Mechanistic aspects of microbial invasion of wood

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G. MOWE Mechanistic aspects of microbial invasion of wood.

Work described in this thesis was undertaken to determine the effect of wood on behavioural aspects and growth of fungi and bacteria in pure culture and soil.

It was shown that sapwood blocks of lime (Tilia vulgaris) and pine (Pinus sylvestris) strongly influenced hyphal extension patterns of several wood inhabiting fungi when placed to one side of developing colonies, by eliciting either positive or negative chemotropic responses. A method was developed to measure and quantify these responses and statistically analyse them. When such fungi were grown in the presence of wood blocks treated with organic or CCA preservatives, only those preservatives with a volatile component influenced tropic responses. CCA treated blocks, even when leached, had no significant effect on the chemotropic response.

Aqueous extracts of sapwood of several wood species were shown to elicit positive chemotactic responses in a number of flagellate wood and soil inhabiting bacteria. Chemotaxis was also demonstrated to amino acids and sugars, such responses being greater, at the concentrations used in this investigation, to compounds with a nitrogen component.

Nitrogen contents of soils at IRG test sites were determined and found not to correlate with published decay estimates. It was considered that refinement and standardisation of soil sampling procedures was required in further studies.

Both fungal hyphal lengths and bacterial numbers were shown to increase in soil within 3mm of decaying wood, and such increases correlated with mass loss and nitrogen content of wood. Dehydrogenase activity in soil was significantly correlated with biomass presence, and both biomass increases and enzyme activity were inhibited in the presence of pine and in wood impregnated with CCA.

The presence of plastic barriers about wood in soil inhibited mass loss, nitrogen accumulation and dehydrogenase activity in wood and dehydrogenase activity in soil contiguous with such barriers. It was proposed that barriers acted as a prophylactic against the chemostimulation of soil microbial populations and microbial transfer from soil to wood.

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Contents

Chapter 1	Introduction	1
Chapter 2	Tropic responses of fungi to unpreserved and preserved wood.	15
2.1	Tropic responses of fungi to wood:	
2.1.1.	Introduction	16
2.1.2.	Materials and methods	24
2.1.3.	Results	27
2.1.4.	Discussion	49
2.2	Tropic responses of fungi to preserved wood.	
2.2.1.	Introduction	54
2.2.2.	Materials and methods	56
2.2.3.	Results	58
2.2.4.	Discussion	67
2.3.	General conclusions	72
Chapter 3	Bacterial chemotaxis	73
3.1.	Introduction	74
3.2.	Materials and methods	81
3.3.	Results	87
3.4.	Discussion	92
Chapter 4	Nitrogen content of soil in relation to wood decay.	102
4.1.	Introduction	103
4.2.	Materials and methods	112
4.2.1.	Comparative study of methods for soil nitrogen determination	112
4.2.2.	Nitrogen content of soils adjacent to and distant from wood in service.	117
4.2.3.	Nitrogen contents of soils at I.R.G. field sites.	118
4.3.	Results	119
4.4.	Discussion	128

Chapter 5	Changes in microbial populations in soil adjacent to wood.	135
5.1.	Introduction	136
5.2.	Materials and methods	143
5.3.	Results	151
5.4.	Discussion	185
Chapter 6	Effects of physical barriers on decay of wood in soil contact	195
6.1.	Introduction	196
6.2.	Materials and methods	198
6.3.	Results	203
6.4.	Discussion	218
Chapter 7	General discussion	223
	References	237

Chapter 1.

Introduction.

Introduction

Very large quantities of organic materials are produced annually in terrestrial ecosystems by plant growth, varying proportions of which, depending on biome type, are deposited as litter. Bray and Gorham (1964) estimated that for temperate forests four tonnes of litter per hectare are produced yearly, of which as much as 50% consists of woody material. Such material is recycled within specific time periods, although persistence of organic material in soil may also occur, often for considerable periods of time, resulting from special adverse conditions, such as permanent waterlogging. Few naturally occurring organic materials are inherently resistant to breakdown, however, and generally complete substrate mineralisation occurs (Jenkinson, 1981).

The decomposition of litter by microbial action has evolved as the predominant process by which nutrients are recycled within soil systems and rates at which litter substrates are mineralised depend on prevailing ecological conditions; steady rates develop, however, which are characteristic of certain stable biomes. Litter decomposition processes in temperate soils are dominated by fungi and bacteria. Soil fauna aid the microbial processes by comminuting large litter particles resulting in increased surface areas of substrate available for invasion and colonisation, whilst effecting only limited changes in the chemical status of the substrate itself (Jensen, 1974).

The mycelial growth form of fungi allows these

organisms to invade wood substrate at both a cellular and molecular level. Modes of penetration and specific patterns of substrate depolymerisation lead to characteristic types of decay. The major decay types delimited by these criteria have attracted considerable research interest, and fungi which effect decomposition can be classified on the physiology of the decay organisms and the resulting micromorphology of the decay produced. Until the work of Savory (1954), cellulolytic and lignocellulolytic Basidiomycete fungi were considered to be the sole causative agents of large scale fungal degradation of timber. Savory (1954 op. cit.), however, demonstrated that Ascomycetes and Fungi Imperfecti were implicated in surface decay of timber, decay being restricted to S_2 layer of the secondary cell walls of tracheids and fibres and moderated by cellulase activity. This decay form resulted in surface softening of timber and he proposed the term 'soft rot' to describe such decay.

Subsequent to the observations of Savory, analyses of soft rot micromorphology (Findlay, 1970; Lundstrom, 1972; Hale & Eaton, 1981) and physiology (Nilsson, 1974) have elucidated the detailed relationship between decay fungi and wood substrate. The importance of soft rot decay in terrestrial ecosystems is now recognised, especially in wood treated with copper chrome arsenic preservative.

Behaviour patterns of microbial communities are regulated by a multitude of exogenous environmental factors and restrictions conferred on them by constitutive necessities such as dormancy. Although nutritional requirements vary only to a

small degree between groups of organisms, their ability to depolymerise complex substrates encompasses a wider spectrum. Classifications of soil microorganisms based on their nutritional abilities have been in existence for many years. Winogradsky (1924) divided soil bacteria into zymogenous and autochthonous species. Zymogenous groups are opportunists which exist in soil under conditions of bacteriostasis. These flourish when soil is amended with easily metabolised substrates, their numbers rapidly decreasing when such substrates have been utilised. Autochthonous species maintain more level numbers, utilising recalcitrant, complex polymeric substrates and vegetative propagules predominate.

A similar ecological scheme for fungi was postulated by Garrett (1963). Initial colonisers of a new resource in soil were considered to be those fungi which could readily assimilate non-polymer substrates and produce rapid growth to exploit the available resource. Sporulation and dispersal then took place when resources became depleted. Further on in the decay process, cellulolytic Ascomycetes and Fungi Imperfecti and lignolytic Basidiomycetes became dominant, causing the bulk of the decay. Associated with the slower growing macrofungi were a second group of secondary sugar fungi. Frankland (1976), however, investigating Pteridium petiole decomposition found primary colonisers to be cellulolytic and lignolytic basidiomycetes, and microfungi did not become prevalent until the fourth year of decay, presumably assimilating soluble sugars resulting from the hydrolytic action of basidiomycete cellulases. Caution must be exercised, however, in

nutritionally based classifications; Park (1976) has shown that fungi earlier thought to be non-cellulolytic, do, in fact, produce cellulases under certain conditions.

A great deal of attention has been given to microbial succession in sapwood exposed to soil to determine the ecology of wood decay in the soil situation. Detailed analyses of microbial succession in both an untreated and copper chrome arsenic treated hardwood (birch), and a softwood (Scots pine), have been undertaken by Clubbe & Levy (1982). The successional events for untreated hardwood and softwood were shown to be similar; however, the time course for the decay of the softwood was longer with basidiomycetes the climax microflora in both. Initial colonisers were bacteria which diminished in numbers during the year. These were followed sequentially by primary molds, 'soft rots' and basidiomycetes with secondary molds increasing in importance with the establishment of gross decay. The effect of treating sapwood of both species with copper chrome arsenic was to eliminate the establishment of basidiomycetes the climax mycoflora and the substitution of soft rot fungi in this role, and to confer greater protection on the softwood to soft rot decay.

Substrate decomposition processes are mediated by enzymes. Nutrients released during such processes are assimilated and immobilised by degrading organisms and are utilised for growth and energy requirements. By far the largest proportion of plant material is composed of complex carbohydrate substrates which are readily degraded and assimilated by decay organisms. These substrates not only

supply material for the synthesis of new microbial biomass, but are also oxidised to provide energy for the anabolic processes. Because of its relative abundance in the soil environment, carbon tends not to be a limiting factor during decomposition and is readily mineralised to CO_2.

Not all nutrient elements are present in plant materials in such large quantities, and during decomposition growth of decay organisms may be limited by nutrient deficiences. Carbon utilisation is moderated by the proportion of other nutrient elements present and restricted if these elements are present in limited amounts. One indicator of the degree to which carbon may be utilised is the ratio of the amount of carbon present in the resource to that of other nutrient elements. A large ratio is indicative of excess carbon, and its utilisation is limited initially by the restricted amounts of other nutrient elements present. However, as the latter are present in limited quantities, they are immobilised and conserved for synthesis by microorganisms present, whereas the proportion of the carbon utilised for energy is volatilised as CO_2 and lost from the system. The continued loss of carbon via respiration and the retention of nutrient elements via microbial immobilisation narrows the carbon : nutrient element ratio, until a ratio is reached where the nutrient element is no longer a limiting factor, and nett mineralisation of that element can occur.

Nitrogen is a nutrient element essential to all biological systems being a constituent of protein and nucleic acid polymers, and is present in limited quantities both in

litter and in soil in the form of proteins, nucleic acids, amino acids and inorganic forms (Alexander 1977). Demands for nitrogen by microorganisms are at their highest during periods when the physical conditions of the environment are optimal for rapid growth, provided other nutritional parameters are satisfied. Fungal and bacterial cells not under environmental or nutritional stress contain, on a dry mass basis, approximately 3 - 4% and 8 - 15% nitrogen, and C : N ratios of 10 : 1 and 4 : 1 respectively; however, such conditions rarely prevail in natural situations. Speculating on these figures it would be necessary for a mycological community actively decomposing a plant resource to assimilate carbon and nitrogen in the ratio of 10 : 1 to satisfy optimal growth demands and a further amount of carbon for the provision of energy. Estimates for the proportion of carbon assimilated to the amount volatilised as CO_2 during respiration vary between 40% and 66%, the remainder being assimilated. Assuming a median figure of 50% then for 20 units of carbon utilised, 10 units are mineralised as CO_2, and 10 units synthesised into new cell constituents; concomitant with this is an assimilation of 1 unit of nitrogen. At or above this theoretical C : N ratio of 20 : 1, all substrate nitrogen mobilised by decay organisms will be utilised as microbial biomass; below this figure nitrogen would be in excess of requirements and mineralised. These figures assume that nitrogen present in the plant resource is in a potentially available form which might not be the case. In terms of resource quality, plant materials are decomposed at accelerated rates in response to increases in

the amount and availability of their nitrogen contents. This is supported by experimental data which demonstrates that supplementary nitrogen added to plant material increases rates of decomposition (Findlay, 1934; Allison, 1960), and that plant materials with naturally high nitrogen levels tend to be mineralised more readily than those with lower nitrogen concentrations (Levi & Cowling, 1968; Alexander, 1977).

The economic importance of wood to man has meant that processes which moderate decomposition have attracted intensive interest. Wood, as a plant material, is particularly low in nitrogen, with high carbon : nitrogen ratios. Wood of standing trees is not a homogenous material, and variation in nitrogen contents of tissues and cells is observed. Nitrogen content of tissues varies with the stage of development, physiological status and function at maturity; however, it rarely exceeds 0•2% and is generally lower (Merrill & Cowling, 1966). In compensation for poor resource quality, many wood decomposing basidiomycetes appear to have evolved adaptive physiologies, utilising nitrogen poor substrates by a preferential distribution of nitrogen to essential metabolic pathways. Such allocation allows for continued decomposition of the resource, even though the nitrogen content of the mycelium is depleted.

Whereas basidiomycetes can utilise wood resources with inherently low levels of nitrogen, soft rot fungi have greater demands for nitrogen (Savory, 1954; Duncan, 1965; Levi & Cowling, 1969; Lundstrom, 1972; Butcher & Drysdale, 1974). Mechanisms which determine increases of nitrogen in wood in soil whereby soft rot organisms can become active decomposers

have been postulated by a number of authors. Levy (1968) proposed a passive, abiotic movement of soluble compounds of nitrogen into wood in soil in response to a 'wick' action occurring in the stake in the soil. Increases in nitrogen within wood due to the activities of nitrogen fixing bacteria have also been suggested and this capability has been demonstrated by various authors (Sharp & Millbank, 1973; Levy et al, 1974; Baines & Millbank, 1976). King et al (1976) found nitrogen increases in wood only in areas where there was a microbial presence; areas of the wood uncolonised, but wetted by soil solution showed no such increases. This led King to suggest that nitrogen increases were mainly of microbiological origin.

King et al op. cit. demonstrated that during drying of green wood, nutrients including soluble nitrogenous compounds which are present in only limited amounts in green wood, migrate to and are concentrated at the wood surface as water evaporates at the wood - air interface. Wood surfaces, to depths of 5mm, are by this process of redistribution enriched with an increased nutrient status. Examination of matched sapwood blocks of sitka spruce, with and without nutrient rich surfaces, which had been buried in soil for periods of 12 weeks, indicated that the degree of soft rot cavity formation was greater in nutrient rich material. Waite and King (1979) further demonstrated that the presence of a nutrient rich surface accelerated decay rates compared to wood where nutrients were depleted. It was also noted that there was a continuous input of nitrogen to blocks during

decomposition processes, with a significant correlation between mass loss and nitrogen content, irrespective of wood species studied.

