.

APPENDIX 10.2E

Source of variation	S.S.	df	M.S.	F	
Reps	$.1204 \times 10^{12}$	1	$.1204 \times 10^{12}$	2.471	
Season	$.1128 \times 10^{13}$	1	$.1128 \times 10^{13}$	23.14	**
Age	$.8858 \times 10^{12}$	3	$.2953 \times 10^{12}$	6.058	**
Position	$.2269 \times 10^{11}$	1	$.2269 \times 10^{11}$	0.465	
Depth	$.8928 \times 10^{12}$	3	$.2976 \times 10^{12}$	6.106	**
Reps x Season	$.7676 \times 10^{9}$	1	$.7676 \times 10^{9}$	0.016	
Reps x Age	$.3345 \times 10^{12}$	3	$.1115 \times 10^{12}$	2.288	
Reps x Position	$.1151 \times 10^{12}$	1	$.1151 \times 10^{12}$	2.361	
Reps x Depth	$.3793 \times 10^{12}$	3	$.1264 \times 10^{12}$	2.594	
Season x Age	$.3278 \times 10^{12}$	3	$.1093 \times 10^{12}$	2.242	
Season x Position	$.1753 \times 10^{11}$	1	$.1753 \times 10^{11}$	0.359	
Season x Depth	$.1644 \times 10^{12}$	3	$.5482 \times 10^{11}$	1.125	
Age x Position	$.3659 \times 10^{12}$	3	$.1220 \times 10^{12}$	2.503	
Age x Depth	$.3116 \times 10^{12}$	9	$.3462 \times 10^{11}$	0.710	
Position x Depth	$.2270 \times 10^{12}$	3	$.7568 \times 10^{11}$	1.553	
Error		88			
Total	$.9582 \times 10^{13}$	127	$.7545 \times 10^{11}$		

C.F. = $.6988 \times 10^{13}$

Analysis of variance to show factors causing differences in numbers of actinomycete propagules found in soil from Tasman Forest.

APPENDIX 10.2F

Source of variation	S.S.	df	M.S.	F
Reps	$.4569 \times 10^{13}$	1	$.4569 \times 10^{13}$	0.105
Season	$.1043 \times 10^{13}$	1	$.1043 \times 10^{13}$	0.002
Age	$.9741 \times 10^{14}$	3	$.3247 \times 10^{14}$	0.747
Position	$.1530 \times 10^{15}$	1	$.3247 \times 10^{14}$	3.522
Depth	$.5155 \times 10^{15}$	3	$.1718 \times 10^{15}$	3.955 *
Reps x Season	$.1549 \times 10^{12}$	1	$.1549 \times 10^{12}$	0.003
Reps x Age	$.1303 \times 10^{15}$	3	$.4345 \times 10^{14}$	0.999
Reps x Position	$.1844 \times 10^{14}$	1	$.1844 \times 10^{14}$	0.424
Reps x Depth	$.4140 \times 10^{14}$	3	$.1380 \times 10^{14}$	0.318
Season x Age	$.1992 \times 10^{15}$	3	$.6640 \times 10^{14}$	1.528
Season x Position	$.1710 \times 10^{14}$	1	$.1710 \times 10^{14}$	0.393
Season x Depth	$.1481 \times 10^{15}$	3	$.4936 \times 10^{14}$	1.136
Age x Position	$.7682 \times 10^{13}$	3	$.2561 \times 10^{13}$	0.059
Age x Depth	$.2786 \times 10^{15}$	9	$.3096 \times 10^{14}$	0.713
Position x Depth	$.2721 \times 10^{15}$	3	$.9069 \times 10^{14}$	2.087
Error		88		
Total	$.5708 \times 10^{16}$	127	$.4495 \times 10^{14}$	

C.F. = $.2165 \times 10^{16}$

Analysis of variance to show factors causing differences in numbers of micro-organisms found in soil from Tasman Forest.

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