Pure culture studies indicated that fungi (King & Waite, 1979) and bacteria (King et al, 1980) could contribute significant amounts of nitrogen to wood. These observations led King to propose that soft rot decay of wood in soil is mediated by a continual microbial biomass transfer from soil to wood, increasing the nutrient status of the wood and thereby satisfying the exacting nutritional requirements of soft rot fungi. Pine needle decomposition studies undertaken by Berg & Soderstrom (1979) also noted a contemporaneous increase in nitrogen during decay. Henningsson & Nilsson (1976) reported enhanced nitrogen contents of preserved transmission poles in Sweden, and Friis Hansen (1976) noted that where transmission poles were not in contact with soil because of the presence of a rock backfill, decay did not take place.

Soft rot fungi are the major agents for the failure of copper chrome arsenic treated wood in soil. Henningsson (1976) demonstrated that the availability of nitrogenous compounds in laboratory media or in wood, increased the microbial tolerance to both copper and arsenic, and Levi (1969) observed solubilisation of C.C.A. by microbial biomass present in wood. The importance of soluble nutrients on the effectiveness of C.C.A. preservative was studied by King et al (1981) using materials with high and low nutrient status. The presence of soluble nutrient containing nitrogen lowered the toxic threshold at which buried blocks were protected and in

undecayed blocks with high preservative loadings no increases occurred in nitrogen contents.

Details of microbial succession patterns in both copper chrome arsenic treated and untreated wood in soil are now well understood; however, explanations as to how such organisms occur in wood in the first instance are unclear, and initial interactive effects between wood and decay organisms have received little attention.

An analagous system in soil which has received much attention is the plant root rhizosphere, and even here determinants which govern rhizosphere development are unclear. Qualitative and quantitative differences between rhizosphere populations and the general microflora distributed throughout the soil indicate that not all soil microorganisms make the transition from the soil to the rhizosphere. The concept that during root development there is random coincidence of root with soil microflora leading to enhanced populations of select groups better able to compete in the root zone environment can explain how these differences in populations arise.

Motile bacteria have been demonstrated, in vitro, to accumulate in areas which have high nutritional status (Berg, 1975), and move to these areas in response to sensed gradients in soluble nutrient concentrations (Adler, 1973). Moreover, recent work (Currier, 1980; Bowra & Dilworth, 1981; Gotz et al, 1982) has demonstrated that Rhizobium spp. respond chemotactically to components of root exudates. The significance of this response in rhizosphere development remains unassessed, but the possibility arises that chemotactic responses shown by

Rhizobium may be of fundamental importance in establishment of contact between root and bacterium, prior to nodule formation.

Smith (1980) proposed that decay of wood in soil, as measured by loss in tensile strength, occurred in two stages, an induction phase and a decay phase. The induction phase consisted of that phase during which stasis release of microbial populations, initiated by nutrient diffusion from wood to soil, occurred. The duration of this phase was extended by the presence of copper chrome arsenic preservative, which might either interfere with stasis release or necessitate inactivation of the preservative by microorganisms and / or microbial products. Following the induction phase decay proceeded at a more or less constant rate, again this decay phase was extended by increased preservative presence.

It is now established that the presence of green or heat treated wood in proximity to fungal colonies can result in inhibition or stimulation of fungal growth in response to volatiles evaporating from the wood surface (Fries, 1973). Volatiles present in root tissue of Pinus sylvestris L. have been demonstrated to have profound effects on growth patterns of several mycorrhizal and parasitic fungi (Melin & Krupa, 1971; Krupa & Nylund, 1972), and these authors suggest that such volatiles may be important in conferring disease resistance to root systems of pine in soil.

Smith (1980, op cit.) concluded from his work that mechanisms determining microbial succession in wood in soil were poorly understood. Furthermore, he considered that a considerable amount of work had been undertaken on decay, but

little on factors which initiate the decay process. The importance of soluble nutrients on decay of wood in soil has been demonstrated by King et al (1981). These authors noted that wood in soil, especially wood with high soluble nutrient surfaces, stimulated soil aggregate formation, indicative of a large microbial presence at the wood - soil interface, and that mycelial integrity was maintained between soil peds and decomposing wood.

Preliminary experiments had shown that directional growth was demonstrated by Chaetomium globosum Kunze towards wood in pure culture. In view of this and of the chemotactic behaviour demonstrated by bacteria, especially those responses shown by soil inhabiting Rhizobium spp. to root exudate components, and also the effects that root volatiles exert on growth patterns of mycorrhizal fungi noted from the literature, it was hypothesised that far from being inert and passive, wood volatiles and nutrients exert considerable modifying influences on behavioural patterns of soil microorganisms. It was also hypothesised that these effects are not restricted to those organisms at the wood - soil interface, but also on those at some distance into the soil, leading to the development of soil aggregate formation at the wood - soil interface and associated continual nitrogen input to the decomposing wood, noted by King, Smith & Bruce (1980).

This thesis examines these aspects under the following headings:

1. An assessment of the influence of wood volatiles on growth patterns of fungi.

2. The effects commonly used wood preservatives may have on volatile influences on fungal growth.
3. Determination of bacterial chemotactic behaviour to water soluble extracts of wood.
4. Analyses of soil nitrogen content to establish its influence on rates of decay of wood in soil.
5. Determination of microbial activity and biomass in soil adjacent to wood during the decomposition process over a period of time.
6. Determination of the effectiveness of physical barriers at the groundline as prophylactic measures against decay.

Chapter 2.

Tropic responses of fungi to unpreserved and preserved wood.

2.1. Tropic responses of fungi to wood

2.1.1. Introduction

The mycelial growth habit of fungi ensures that the majority of aerial hyphae are in intimate contact with a gaseous atmosphere, and compounds which have a vapour pressure at temperatures suitable for biological activity are able to diffuse through these atmospheres to metabolically active organisms. The extended cylindrical nature of fungal vegetative hyphae creates a large surface area to volume ratio through which gaseous exchange can be effectively carried out. It was noted by Fries (1973) that many compounds which are insoluble in water, but are lipophilic, may be transferred from source to plasma membranes of cells by the mechanisms of diffusion in the gas phase.

It has been realised for many years that volatile substances effect the growth of fungi. Meissel (1943) and Meissel & Trofimova (1946) demonstrated that the fungus Endomyces magnusii, a thiamine auxotroph, could obtain thiazole, an essential precursor for thiamine, through the gas phase and thereby promote growth. Thiazole is produced by a number of fungi, including yeasts, which could act as a source of this essential metabolite during the development and growth of Endomyces.

Suolhati (1951) showed that wood, especially air and heat dried wood, when suspended in the air above a growing fungal culture in Erlenmeyer flasks, stimulated aerial hyphal

production and extension. He noted that not all fungal species tested responded positively, but that many wood decay fungi were particularly responsive. From further investigations he concluded that the active volatiles evaporating from the wood probably derived from unsaturated fatty acids.

Fries (1960) investigated the effect of nonanoic acid and nonanal, formed as autoxidation breakdown products of the unsaturated fatty acid oleic acid known to be present in wood, on four wood decomposing fungi: Stereum sanguinolentum, Daedalea unicolor, Polyporus cervinus and Polyporus applanatus. The test substances were either added to liquid nutrient medium inoculated with the fungus and growth measured as dry mass production, or in the gas phase. In the gas phase experiment a quantity of the substance in a small aluminium container was placed at the periphery of a petri dish in which the fungus was centrally inoculated onto a solid nutrient agar. In all cases nonanoic acid had no effect on growth. Nonanal stimulated growth of Stereum sanguinolentum in liquid culture mainly by the stimulated growth of aerial hyphae. In the gas phase nonanal stimulated all four fungi, either by increasing their rates of growth or increased production of aerial hyphae.

Further work by Fries (1961) demonstrated that nonanal stimulated aerial hyphal development of several other hymenomycetes when delivered in the gas phase. Other homologous aldehydes were tested, of which heptanal was as active as nonanal, then decreasing sequentially in effect from octanal, hexanal and pentanal; the higher homologues, decanal and undecanal caused either stimulation or inhibition depending

on their concentrations. Interestingly, Fries noted that Polyporus applanatus demonstrated preferential growth on the side of the colony towards the cup containing the nonanal.

Glasare (1970) investigated volatiles in extracts of Pinus sylvestris heated for 25 hours at 110°C and the results showed the presence of several unsaturated fatty acids. He exposed Stereum sanguinolentum to a series of acids, including those identified as being present in heated pine in gas and liquid phases employing the methods of Fries (1961, op. cit.); those acids which had between 4 and 9 carbons stimulated growth. Glasare also attached pieces of heat treated pine to the lids of petri dishes containing growing colonies of 14 wood decay fungi. The presence of wood stimulated the growth of 12 of the fungi tested, including two ascomycetes, Chaetomium funicolor and Ceratocystis minor. A non-acidic fraction of the wood extract was also shown to be active causing morphological changes in cultures of Coniophora cerebella.

Rice (1970) investigated the effects of green and heat dried red pine sapwood, Pinus resinosa, and wood volatile components on the growth of Fomes annosus. Tests were carried out by suspending either wood blocks or glass fibre soaked with test substances over float cultures of the fungus on nutrient medium in Erlenmeyer flasks plugged with cotton wool. The mycelial mat was removed after 12 day incubation in the dark at 24°C, dried to constant mass and weighed. Rice noted that Fomes annosus responded to both green and heat treated sapwood by increased production of aerial hyphae. Wood pretreated with substances which increased oxidation of

fatty acids induced greater stimulation, whilst those blocks impregnated with an oxidation inhibitor effected less stimulation. Fomes was not responsive to any of the aldehydes tested and he concluded that either an unidentified non-aldehyde or an as yet untested aldehyde was responsible for the increases in aerial hyphal production. Rice also found that there were no indications which showed that the volatiles acted as carbon or nitrogen nutrient sources, but functioned by stimulating the fungus to utilise existing nutrient sources in greater production of aerial hyphae. Rice tested other hardwoods to determine if any induced stimulation in growth. Of those tested, Populus tremuloides, Quercus rubra, Betulus papyrifera and Juglans nigra initiated a very active response and Acer sacchanum was somewhat less effective.

In quantitative terms, volatiles of greatest importance which evaporate from green wood are the terpenes, other compounds being present in only trace amounts (Rice, 1970; Flodin & Andersson, 1977). Terpene compounds are generally produced by plants and are characterised by carbon skeletons constructed from C_5 isoprene units. Different terpenes will therefore occur with their carbon skeletons varying in multiples of five, e.g. monoterpenes C_{10}, sesquiterpenes C_{15} and diterpenes C_{20}. Mono and sesquiterpenes are common constituents of plant oils, e.g. eucalyptus oil (C_{15}), celery oil (C_{15}) and various flower oils (C_{15}) pinene (C_{10}) being the main constituent of turpentine oil secreted by conifers. Many workers have studied the effects of terpenes on fungal growth (Hintikka, 1970; Melin & Krupa, 1971; DeGroot, 1972; Krupa &

Nylund, 1972; Fries, 1973; Flodin, 1979), with a large degree of variability in responses observed.

Hintikka (1970) tested terpene tolerance of 16 species of hymenomycetes which naturally occur in coniferous wood and of 22 species which occur in deciduous wood. This was done by exposing growing cultures of the organism to saturated atmospheres of individual terpenes. She showed that most of the fungi from coniferous habitat were tolerant of saturated atmospheres of terpenes, whereas fungi from deciduous habitat were inhibited. The presence of terpenes in wood may, therefore, have a selective effect on organisms wishing to invade wood. Melin & Krupa (1971) demonstrated that all the terpenes tested when delivered in the gas phase in the saturated atmosphere of a sealed petri dish inhibited extension growth of the two mycorrhizal fungi Boletus variegatus and Rhizopogon roseolus. In a similar experiment Krupa & Nylund (1972) showed that terpenes inhibited the growth extension of two root pathogenic fungi Phytophthora cinnamoni and Fomes annosus although there was a great disparity in the severity of inhibition caused by individual terpenes between the two organisms.

In contrast to this Fries (1973) not only measured linear extension of hyphae but also the dry matter production of fungi exposed to terpenes. The method used was similar to other workers (Glasare, 1970; Melin & Krupa, 1971; Krupa & Nylund, 1972), in which a cup containing the test substance was introduced into a petri dish containing a growing culture of the fungus, the dish then being sealed. Fries showed that linear growth in most cases was inhibited; some fungi, however,

were stimulated by terpene presence, not only in linear terms but also by increased production of dry mass. Flodin (1979) reported that dry matter production of Fomes annosus was inhibited in the presence of three terpenes commonly found in coniferous wood, and pinene and 3-carene, whereas the production of enzymes by the organism was stimulated.

Terpenes have in general then been found to inhibit fungal growth (Cobb et. al., 1968; Hintikka, 1970; DeGroot, 1972; Flodin & Fries, 1978; Flodin, 1979). There are, however, reports which demonstrate stimulatory activity of terpenes (Rice, 1970; Fries, 1973). The major component volatiles of fresh Pinus sylvestris L. sapwood are monoterpenes, with pinene and 3-carene quantitatively the most important (Flodin & Andersson, 1977). Heat treated wood showed a marked decrease in the amount of terpenes volatilising from the wood with a decrease of 99% compared with green wood. Autoxidation products such as low molecular mass aldehydes, e.g. hexanal, and ketones e.g. furfural, were much more prevalent in the heated wood, being in relatively high concentrations. Both these products have been shown to have phytoactive effects on fungal propagules; hexanal having been shown to stimulate growth of Stereum sanguinolentum (Flodin & Fries, 1978) and furfural to stimulate spore germination in Neurospora crassa (Emerson, 1948).

King & Waite (1979) demonstrated, in pure culture experiments, that several wood decay fungi increase the nitrogen content of wood blocks during the decay process. The soft rot fungus Chaetomium globosum was seen to maintain mycelial integrity between wood blocks and inocula for the duration of

the 9 week experiment. Interestingly, it was noted that aerial hyphae of Chaetomium globosum showed preferential development on the side of the inocula nearest to the block. In soft rot studies using blocks buried in soil, Waite & King (1979) again showed nitrogen increases in blocks during the decay period, but on removal of wood blocks from soil it was noted that decaying blocks had a covering of adhering soil ramified with fine hyphal strands. Those observations led King, Smith & Bruce (1980) to postulate that during soft rot decay of wood in soil biotic connections between wood and soil are maintained, and that wood may influence the direction of hyphal orientation.

Orientation or directional growth of fungal hyphae hasnot received much comment in the past. A great deal of work has been undertaken on overall growth stimulation or inhibition by wood volatiles, but amongst these workers only Fries (1961, op. cit.) and Rice (1970, op. cit.) noted growth in the direction of the source of the volatiles. Fries observed a strong orientation of growth by Polyporus applanatus towards the cup containing the test substance, and Rice, using Fomes annosus, concluded that stimulation of growth increased 'along the concentration gradient towards the source of the active volatiles'.

Hrib & Rypacek (1977) using callus tissue of Picea excelsa as the bait demonstrated negative chemotropic behaviour in the rhizomorph development of Poria vaillantii. Inocula of the fungus were placed in the centre of petri dishes with spruce callus tissue 30mm from the inocula. After 2 - 3 days, the inocula demonstrated fine hyphal development

equally distributed around the inocula. Later, however, initiation of dense bundles of aerial hyphae occurred which grew in a direction 45^{o} - 90^{o} clockwise from the direction of the callus; they termed this oriented growth 'right handed negative chemotropism'.

The continual input of nitrogen to wood in soil observed by King et. al. (1980), if due to microbial biomass transfer, suggests a movement of microorganisms from soil to wood over an extended time period. A random coincidence of microorganisms with the wood surface followed by colonisation of the wood, using wood nutrients for growth and development would cause an initial increase of nitrogen in the wood during early decay stages, but limited increases later on.

In view of the marked effects wood volatiles have on growth of numerous fungi in terms of inhibition or stimulation, and the observations of Fries (1961), Rice (1970) and Hrib & Rypacek (1977) that directional growth had occurred on occasions, an investigation was undertaken, designed to determine if directional growth was part of the behavioural repertoire of wood invading microorganisms.

As it had been demonstrated that dried, rather than fresh, wood elicited greater responses from fungi (Suolhati, 1951), and considerable changes occurred in volatile composition evaporating from wood after heat drying (Flodin & Andersson, 1977), it was decided to investigate the effect various drying regimes might exert on any directional responses produced by fungi.

Supplementary experiments were undertaken:

1. To determine distances over which chemotropic responses could be elicited if these occurred.
2. To determine any effect sterilising the blocks by autoclaving (121°C. 20 minutes) might have on growth responses.

2.1.2. Material and methods

2.1.2.1. Preparation of wood blocks

Freshly felled, quartersawn planks of two wood species Pinus sylvestris L. and Tilia vulgaris Hayne were either stored in polythene bags at -18°C or dried in a fan oven at 40°C to 12% moisture content before being stored in a ventilated cabinet under ambient room conditions for periods of not less than 6 months. Blocks measuring 10 x 10 x 5mm, prepared from the sapwood of both fresh and dried wood, were cut so that 10 x 5mm faces were in tangential, longitudinal and transverse planes respectively, and the 10 x 10mm faces were in radial longitudinal section. Half the fresh blocks and half the blocks dried at 40°C were further heated in an oven at 102°C for 12 hours, giving four treatments for both wood species:- fresh blocks; fresh blocks heat treated at 102°C; blocks heat treated to 40°C and blocks heat treated at 40°C then further heat treated to 102°C.

2.1.2.2. Exposure of fungi to wood

Nine fungi were used:-

1. Coriolus versicolor (L. ex Fr.) Quel. FPRL 28A.
2. Serpula lacrymans (Fries) Karst. FPRL 12C.
3. Coniophora puteana (Schum ex Fr.) Karst FPRL 11E.
4. Chaetomium globosum Kunze FPRL 70K.
5. Humicola grisea Traen
6. Ceratocystis picea (Munich) Bakshi FPRL 5-9, 5F.
7. Phialophora mutabilis
8. Phialophora fastigiata (Lagerberg et Melin)
9. Trichoderma viride Pers. ex Fr.

They were grown on 90mm petri dishes containing 20cm^3 malt extract agar (Oxoid) at 3% strength. The plates were inoculated at their centres with 5mm cores of each organism and incubated at 25°C except for S. lacrymans which was incubated at 22°C. Plugs of each culture (5mm diameter) were removed (using a sterile cork borer) from the margins of growing colonies and transferred to 5mm discs of sterile filter paper on the surface of Czapek Dox agar in a petri dish. A support constructed from 2mm diameter pliable plastic coated wire formed into a Z shape was positioned at a distance of 12·5mm from the centre of each inoculum (Fig. 2.1.). The wood blocks were placed on these supports with a 10 x 10mm radial face in contact with the support and a 10 x 5mm transverse face towards the inoculum. Five replicate plates for each wood type and heat treatment were exposed to each test fungus and incubated in the dark at 25°C, except for S. lacrymans plates which were incubated at 22°C. Controls were arranged identically, except that blocks

of moist sponge or glass of the same dimensions replaced the wood block.

Two supplementary experiments were undertaken, both using C. globosum, chosen as the test organism because of the marked growth responses it demonstrated in trial experiments. In the first experiment, lime sapwood blocks heat treated at 40°C were placed a range of distances, increasing in 5mm increments, from 5-40mm from inocula to determine the effect of increasing distance on growth responses. Secondly, sapwood blocks of lime, pine and eucalyptus (Eucalyptus regnans Muell.) were dried at 40°C, half of these were then autoclaved at 120°C for 20 minutes. Inocula of C. globosum were exposed to both autoclaved and unautoclaved blocks of each wood species to determine any effects sterilisation by autoclaving might have on growth responses. Five replicate plates were set up in both experiments for each variation in distance or combination of block treatment and wood type.

2.1.2.3. Measurement of growth

Eight consecutive radial measurements, Y_1 - Y_8, were taken at 45° intervals in anticlockwise order starting at Y_1 from centres of inocula to limits of hyphal extension (Fig. 2.2.). Growth was monitored and measurements always taken prior to establishment of any hyphal contact with the block if this was about to occur. Times at which measurements were made varied due to growth rate differences between organisms, and ranged from 25 hours for T. viride to 120 hours for S. lacrymans.

2.1.3. Results

2.1.3.1. Growth model

Fungal colonies on agar plates generally grow with radial symmetry, such colony radii measured at regular intervals when plotted in sequence against measurement intervals follow straight lines parallel to X axes. The radii of colonies distorted by stimuli when similarly plotted approximately describe a sine wave. Radii of colonies were therefore plotted in sequence against measurement intervals and the sine wave of best fit calculated by least squares; examples for unstimulated and stimulated colonies of C. globosum are shown in Fig. 2.3 (1),(2).

Sine waves thus calculated were used for growth analysis by describing them mathematically in the form $Y = \gamma + \alpha \sin (X+\beta)$. γ represents the mean colony radius and its magnitude used as an indicator of growth stimulation or inhibition. α, the amplitude of the sine wave, indicates the degree to which the colony exhibits eccentric or differential, rather than circular, growth, and β the phase, the location at which the model calculates where maximum differential growth occurs and is expressed as the number of degrees in a clockwise direction from Y_3, the position of the bait. Finally, X is the number of degrees in an anticlockwise direction from 0° (Y_1) to the point at which Y is measured.

The model can also be expressed in the form $Y = \gamma \left[1 + \delta \sin (X+\beta)\right]$ where $\delta = \frac{\alpha}{\gamma}$, thereby relates differential

growth to mean colony radius to give a dimensionless value useful in comparing the magnitude of directional responses between fungi to same stimuli or the same fungus to different stimuli.

2.1.3.2. Mean colony radii, differential growth and phase of fungal colonies.

Differences in mean colony radii between fungi exposed to wood and controls are presented in Table 2.1.; C. picea, P. fastigiata and P. mutabilis were not recorded because of absence of growth after a minimum of 14 days. In all, 4000 measurements were taken, necessitating computer analysis and the results indicated that hyphal extension of the organisms C. globosum, S. lacrymans and C. puteana was significantly stimulated or inhibited in the presence of wood, especially heat treated wood. C. versicolor and H. grisea were unaffected by wood presence, and hyphal extension of T. viride, although not shown in the results of Table 2.1. to be significantly influenced, showed marked orientation towards blocks. Differential growth results, for those organisms which demonstrated asymmetric growth, are presented in Table 2.2. Those organisms which demonstrated asymmetric growth showed either orientation of hyphal extension towards wood baits (Figs. 2.4. & 2.5.), or alternatively growth in an opposite direction (Fig. 2.6.). Phase data (Table 2.3.) generally varied between $\pm$ 15° from the direction of the bait (Y_3) where hyphal extension towards baits occurred, or between $\pm$ 15° from

the direction directly opposite the bait (Y_7) where hyphal extension was away from blocks.

2.1.3.3. Statistical treatment of results

A planned comparison between the combination of eight treatments and controls was used to determine the significance of data for mean colony radii and differential growth. Individual 't'-tests were performed for the five replicates of each eight treatments and controls for both mean colony radii and differential growth, the results of which are shown as superscripts in Tables 2.1. and 2.2.

A factorial analysis was undertaken for mean colony radii and differential growth to determine which effects, either on their own or by interaction with each other, produced a particularly significant growth response. Factors investigated were A. Differences caused by wood type, B. Differences between wood dried at 40°C and material not exposed to this temperature, and C. Wood dried at 102°C, compared to wood not elevated to this temperature. Interactive effects were investigated for combinations of AB, AC and BC and finally for combinations of all three conditions, ABC.

The phase data for each five replicate treatments were analysed (Batschelet, 1981), and mean angles calculated (Table 2.3.). The location of the mean angle does not indicate the degree to which the sample is clustered about that direction, and a measure of dispersion about the mean is necessary. The mean angular deviation indicates the degree of

dispersion around the location of the mean angle, the smaller its value, the greater is the preference for growth in that direction. Conversely, a high value for mean angular deviation indicates a more random dispersal about the mean location, with no preferred direction of growth. An example for stimulated and unstimulated colonies of C. globosum is shown in Fig. 2.7. indicating location of the phase and mean angular deviation around this mean.

The planned comparison for the determination of significance of data of mean colony radii and differential growth compared with data for controls is presented in Table 2.4. Mean colony radii and differential growth were both significant at P = 0·001 for C. globosum; mean colony radius was significant at P = 0·001 for S. lacrymans, but not significant for differential growth; both mean colony radius and differential growth was significant at P = 0·001 for C. puteana; mean colony radius was not significant for T. viride whereas differential growth was significant at P = 0·01.

Factorial analyses for mean colony radii are presented in Table 2.5. and for differential growth in Table 2.6.; both tables indicate that significant differences exist between organisms in the nature and degree of their responses.

Effects on mean colony radius

The degree to which hyphal extension was influenced in responsive organisms was dependent on differences in the drying regime and in variations due to wood type. C. globosum

and T. viride were unaffected by differences between wood species, but showed greater stimulation to heated rather than fresh wood. The two basidiomycetes showed marked differences in their responses to lime and pine. S. lacrymans was strongly inhibited by volatiles from heated lime, but unaffected by fresh wood, whereas C. puteana was marginally stimulated by the presence of pine, whether fresh or dried.

Effects on differential growth

The positioning of baits to one side of inocula caused hyphal extension to proliferate in one direction rather than uniformly in all directions; this directionality occurred either towards or away from wood blocks. C. globosum and T. viride demonstrated greater hyphal extension in the direction of volatile source regardless of wood type, but more markedly in the presence of dried rather than fresh wood. C. globosum demonstrated this to a remarkable extent (Figs. 2.4. and 2.5.), with dense production of aerial hyphae on sides of inocula facing blocks; hyphae on sides of inocula away from blocks demonstrated curvature, thereby redirecting growth towards baits. Hyphae of S. lacrymans grew preferentially on sides of inocula away from dried lime (Fig. 2.6.); the response to pine blocks was, however, varied, with hyphal extension being towards blocks in some replicate plates and away in others. C. puteana showed slight preferential hyphal development on the sides of inocula towards dried pine. This response was, however, variable; in some replicates there was dense production of aerial hyphae, whereas in others aerial

hyphal production was very limited.

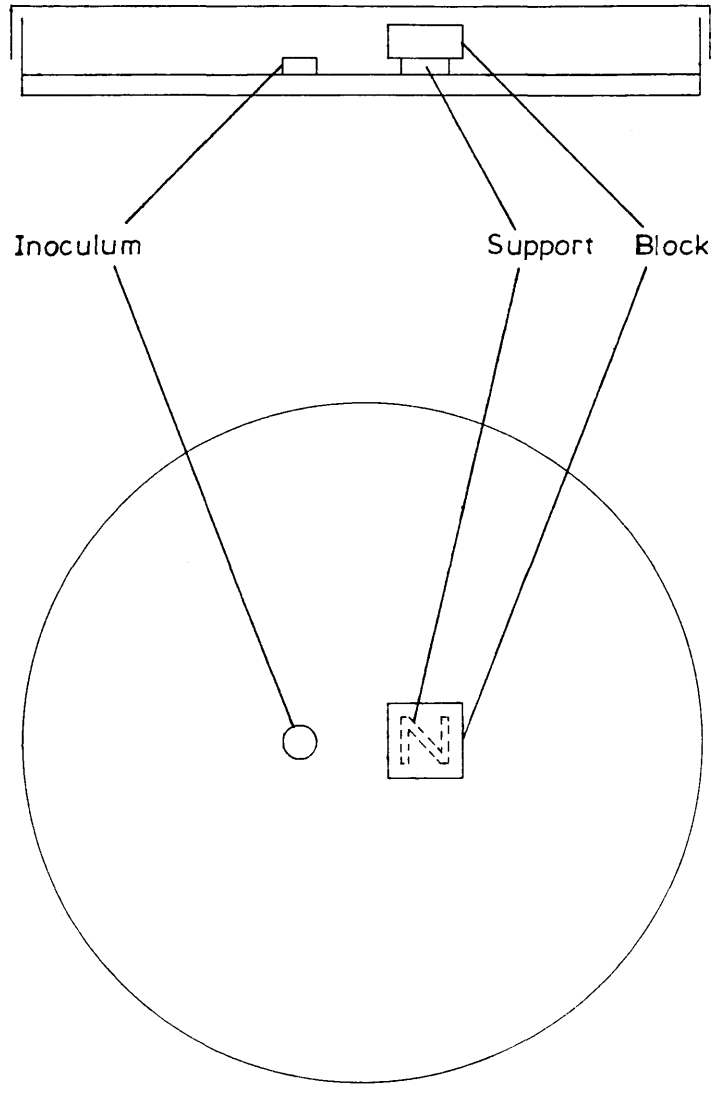
Delta values

Delta values for all fungi are presented in Table 2.7. Values were consistent for the two non-basidiomycetes in the presence of either heat processed pine or lime. Values for C. globosum, an organism which demonstrated a very strong response, averaged 0·5 for dried wood; in the presence of fresh wood, however, the response was diminished, with a delta value of only 0·3. For T. viride the value averaged marginally above 0·1 in the presence of heated wood, whereas values for green wood and control values were insignificant. The two basidiomycetes showed greater variability in values obtained. In S. lacrymans the values were negative for all heated lime blocks indicating hyphal extension away from blocks; however, for pine blocks values varied, growth taking place towards green wood but away from two of the three heat treatments. C. puteana demonstrated consistent values above 0·1 towards heated pine, but variable responses to heated lime.

Variation in distance

Measurable growth was shown by C. globosum up to distances of 30mm from blocks (Table 2.8.), and hyphal contact was established with blocks placed 5mm from inocula. The results indicated that positive hyphal extension towards blocks occurs up to 30mm distant, although differential growth decreases as the distances increase. At distances above 30mm, aerial growth was limited but still directed towards the blocks.

SIDE VIEW



PLAN

FIG. 2.1. Arrangement for exposure of fungal inocula to wood blocks

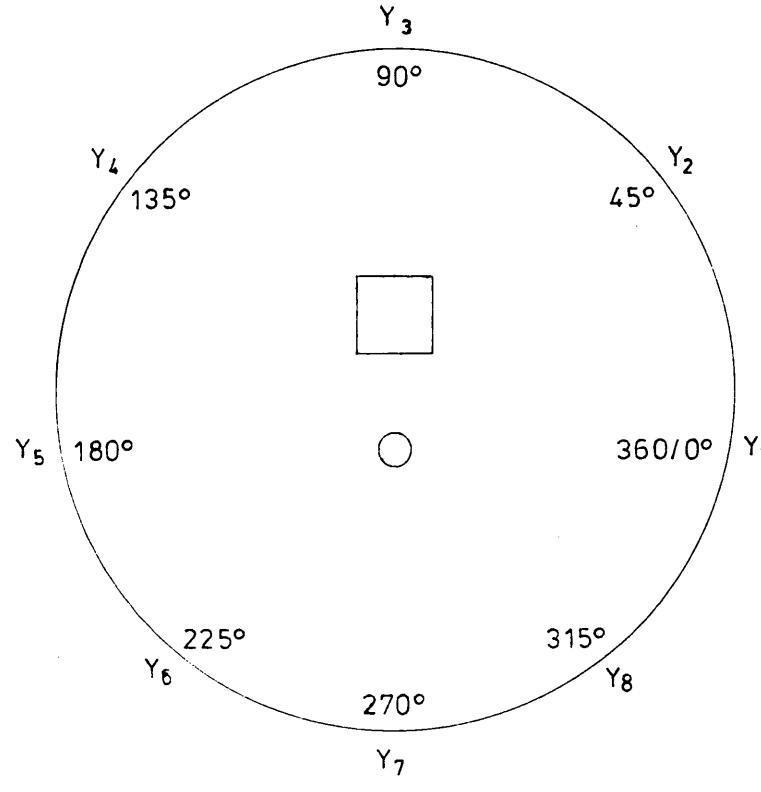


FIG. 2.2. Arrangement of measurement intervals and the sequence in which measurements were taken

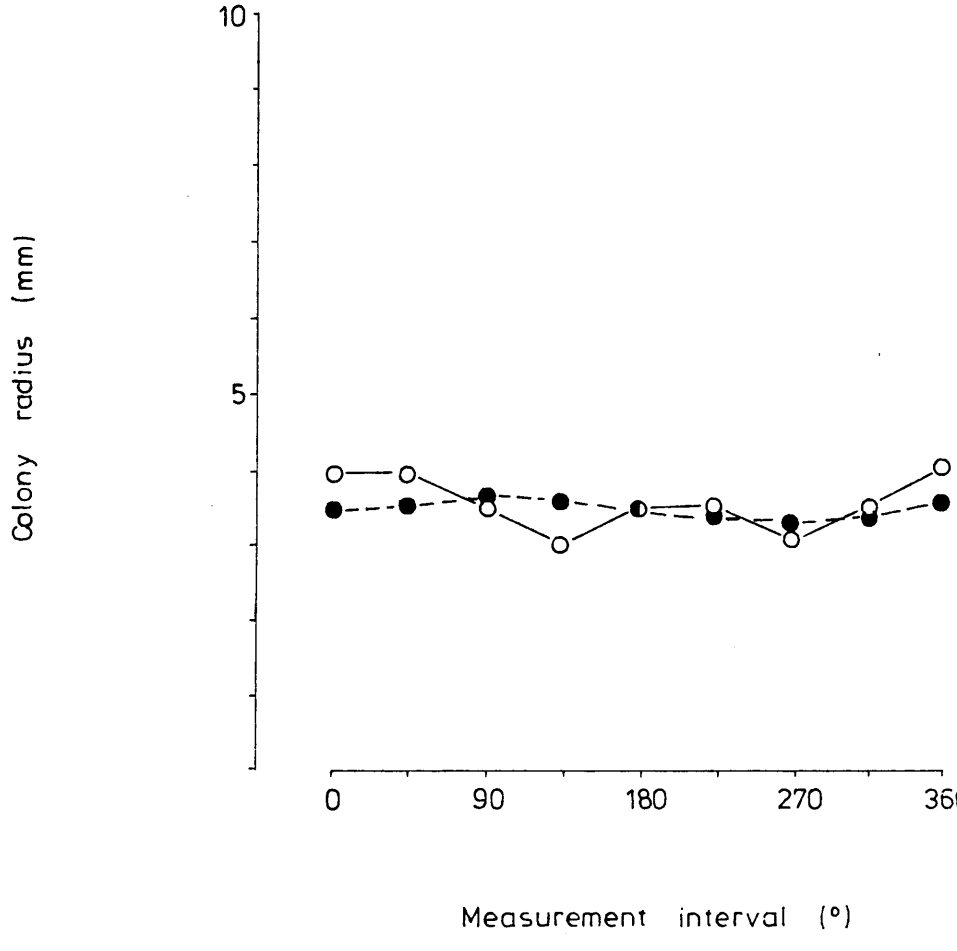


FIG. 2.3.(1). Growth pattern of Chaetomium globusum grown in the presence of a sponge block placed at 90° position to the inoculum. The points show the colony radius measured from the centre of the inoculum to the limit of hyphal extension at the degree interval indicated. o represents actual measurements and ● represents the sine wave of best fit.

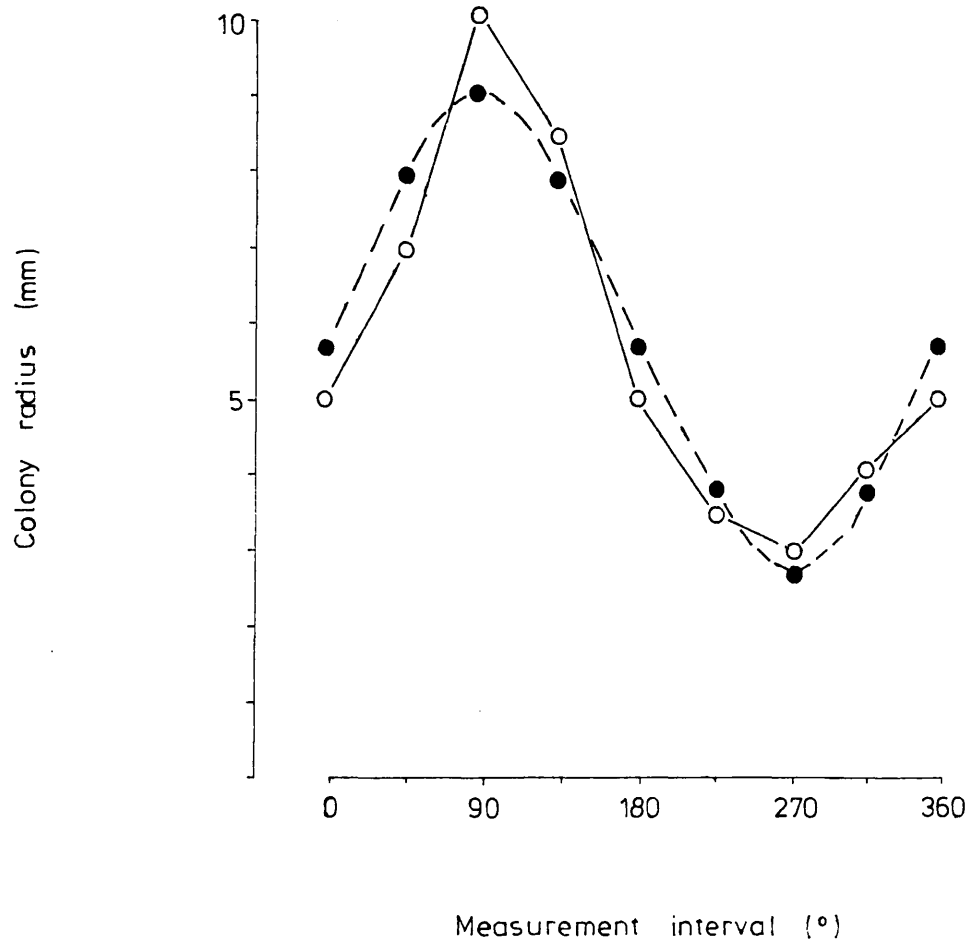


FIG. 2.3.(2). Growth pattern of Chaetomium globosum grown in the presence of a heat-dried lime sapwood block placed at 90° position to the inoculum. The points show the colony radius measured from the centre of the inoculum to the limit of hyphal extension at the degree angles indicated. O represents actual measurements and ● represents the sine wave of best fit.

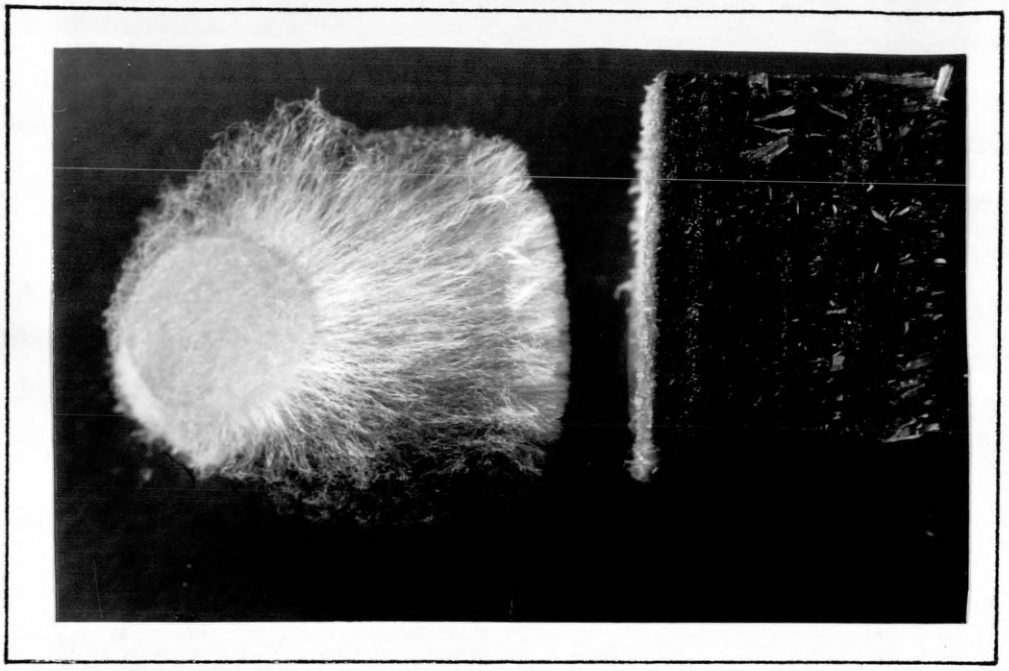


Fig. 2.4(a). Oriented hyphal extension of Chaetomium globosum towards heat dried (40°-102°C) lime sapwood after 25 hours incubation at 25°C.

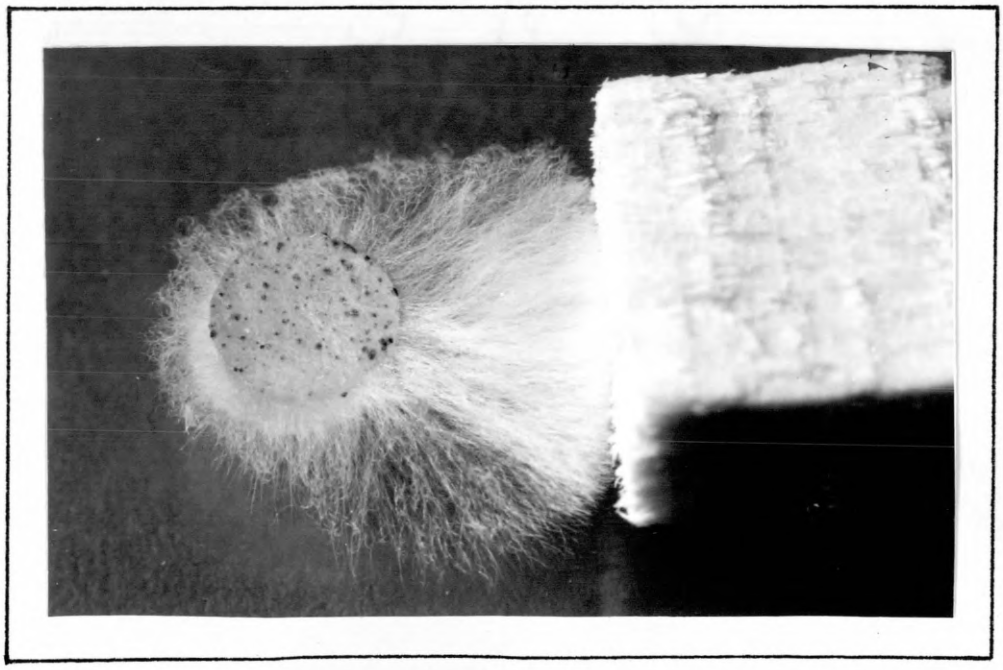


Fig. 2.4(b). Oriented hyphal extension of Chaetomium globosum towards heat dried (40°C) lime sapwood after 31 hours incubation at 25°C.

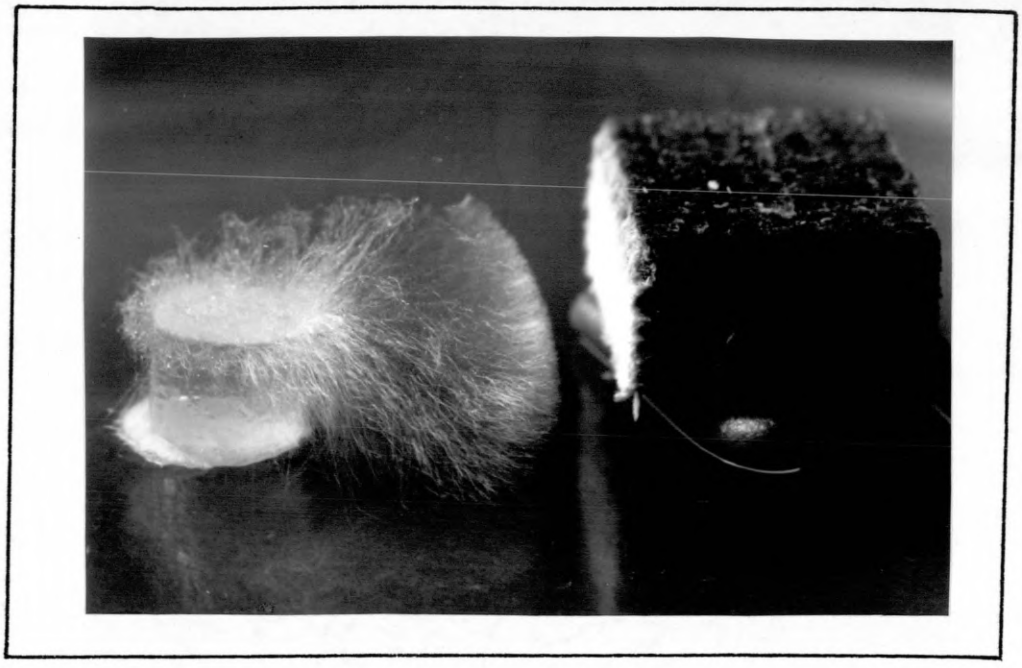


Fig. 2.5. Oriented hyphal extension of Chaetomium globosum towards heat-dried (40°C) lime sapwood after 30 hours incubation at 25°C.

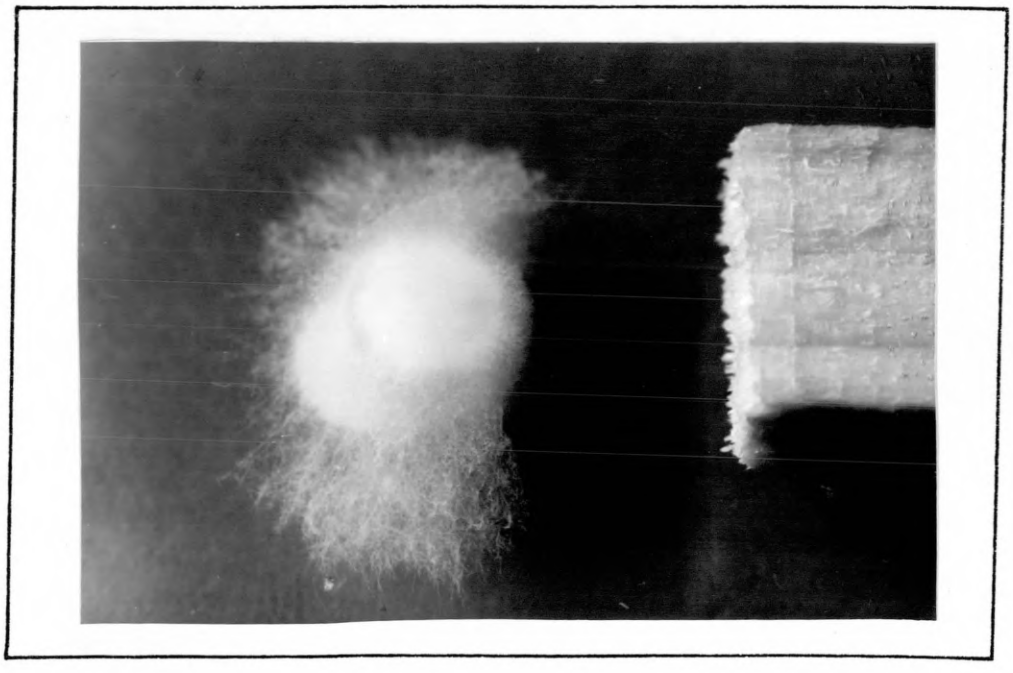
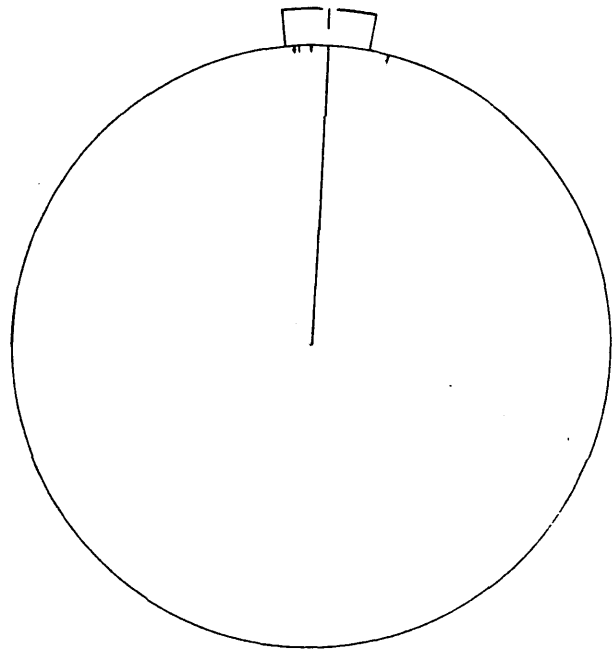


Fig. 2.6. Inhibition of hyphal development of Serpula lacrymans on side of inoculum towards heat-dried (40°C) lime sapwood after 50 hours incubation at 22°C.

(i) Pine dried at 40°C.

Phase: 3° ± 8°



(ii) Inert sponge control

Phase: 43° ± 60°

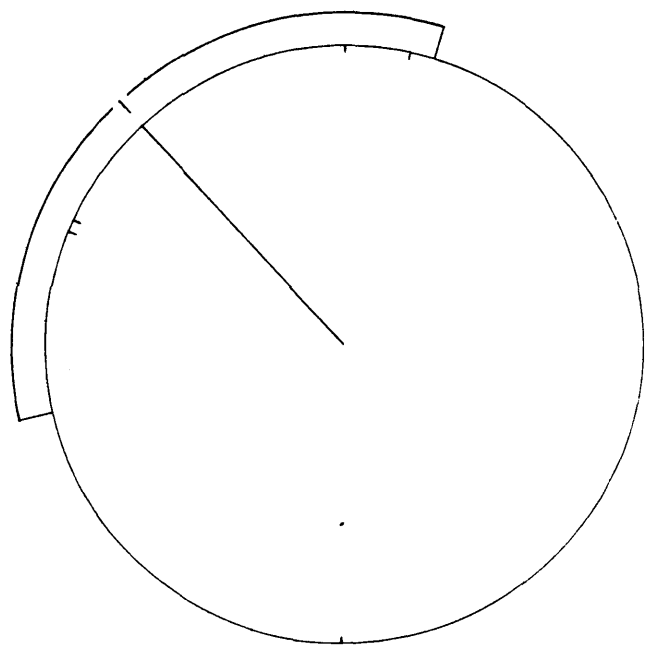


FIG. 2.7. Direction of differential growth and mean angular deviation for five replicate plates of Chaetomium globosum grown in the presence of (i) Pine dried at 40°C., and (ii) Inert sponge control, both at 360/0° position.

Table 2.1. Mean radii of fungal colonies exposed to either fresh or variously dried lime and pine sapwood blocks and inert controls. Figures represent means of five replicates and their standard deviations.

Organism	Wood type	Colony radius (mm) with indicated wood treatment †				
		Fresh	Fresh ⟶102°C	40°C	40°C⟶102°C	Control
Chaetomium globosum	Lime	5·3 ± 0·45	5·7 ± 0·18	6·0 ± 0·09	5·8 ± 0·67	4·2 ± 0·38
	Pine	4·4 ± 0·49[a]	5·7 ± 0·08	5·8 ± 0·30	6·0 ± 0·31	
Serpula lacrymans	Lime	6·7 ± 0·54[a]	4·4 ± 0·25	3·7 ± 0·23	4·3 ± 0·51	6·7 ± 0·25
	Pine	6·8 ± 0·26[a]	6·6 ± 0·32[a]	5·1 ± 0·26	5·6 ± 0·93	
Coniophora puteana	Lime	4·8 ± 0·86[a]	4·8 ± 0·60[a]	4·8 ± 0·37[a]	4·7 ± 0·34[a]	4·4 ± 0·38
	Pine	5·3 ± 0·09	4·6 ± 0·44[a]	5·2 ± 0·21	5·5 ± 0·14	
Trichoderma viride	Lime	9·5 ± 0·32	9·7 ± 0·37[a]	10·8 ± 0·41	10·0 ± 0·43[a]	10·0 ± 0·06
	Pine	9·4 ± 0·25	10·4 ± 0·64[a]	10·5 ± 0·27	10·5 ± 0·64[a]	
Coriolus versicolor	Lime	11·0 ± 0·71[a]	11·0 ± 0·35[a]	12·2 ± 0·44[a]	11·5 ± 0·35[a]	11·6 ± 0·74
	Pine	12·0 ± 0·60[a]	12·0 ± 0·70[a]	11·4 ± 0·54[a]	11·2 ± 0·76[a]	
Humicola grisea	Lime	6·8 ± 0·27[a]	6·6 ± 0·22[a]	6·9 ± 0·42[a]	7·3 ± 0·27[a]	7·0 ± 0·35
	Pine	6·8 ± 0·44[a]	6·8 ± 0·26[a]	6·3 ± 0·57[a]	6·1 ± 0·22[a]	

† Values marked with a superscript a are not significantly different from the control at the 5% level

Table 2.2. Mean differential growth values (α) of fungal colonies exposed to either fresh or variously dried lime and pine sapwood blocks and inert controls. Figures represent means of five replicates and their standard deviations. Negative values indicate growth away from the block.

Organism	Wood type	Value of α (mm) with indicated wood treatment †				
		Fresh	Fresh→102°C	40°C	40°C→102°C	Control
Chaetomium globosum	Lime	1·6±0·18[a]	3·1±0·32	3·3±0·26	2·6±0·33	1·3±0·38
	Pine	1·2±0·30[a]	3·0±0·29	3·1±0·56	3·6±0·21	
Serpula lacrymans	Lime	0·6±0·27	-1·9±0·33	-0·5±0·67	-0·9±0·24	-0·1±0·14
	Pine	0·7±0·40	0·0±0·96[a]	-0·9±0·28	-0·8±0·61	
Coniophora puteana	Lime	0·1±0·20[a]	0·3±0·50[a]	0·8±0·23	0·2±0·22[a]	-0·1±0·28
	Pine	0·3±0·23[a]	0·6±0·17	1·0±0·19	1·4±0·36	
Trichoderma viride	Lime	0·3±0·29[a]	1·2±0·42	1·3±0·22	1·3±0·15	0·4±0·44
	Pine	0·5±0·63[a]	1·0±0·43[a]	1·3±0·32	1·1±0·63[a]	

† Values marked with a superscript [a] are not significantly different from the control at 5% level

Table 2.3. Phase data (β)† of fungal colonies exposed to either fresh or variously dried lime and pine sapwood blocks and inert controls. Figures represent means of five replicates and their mean angular deviations.

Organism	Wood type	Value of β (°) with indicated wood treatment				
		Fresh	Fresh 102°C	40°C	40°C 102°C	Control
Chaetomium globosum	Lime	18±8	2±8	7±8	6±8	43±60
	Pine	2±8	5±8	3±8	3±8	
Serpula lacrymans	Lime	4±32	-6±11	-37±52	-8±8	86±44
	Pine	4±23	-55±58	-21±8	-20±45	
Coniophora puteana	Lime	29±65	5±67	8±8	57±41	-74±56
	Pine	50±23	12±14	13±16	14±8	
Trichoderma viride	Lime	28±51	7±26	1±8	5±8	17±61
	Pine	27±59	10±26	10±24	18±29	

† Expressed as the number of degrees the direction of differential growth varies from 0° (Y_3) when values are positive, and from 180° (Y_7) when values are negative

Table 2.4. t values† for planned comparisons showing significances of differences between the growth parameters, mean colony radius and differential growth, of fungi exposed to wood baits and inert controls.

Organism	Mean colony radius		Differential growth	
	t value	significance	t value	significance
Chaetomium globosum	8·05	p= 0·001	8·97	p= 0·001
Serpula lacrymans	-6·28	p= 0·001	-1·68	not significant
Coniophora puteana	2·89	p=0·01	4·87	p=0·001
Trichoderma viride	0·42	not significant	3·07	p=0·01

† Significant positive values indicate stimulation of mean colony radius or differential growth towards blocks, negative values indicate growth inhibition or differential growth away from blocks

Table 2.5. F values of Factorial analysis for determining effects of A: wood type, B: wood dried at 40°C compared with wood never dried at 40°C, C: wood heated to 102°C compared with wood never heated to 102°C and the interactive effects between these parameters on mean colony radii.

Organism	A	B	C	AB	AC	BC	ABC
Chaetomium globosum	3·7	27·0 **	11·5 *	3·5	9·8 *	11·9 *	0·95
Serpula lacrymans	70·0 **	97·5 **	5·1	0·7	11·8 *	37·3 **	12·9 **
Coniophora puteana	7·1	2·1	1·2	2·1	0·2	3·8	4·5
Trichoderma viride	3·2	26·2 **	0·53	0·61	8·3 *	14·1 **	0·00

* and ** indicates significance at 1% and 0·1% levels respectively

Table 2.6. F values of factorial analysis for determining effects of A: wood type, B: wood dried at 40°C compared with wood never dried at 40°C, C: wood heated to 102°C compared with wood never heated to 102°C and the interactive effects between these parameters on differential growth.

Organism	A	B	C	AB	AC	BC	ABC
Chaetomium globosum	0·4	85·9 **	55·9 **	9·4 *	11·9 *	73·9 **	6·6
Serpula lacrymans	7·0	13·3 **	26·0 **	12·5 *	10·3 *	20·0 **	3·6
Coniophora puteana	24·8 **	31·5 **	0·86	5·28	8·39 *	4·76	7·2
Trichoderma viride	0·14	13·6 **	6·7	0·09	1·16	7·9 *	0·09

* and ** indicates significance at 1% and 0·1% levels respectively

Table 2.7. Ratio of differential growth : mean colony radius (δ) of fungal colonies exposed to either fresh or variously dried lime and pine sapwood blocks and inert controls. Figures represent means of five replicates.

Organism	Wood type	Treatment				
		Fresh	Fresh 102°C	40°C	40°C 102°C	Control
Chaetomium globosum	Lime	0·29	0·55	0·55	0·45	0·11
	Pine	0·29	0·52	0·54	0·60	
Serpula lacrymans	Lime	0·09	0·43	0·14	0·20	0·01
	Pine	0·11	0·00	0·18	0·15	
Coniophora puteana	Lime	0·02	0·07	0·17	0·04	0·02
	Pine	0·06	0·13	0·19	0·25	
Trichoderma viride	Lime	0·03	0·12	0·12	0·13	0·04
	Pine	0·05	0·10	0·12	0·11	

Table 2.8. Mean colony radii, differential growth and delta value for Chaetomium globosum exposed to lime sapwood blocks dried at 40°C at varying distances between inocula and blocks. Figures represent means of five replicates.

Distance between inocula and blocks (mm)	Mean colony radius (mm)	Differential growth (mm)	Delta value
5†	4·4	2·1	0·48
10	4·7	2·7	0·57
15	4·7	2·6	0·55
20	4·4	2·2	0·50
25	4·9	1·5	0·31
30	4·5	1·7	0·38
35‡	—	—	—
40‡	—	—	—

† hyphal contact with block

‡ limited production of aerial hyphae

Table 2.9. Mean colony radii (mm), differential growth (mm) and delta value for Chaetomium globosum exposed to autoclaved and 40°C dried sapwood blocks of lime, pine and eucalypt and inert controls. Figures represent means of five replicates.

Wood type	Treatment	Mean colony radii	Differential growth	Delta value
Lime	Autoclaved	5·7	3·5	0·61
	40°C	5·1	2·8	0·56
Pine	Autoclaved	5·5	2·5	0·46
	40°C	5·3	2·7	0·51
Eucalypt	Autoclaved	6·1	2·7	0·44
	40°C	5·3	2·5	0·47
Control		2·9	0·0	—

Autoclaved material

Values for mean colony radii, differential growth and delta values for C. globosum grown in the presence of both autoclaved (120°C for 20 minutes) and 40°C dried lime, pine and eucalyptus blocks are shown in Table 2.9. The results indicate that hyphal extension was towards all wood species, regardless of treatment, with no significant growth differences being demonstrated in the presence of autoclaved material.

2.1.4. Discussion

Gross stimulation of fungal growth in response to volatiles emanating from heated pine wood has been shown by several workers (Glasare, 1970; Flodin & Fries, 1978); however, it has also been shown that fresh pine or pine extracts such as terpenes inhibit growth (Shrimpton & Whitney, 1968; Cobb et. al., 1968; Hintikka, 1970; DeGroot, 1972). In contrast with this Fries (1973) showed linear growth stimulation by some fungi to several terpenes. The experimental design used by Fries (1961), when he observed preferential growth of P. applanatus towards the vessel containing nonanal, consisted of ventilated petri dishes with containers holding the test compound at the periphery of the dish and a centrally inoculated organism. More recent investigations on the effect of volatiles on fungi have, however, been undertaken in sealed containers in which the atmosphere becomes saturated with volatile vapours, hence organisms are equally stimulated or inhibited, from all directions. Volatile concentration gradients would not be

produced using such experimental systems, hence reducing the likelihood of observing oriented growth.

In experiments used to determine fungal growth responses in the investigation at this laboratory, inocula and blocks were placed centrally in ventilated petri dishes. Consequently, volatiles emanating from wood blocks would decrease in concentration from wood - air interfaces to peripheries of plates where venting to the atmosphere is possible. Such gradients in volatile concentration would allow organisms, if chemotropic, to sense the direction of the source of volatiles.

The results clearly show that when fungi are exposed to eccentrically placed wood blocks, directional growth occurs. Growth on sides of colonies of C. globosum and T. viride closest to wood blocks was stimulated, resulting in increased hyphal length, whereas hyphal extension of S. lacrymans was generally reduced on sides of colonies nearest wood blocks, restricting major growth to a direction away from the baits. In strongly stimulated colonies of C. globosum, curvature of hyphae on those sides of colonies away from wood baits occurred, thereby redirecting their growth towards the stimulus. Upon contact of hyphae and wood block, no further directional growth took place, although mycelial integrity between inoculum and wood block was maintained, and it is tempting to speculate that feedback mechanisms may be functioning.

Where previous workers have noted the occurrence of directional growth, the phenomenon has been largely ignored

and not measured or statistically analysed. From our observations it can be concluded that growth responses of some fungi to wood are not simply those of stimulation or inhibition, but rather tropic, in which changes of direction of growth take place in response to stimuli. The model devised and developed in this chapter effectively analyses and evaluates directional growth in fungi, and conclusively shows that tropic responses are not aberrant manifestations, but rather normal responses in fungal behavioural patterns when responsive organisms are exposed to wood volatiles.

The importance of these responses in the natural environment are unassessed to date, but may be of fundamental importance. Wood decay in soil is a natural extension of the normal decay process of plant litter decomposition, in which wood decays mainly due to fungal attack. Rates and time periods required for such processes are dependent on numerous variable factors inherent in wood and soils themselves; predominant amongst these must be the ability of decay organisms to make contact with nutrient resources such as wood.

Gases in soil are generally enclosed, in continuous phase, by a matrix of solid soil particles with many interconnecting pores. Gases move through soil mainly by diffusion (Brady, 1974). In the situation where wood is emplaced in soil, there is a possibility that volatiles emanating from the wood surface diffuse into the soil, so forming a gradient in concentration, most concentrated at the wood - soil interface, and diminishing with increasing distance

into the soil. Microorganisms may respond to such gradients by oriented growth, and in this respect measurement of linear growth is of more significance than that of dry weight, which is a commonly used parameter in other studies on the effect of wood volatiles on fungal growth.

Distances into the soil from the wood - soil interface to which volatiles may penetrate will depend on many wood and soil variables, such as soil temperature and structure, moisture content and the degree of volatilisation at the wood surface. In pure culture studies undertaken in this investigation, C. globosum demonstrated marked oriented growth towards baits at a distance 30mm from inocula. The major effect of increasing distance from 30mm to 40mm was a diminution in the production of aerial hyphae, although orientation in growth remained.

Mechanisms which enhance the likelihood of organisms encountering a nutrient resource would be highly advantageous in biologically competetive environments such as soil. Organisms that respond by directional growth to wood in soil would have an advantage over non-chemotropic organisms, as such mechanisms would reduce the reliance on random coincidence between fungi and substrate prior to invasion and colonisation. Wood volatiles may have a selective effect on invading mycoflora, variations in volatile emission from different wood species affecting the diverse soil mycoflora in a discriminate manner. In this context, oriented growth responses to wood emplaced in soil would be of considerable ecological advantage for wood invading fungi, and wood volatiles may be

of prime importance as determinants of microbial invasion of wood in this environment.

2.2. Tropic responses of fungi to preserved wood

2.2.1. Introduction

Untreated wood in the soil environment is inevitably susceptible to decay, although some timbers may be considered durable because of the slow rates at which decay processes take place in these species. Non-durable timbers cannot perform adequately in service due to decay susceptibility, thus many methods have been developed to increase longevity. The most widely adopted process to extend the service life of timber is impregnation with chemicals toxic to those microorganisms which effect its decomposition. In view of the tropic responses demonstrated by wood decay fungi in the previous section, and the fact that wood in service is used in a preserved state, it was thought interesting to investigate the influence preservatives impregnated into wood blocks to toxic levels might have on such tropic responses.

Three main classes of preservative are described in British Standard 1282 : 1975:

a. Tar oil type. Type TO

Tar oil types, e.g. coal tars, have long been used as wood preservatives; however, the lighter distillates, which include creosotes, are now most commonly used due to lower viscosity and better penetration.

b. Organic solvent type. Type OS

These include organic biocides usually dissolved

in petroleum oil distillate; the solvent evaporates off soon after treatment. Commonly used preservatives of this type include: Tributyltin oxide (TBTO), Pentachlorophenol (PCP), Copper napthenate (CuN) and Zinc napthenate (ZnN).

c. Water borne type. Type WB

These include solutions of heavy metal ions which have biocidal properties against agents of decay, e.g. copper as a fungicide, arsenic as an insecticide. Other compounds may be added to the formulation to increase solubility of the toxic agent or to aid in its fixation to wood material. The world market in water borne preservatives is now dominated by copper chrome arsenic formulations using dichromate as the fixing agent.

Fungicides of all three classes were initially included in this investigation. As it had been demonstrated that fungi respond to volatile elements evaporating from untreated wood, those preservatives with volatile compounds were of particular interest. Odourous emanations from creosoted materials are well known and both freshly creosoted and creosoted material from 20 year old transmission poles were included in some experiments. Tributyltin oxide and pentachlorophenol have been shown to be lost to the environment via the gas phase (Morgan & Purslow, 1973), and the napthenate component of both copper and zinc napthenates volatilises from treated wood (Richardson, 1978). It was

therefore decided to include these five preservatives in the investigation, not only because of their volatile components, but also because of their widespread use. Although it has no vapour pressure, copper chrome arsenic was also included, not only because of its ubiquitousness, but also because heavy metals have been demonstrated to accelerate the oxidation of unsaturated fatty acids into volatile aldehyde and ketone units (Rice, 1970). Such compounds have been shown to be present in wood, especially when dried, and are known to stimulate fungal growth (Flodin & Andersson, 1978).

In the external environment, or prior to laboratory testing, preservative treated wood is subjected to leaching, consequently leached blocks were included to investigate the influence of leaching procedures on tropic responses.

2.2.2. Materials and methods

Wood blocks measuring 10 x 10 x 5mm were prepared from sapwood of lime (Tilia vulgaris Hayne) and pine (Pinus sylvestris L.) and cut with 10 x 10mm faces in radial longitudinal section. Blocks, oven dried at 102°C for 12 hours and allowed to cool in a dessicator, were impregnated to toxic concentrations with preservatives by placing in a vacuum dessicator, and a vacuum was drawn for 30 minutes. Preservatives, dissolved in a suitable solvent (Table 2.10), were introduced and the blocks allowed to soak, submerged under atmospheric pressure for 2 hours following the release of any residual vacuum. All test blocks were slowly dried

over a 4 week period according to the method in BS 5218 : 1975 (EN 21); blocks impregnated with organic preservatives were placed on 10 x 5mm faces in petri dishes and turned through 180° every third day. Dishes were kept closed for one week, partially open for the second week, and fully open for a further two weeks. Blocks treated with copper chrome arsenic were stored in a large glass vessel, with a small quantity of xylene in a glass container to prevent mould growth; the glass vessel was kept closed for two weeks, partially open for another week, and fully open for a further week. Creosoted material of freshly impregnated 10mm diameter beech dowel cut into 5mm thick discs, and 10 x 10 x 5mm Scots pine sapwood blocks cut from a 20 year old creosote treated transmission pole. Control blocks consisted of heat dried blocks impregnated with either solvent or water alone and untreated blocks which had only been heat dried. All blocks were stored in closed sterile glass petri dishes until used.

Fungi chosen were those which had demonstrated growth responses to untreated heat dried wood blocks in earlier experiments:

1. Chaetomium globosum Kunze FPRL 70K
2. Trichoderma viride Pers. ex Fr.
3. Serpula lacrymans (Fries) Karst. FPRL 12C
4. Coniophora puteana (Schum ex Fr.) Karst. FPRL 11E

Cultures of these organisms were maintained on 3% malt extract agar (Oxoid) in the dark at 25°C, except for S. lacrymans which was incubated at 22°C. Exposure of fungi to wood blocks was as described in section 2.1.2.2., except that blocks were

placed 22·5mm from centres of inocula of T. viride due to rapid growth rate of this organism. Five replicate plates of each combination of wood type and preservative formulation, plus five replicate plates for each control, were prepared for each fungus. Plates were incubated in the dark at 25°C, except for S. lacrymans, which was incubated in the dark at 22°C. Creosoted material was exposed to C. globosum and S. lacrymans only.

Hyphal extension patterns were monitored and measurements taken prior to establishment of hyphal contact between organisms and blocks if this was about to occur. The methods used to measure growth and analyse growth patterns has been described in the previous section. Measurements were made of aerial hyphae, except for C. puteana which produced only basal mycelium in the presence of blocks impregnated with organic preservatives.

Effects of prior leaching of blocks on funal growth was determined by submitting one set of blocks, of each combination of wood type and preservative plus control, to aqueous extraction for eight hours in a soxhlet apparatus. Leached blocks were stored in ventilated glass containers for five days under ambient laboratory conditions prior to use. Inocula of C. globosum were exposed to leached blocks and measurement and analysis of growth was as described for unleached material.

2.2.3. Results

Times at which measurements were taken varied between organisms, and are shown with results for mean colony radii in Table 2.11. The results show that preservatives vary in their effects on overall growth. Copper chrome arsenic has no measurable inhibitory or stimulatory effect on hyphal extension of any organism. Tributyltin oxide demonstrated a marked inhibitory effect on all organisms. Copper napthenate and pentachlorophenol were inconsistent in responses elicited from organisms; copper napthenate inhibited hyphal extension of C. globosum and T. viride when present in pine, whereas pentachlorophenol inhibited hyphal extension of C. globosum in both pine and lime, and of T. viride only in lime. Zinc napthenate elicited no inhibiting effects on mean colony radii of any organism, regardless of wood type. The low mean colony radii results for control plates of C. puteana reflect the production of aerial hyphae by this organism when exposed to unpreserved wood, whereas when exposed to preserved wood a greater diameter of surface mycelium was produced, but lacking aerial hyphae. Results are not presented for creosoted material exposed to C. globosum and S. lacrymans because of lack of measurable growth by the fungi after a minimum of 10 days.

Results for differential growth are shown in Table 2.12.; these show that oriented hyphal extension was demonstrated by all organisms to both treated and untreated blocks. Water borne copper chrome arsenic is generally neutral in that oriented growth responses towards controls were unaffected by copper chrome arsenic presence, except for S. lacrymans which

showed marginal preferential growth towards copper chrome arsenic treated pine, whereas growth was away from the pine controls. Hyphal extension was inhibited in all organisms on sides of inocula facing blocks of both wood types treated with tributyltin oxide, resulting in growth taking place only in a direction away from blocks. Copper and zinc napthenate inhibit differential growth of C. globosum only in pine, whereas pentachlorophenol causes inhibition in both pine and lime, and zinc napthenate and pentachlorophenol both inhibit differential growth of T. viride when impregnated in lime.

S. lacrymans demonstrated directional growth away from untreated blocks and this response was maintained to all preservatives and wood types except for copper chrome arsenic treated pine; however, growth away was increased in pine treated with both copper and zinc napthenate. C. puteana demonstrated variable and limited oriented growth responses to controls and copper chrome arsenic treated blocks, where an organic preservative was present; however, only basal mycelium was produced which was oriented away from treated blocks.

Leaching of blocks prior to use resulted in only minor modifications in hyphal extension patterns of C. globosum as opposed to unleached material. Mean colony radii were generally unaffected, although there is somewhat diminished growth in the presence of leached lime treated with copper napthenate, and an increase in lime treated with tributyltin oxide. Mean colony radii remain unaffected in response to leached blocks impregnated with copper chrome arsenic, zinc napthenate or pentachlorophenol. Differential

growth also continued to occur and remain unaffected in all treatments of pine; however, diminished responses were observed in lime blocks impregnated with copper or zinc napthenate, and an increase in oriented growth took place towards lime treated with pentachlorophenol or tributyltin oxide.

Phase data (Table 2.12.) indicate that C. globosum exposed to leached or unleached blocks, demonstrated remarkably consistent oriented responses towards all controls and the majority of preservative treatments. The response to tributyltin oxide was strongly oriented away when exposed to unleached blocks, but became erratic and variable in the presence of leached blocks. T. viride demonstrated directed hyphal extension towards most blocks except for pine and lime blocks impregnated with tributyltin oxide, where orientation was strongly directed away from the blocks.

Phase data for the two basidiomycetes indicate that oriented growth is generally away from both untreated and preservative treated blocks. The response was more marked in S. lacrymans which demonstrated consistent growth away from all lime blocks; for pine blocks growth was towards copper chrome arsenic treated blocks and random in three controls. C. puteana demonstrated more widely dispersed directional growth, but growth away from tributyltin oxide treated blocks was consistent with only minor angular deviation.

Values for delta are shown in Table 2.14.; values over 0·1 were considered to be significant, with a higher value indicating a greater magnitude of differential growth.

Table 2.10. Preservatives, solvents and concentrations of treating solutions.

Preservative		Solvent	Concentration (%W/W)
Copper napthenate	(CuN)	60-80 Petroleum Ether	5·00
Zinc napthenate	(ZnN)	60-80 Petroleum Ether	2·99
Tributyltin oxide	(TBTO)	60-80 Petroleum Ether	0·12
Pentachlorophenol	(PCP)	Acetone	0·13
Copper chrome arsenate	(CCA)	Water	2·50

Table 2.11. Mean colony radii of fungal colonies exposed to preservative treated oven dried lime and pine sapwood blocks and controls. Figures represent means of five replicates and their standard deviations.

Organism	Wood type	Colony radius (mm) with indicated preservative treatment							
		CuN	ZnN	TBTO	PCP	CCA	PE	ACET	Control
Chaetomium globosum 48 hrs.	Lime†	4·7±0·37	4·9±0·33	5·0±0·36	5·7±0·19	5·7±0·41	6·0±0·32	6·0±0·26	6·5±0·73
	Pine†	4·2±0·24	5·3±0·53	3·8±1·13	4·5±0·33	5·6±0·43	5·7±0·32	5·4±0·65	5·7±0·39
Chaetomium globosum 45 hrs.	Lime	5·9±0·34	5·5±0·14	3·8±0·22	5·2±0·24	6·0±0·44	5·6±0·24	6·1±0·24	6·2±0·38
	Pine	4·7±0·14	5·0±0·32	2·9±0·53	3·9±0·55	5·3±0·22	5·7±0·46	6·5±0·28	6·5±0·26
Serpula lacrymans 120 hrs.	Lime	4·9±0·23	5·3±0·45	3·3±0·69	5·2±0·41	5·2±0·90	4·9±1·1	5·9±0·72	5·7±0·43
	Pine	5·5±0·36	5·7±0·20	3·4±0·19	4·2±0·51	5·1±1·00	6·5±0·44	5·4±0·71	6·3±0·69
Coniophora puteana 105 hrs.	Lime	7·1±0·64	6·6±0·41	5·9±0·67	5·4±0·98	5·8±0·22	4·5±0·42	5·0±0·21	5·2±0·26
	Pine	7·3±0·66	6·5±0·69	6·7±0·98	5·5±0·85	5·7±0·28	5·1±0·26	5·1±0·14	5·2±0·18
Trichoderma viride 40 hrs.	Lime	15·8±0·67	16·8±0·61	8·5±0·55	14·7±0·51	15·6±0·84	15·8±0·53	16·7±0·82	16·5±2·07
	Pine	14·7±0·24	15·9±0·92	3·5±0·45	14·9±0·49	16·8±0·37	16·6±1·00	15·1±0·62	17·0±0·45

† leached blocks

Table 2.12. Mean differential growth values (∝) of fungal colonies to preservative treated oven dried lime and pine sapwood blocks. Figures represent means of five replicates and their standard deviations. Negative values indicate growth away from blocks.

Organism	Wood type	Value of ∝ (mm) with indicated preservative treatment							
		CuN	ZnN	TBTO	PCP	CCA	PE	ACET	Control
Chaetomium globosum	Lime†	1·5±0·34	1·0±0·40	0·4±0·35	2·7±0·43	2·3±0·58	2·9±0·28	2·9±0·26	2·7±0·47
	Pine†	1·0±0·21	0·5±0·48	-0·6±0·70	1·1±0·37	1·6±0·39	2·5±0·34	2·4±0·44	2·0±0·23
Chaetomium globosum	Lime	2·4±0·34	1·9±0·34	-0·5±0·39	1·4±0·30	2·9±0·69	2·3±0·16	2·6±0·41	2·7±0·42
	Pine	0·8±0·48	1·0±0·39	-0·3±0·40	0·6±0·44	2·0±0·10	2·4±0·55	2·5±0·17	2·6±0·29
Serpula lacrymans	Lime	-1·9±0·32	-2·2±0·25	-0·8±0·60	-0·8±0·30	-1·1±0·54	-1·2±0·45	-1·0±0·64	-2·5±0·51
	Pine	-1·9±0·34	-1·3±0·65	-1·2±0·35	-1·2±0·35	0·6±0·10	-0·4±0·41	-0·8±0·48	-0·8±0·46
Coniophora puteana	Lime	-0·5±0·28	-0·4±0·14	-1·2±0·19	-0·7±0·81	-0·4±0·28	0·8±0·77	-0·3±0·32	0·5±0·26
	Pine	-0·6±0·35	-0·7±0·32	-1·5±0·78	-0·9±1·03	0·4±0·22	-0·4±0·09	-0·1±0·23	-0·1±0·41
Trichoderma viride	Lime	1·1±0·30	0·1±0·65	-5·2±0·77	1·7±0·57	1·4±0·83	2·0±0·51	3·0±0·54	1·8±0·90
	Pine	2·1±0·31	1·2±0·37	-1·4±0·69	1·8±0·30	1·2±0·35	2·4±0·80	1·9±0·62	2·6±0·52

† leached blocks

Table 2.13. Phase data (β)‡ of fungal colonies exposed to preservative treated oven dried lime and pine sapwood blocks. Figures represent means of five replicates and their mean angular deviations.

Organism	Wood type	Value of β (°) with indicated preservative treatment							
		CuN	ZnN	TBTO	PCP	CCA	PE	ACET	Control
Chaetomium globosum	Lime†	12±8	7±4	14±57	2±4	3±3	2±6	0±0	1±5
	Pine†	11±8	31±28	-46±27	3±7	6±7	0±6	4±7	2±11
Chaetomium globosum	Lime	0±8	11±8	-4±23	2±8	4±8	3±8	2±8	0±8
	Pine	25±41	9±14	-15±8	6±24	10±8	0±8	1±8	4±8
Serpula lacrymans	Lime	-15±11	-1±0	-20±20	-1±28	-4±8	-10±28	-0±8	-1±8
	Pine	-8±11	-1±11	-5±8	-19±8	6±23	-25±54	-21±31	-3±43
Coniophora puteana	Lime	-12±27	-25±31	-8±11	-32±45	-20±51	28±40	-26±47	15±29
	Pine	-17±20	-10±28	-7±11	-21±47	33±32	-12±23	-35±56	16±77
Trichoderma viride	Lime	17±11	43±53	-3±8	5±8	13±18	7±18	0±8	13±16
	Pine	3±8	24±8	-4±8	7±11	25±11	8±14	9±11	0±8

† leached blocks

‡ expressed as the number of degrees the direction of differential growth varies from 0° (Y_3) when values are positive, and from 180° (Y_7) when values are negative.

Table 2.14. Mean values of delta (δ)‡ of fungal colonies to preservative treated oven dried lime and pine sapwood blocks. Figures represent means of five replicates. Negative values indicate growth away from blocks.

Organism	Wood type	Value of δ with indicated preservative treatment							
		CuN	ZnN	TBTO	PCP	CCA	PE	ACET	Control
Chaetomium globosum	Lime†	0·33	0·21	0·08	0·46	0·41	0·48	0·48	0·42
	Pine†	0·24	0·10	-0·15	0·24	0·28	0·44	0·43	0·35
Chaetomium globosum	Lime	0·41	0·35	-0·14	0·28	0·48	0·40	0·42	0·44
	Pine	0·16	0·19	-0·09	0·15	0·38	0·43	0·39	0·40
Serpula lacrymans	Lime	-0·38	-0·41	-0·24	-0·15	-0·22	-0·24	-0·18	-0·44
	Pine	-0·34	-0·22	-0·35	-0·28	0·11	-0·06	-0·13	-0·11
Coniophora puteana	Lime	-0·07	-0·05	-0·21	-0·13	-0·05	0·19	-0·06	0·09
	Pine	-0·08	-0·10	-0·23	-0·17	0·05	-0·07	-0·03	0·02
Trichoderma viride	Lime	0·07	0·00	-0·62	0·12	0·09	0·13	0·18	0·11
	Pine	0·15	0·08	-0·41	0·12	0·07	0·14	0·13	0·16

† leached blocks

‡ for definition see text

2.2.4. Discussion

As with the investigation on untreated blocks, great replication was involved in experiments using treated material, with over 3500 measurements taken, obviously necessitating data analysis by computer. The results showed that even when preservatives were present the chemostimulatory nature of wood volatiles on early growth patterns of fungi were not masked, and though the biocides used are very effective, especially out of ground contact, hyphal extension patterns of the fungi *C. globosum* and *T. viride* were still stimulated and those of *S. lacrymans* continued to demonstrate directional responses.

Growth, however, of all organisms was severely limited in the presence of wood blocks impregnated with tributlytin oxide and aerial hyphal development was restricted to those sides of inocula furthest from baits. In many instances no growth at all occurred, and in those in which growth was initiated, hyphae were short and exuded small liquid droplets. Preliminary studies with *C. globosum* indicated that this fungus demonstrated the most marked positive chemotropic responses. In the presence of both fresh and aged creosoted material, however, hyphal extension was severely limited and only minimal growth took place even over extended time periods. Similar preliminary studies with *S. lacrymans* showed similar results, thus further investigations with creosoted materials were not undertaken.

Aqueous extraction of blocks prior to fungal exposure did not significantly modify growth responses of *C. globosum* to either preserved or unpreserved wood. Copper

chrome arsenic, widely used in ground contact and associated in such situation with failure due to soft rot fungi, had no significant inhibitory effects on directional development of fungal hyphae.

The results presented in this chapter show that most preservatives, even in leached wood, do not mask its chemostimulatory nature and section 2.1. demonstrated that the effects volatiles evaporating from wood have on the growth patterns of some wood decay fungi is to elicit preferential growth by organisms towards sources of wood volatiles. Recognition of tropic responses to even preserved wood may, therefore, increase our understanding of the performance of this material in soil contact.

Preservatives are normally thought to act as toxicants to soil microflora and organisms located by random coincidence at the wood - soil interface of newly treated wood in soil would usually be considered to be killed as a consequence of their proximity. Nilsson (1982), however, has shown that viable organisms may be found even in heavily preserved material.

Examination of soil adjacent to decomposing stakes both in the laboratory and in the field, indicates that nitrogen accumulation in the soil contiguous with the wood takes place (King et. al., 1981). It was also noted by these authors that soluble nutrients present in wood stimulated aggregate formation of the soil, indicative of high microbial biomass content, and also accelerated nitrogen transfer even to preserved wood. It is probable, therefore, that microbial

populations in soil surrounding wood is also influenced by such volatiles, and that these influences may not be confined to those microbial populations located at the immediate wood - soil interfaces. Furthermore, soluble nutrients may initiate stasis release of the soil microflora and upon germination of fungal spores volatiles may bring about oriented hyphal extension of germ tubes towards wood.

Soil has a large but relatively inactive microbial population, the activity level being dependent on variable chemical, physical and biological parameters such as moisture content, temperature, exogenous or constitutive dormancy and nutrient status. Furthermore, soil microorganisms are generally is a state of stasis with uncertain inherent soil factors inhibiting and retarding both vegetative hyphal growth and spore germination. Release from stasis only occurs when environmental conditions are favourable for growth and reproduction. Factors moderating stasis release are as yet unclear, but Lynch (1982) implicated nutrient availability as being of some importance in this function. Hardie (1979) showed that spores of C. globosum are stimulated to germinate by aqueous extracts of wood, and Butcher & Nilsson (1982) considered that copper chrome arsenic might not only act as a biocide, but might also preserve wood by masking initiation sites for soft rot cavity formation. Thus decay, but not the growth of fungi in wood, might be inhibited by its use. The possibility therefore exists that when wood is emplaced in soil it may influence soil microflora at some distance into the soil from the wood - soil interface, either by leaching of

soluble materials, e.g. redistributed soluble nutrients, or by volatile emissions.

Preservatives which act only as toxicants may fail to prevent early invasion of wood by microorganisms, as demonstrated by King et. al. (1981), although such organisms may be killed and fail to further colonise the wood. If wood continues to act as a chemostimulant even when preserved, a continual input of microbial biomass would be attracted, and such a mechanism could explain the increased nitrogen noted by King et. al. (1980), that occurs during decay processes of preserved and unpreserved wood in soil. Death and lysis of microbial biomass at wood - soil interfaces may not only result in a build up of nutrient resources, but also either solubilise preservative elements which can then be leached from the system, or immobilise such elements by complexing, thus effectively reducing toxicant availability.

Preservative formulations which inhibit microbial responses to wood, either by masking the chemostimulatory nature of the material or by acting as a repellant against soil microflora, could therefore be important in delaying decomposition processes. Preliminary experiments undertaken to design methods to test chemotropic responses of fungi to wood impregnated with preservatives demonstrated the effectiveness of creosote to act as a growth inhibitor, even 20 year old material effectively prevented hyphal development of C. globosum up to distances of 25mm. Creosote may be particularly effective in this respect, as during volatilisation of some of its lighter constituents, non-volatile toxic materials are left

in the wood; however, the development of preservative formulations which mask the invasive process postulated by King et. al. (1981 op. cit.) provides a possible solution to soft rot failure of wood in soil.

2.3. General conclusions

1) It has been established that some fungi may respond chemotropically to wood volatiles by demonstrating directional growth.
2) A method has been devised to allow quantitative measurement and statistical analysis of these responses to be undertaken.
3) Results of such analyses show that the nature and degree to which tropic responses are demonstrated may be influenced by:-
    (a) species of wood;
    (b) whether the wood is fresh or dried - heat dried wood elicits greater tropic responses than fresh wood, even when heat dried at only 40°C.
4) Tropic responses by C. globosum may be observed at distances up to 35mm from blocks.
5) Responsive fungi maintain their response to wood impregnated with the preservatives copper and zinc napthenate, pentachlorophenol and copper chrome arsenic.
6) The response is severely disrupted by preservatives tributyltin oxide and creosote.
7) Leaching of blocks prior to exposure does not interfere with tropic responses.

Chapter 3.

Bacterial chemotaxis.

3. Bacterial chemotaxis

3.1. Introduction

Soil inhabiting bacteria are found in all major terrestrial ecosystems. Numbers of bacteria in soil generally range from 7×10^6/g in dry tundra soils to 5×10^7 cells/g in tropical forest or savannah soils (Swift et. al., 1979). Numbers vary markedly, however, between horizons within the same soil with the rich organic upper horizons having considerably greater numbers of bacteria than lower mineral horizons, e.g. 3×10^7 in litter down to 3×10^5 1 metre below the soil surface (Campbell, 1977). Bacteria may contribute up to 97% of microbial numbers isolated from tundra and taiga soils, but only 42% in dry prairie soils (Hattori, 1973). Because of their small size, usually less than 5μ, they rarely contribute to more than 50% of microbial biomass in any soil (Alexander, 1977; Campbell, 1977). Exceptions may occur in special situations, such as waterlogging, which favour the development of bacteria with the biochemical apparatus to deal with low oxygen concentrations, or which can derive energy via pathways which do not require oxygen. These organisms proliferate and dominate total counts and biomass calculations under such conditions.

Many environmental factors influence bacterial numbers in soils, and parameters such as pH, temperature, moisture content and nutrient availability initiate changes in numbers as these vary. Environmental factors are inter-related,

however, and variation in one may induce complex patterns of change in the others, though some simple generalisations are possible. The effect of decreasing pH causes a dramatic drop in numbers of bacterial cells, and a concurrent increase in fungal biomass. Bacterial growth is favoured at pH values of 6-7, whereas fungi have growth optima at values below 5. Furthermore, changes in pH not only effect microorganisms directly, but also induce changes in nutrient availability, leaching etc. within the soil.

Moisture contents of soils are critical in determining microbial growth, and all soil inhabiting bacteria may be considered as aquatic, living in water films which surround soil particles. Moisture contents of soils must be above certain minimal values for successful bacterial vegetative growth, and below this value water becomes unavailable due to the strong affinity water molecules have for clay and organic particles. Water availability is also strongly influenced by soil particle size, clay soils retain much larger quantities of moisture than soils of larger particle size such as loams. Bacteria in soil are not uniformly distributed, but restricted to microcolonies on particle surfaces, particularly ones of organic origin. Motile bacteria obviously require a continuous water film to transfer from one, possibly nutrient depleted, microsite to another, and are dependent on the presence of free water for this transfer. Water itself is not toxic, but an overabundance in soil will cause a displacement of air, lowering the amount of available oxygen, sometimes to a point where only anaerobic

respiration or fermentation can take place. Aerobic bacteria proliferate in soils at moisture contents of 75% of the soil moisture holding capacity. Temporary flooding of a well drained soil may have only minimal effects on the soil microflora; however, prolonged waterlogging has a major impact on numbers and type of microorganisms present.

Biochemically mediated chemical reactions are favoured by temperatures between 25°C - 35°C and in temperate regions most soil inhabiting bacteria are mesophiles, although psychrophilic and thermophilic bacteria may predominate in arctic tundra and tropical soils (Alexander, 1977). The main effect of increased temperature is that the rate of substrate utilisation is enhanced by the increased energy within the system; however, above certain temperatures problems occur because of thermal denaturation of extracellular enzymes and substrate turnover will decrease.

The nutrient status of soil has a major influence on microbial numbers, and there is a strong correlation between bacterial numbers and organic content of the soil. Campbell (1977), states that in soils in which organic particles contributed to only 15% of the total particle numbers, more than 50% of bacteria present were associated with the organic fraction. Generally, however, soil may be considered to be a nutrient poor medium and soil microorganisms in a low state of activity. Only when the soil is amended with fresh substrate, especially if soluble, will the activity levels increase markedly. Increased activity is in part due to enhanced metabolic rates of autochthonous flora, and in part to a flush

of activity caused by stasis released in zymogenous flora, bacteria being present in both groups. In natural temperate terrestrial ecosystems many of the environmental factors discussed have seasonal variability, e.g. temperature and input of organic material, therefore populations of microorganisms in such soils have developed to survive wide fluctuations in the physiochemical environment, and are able to invade, colonise and exploit the abundance of organic material which arrives during times of deciduous leaf fall.

Amongst the primary colonisers of plant debris in soil bacteria are an important group, and of those groups of bacteria which are most frequently isolated from soil actinomycetes and coryneforms (including Arthrobacter), spore forming bacilli and pseudomonads account for 90% of total numbers (Campbell, 1977). Gray et. al. (1974), showed that some of these groups are major isolates during ash and oak leaf litter decomposition studies. Of bacterial biomass present in ash litter, 38·6% was attributed to the genus Arthrobacter, 11·5% to Bacillus and 30·2% to Pseudomonas; i.e. 80·3% of the total biomass. For oak litter, 6·5% was attributed to Arthrobacter, 20·6% to Bacillus and 30·6% to Pseudomonas, with 28·2% Enterobacteriaceae also present. It can be seen from these results that in terms of numbers and biomass, bacteria isolated from soil and deciduous litter are restricted to a few groups.

Another area in soil in which there are large numbers of bacteria is the rhizosphere, i.e. that volume of soil influenced by the presence of growing plant roots. In the

rhizosphere bacterial numbers may reach figures of 10^9 - 10^{10} cells/g of dry soil (Campbell, 1977), whereas fungi tend to show no appreciable increase, at least when measured by isolation, apart from a very few restricted genera, e.g. mycorrhizal fungi. Zoospores of several genera, however, are strongly attracted to roots. Motile spores of such genera show movement to the rhizosphere region in response to chemical stimulation (Alexander, 1977).

Not all bacteria make the transition from the soil to the rhizosphere, and there are qualitative as well as quantitative differences between soil and rhizosphere microbial populations. It is possible that after coincidence of growing root with bacterial colonies in soil those bacteria, better adapted to the rhizosphere environment, proliferate at the expense of less well adapted organisms, leading to marked variations in soil and rhizosphere bacterial populations. Plant roots, especially at the growing tip and zone of elongation, exude a wide variety of soluble organic molecules, including all the naturally occurring amino acids and a variety of monosaccharides, organic acids and vitamins. Root exudates of several plants initiate positive chemotactic behaviour in Rhizobium (Gitte , Rai & Patil, 1978; Currier & Strobel, 1976), as well as individual amino acids and carbohydrates (Gotz et. al., 1982).

Chemotaxis, the process by which motile bacteria swim from low to high concentrations of an attractant, or vice versa for a repellent, has been demonstrated qualitatively since Pfeffers experiments in 1880's (Pfeffer, 1884). More

recently the development of a capillary tube method has enabled bacterial chemotaxis to be quantified (Adler, 1973). Both negative and positive responses have been investigated, and a wide range of bacterial taxonomic groupings have been shown to demonstrate directional responses, including Gram negative facultative anaerobic rods, e.g. Escherichia coli (Adler, 1973), Salmonella typhimurium (Adler, 1975); Gram negative aerobic rods, e.g. Pseudomonas (Lynch, 1980; Moench & Konetzka, 1978; Moulton & Montie, 1979), Rhizobium (Bowra & Dilworth, 1981) and Gram positive andospore forming rods, Bacillus subtilis (Ordal & Gibson, 1977; Ordal et. al., 1977; Van der Drift & de Jong, 1974). It is most interesting that bacteria which demonstrate most marked increases in the rhizosphere (Alexander, 1977), are all flagellate organisms, i.e. Pseudomonas spp., Flavobacterium spp., Alcaligenes spp., Agrobacterium spp. and Rhizobium spp.

The importance of bacteria as primary colonisers of both preserved and unpreserved wood in soil is also well recognised (Clubbe & Levy, 1982), although there are few quantitative studies of their involvement in the decay process. Bacteria from several genera are known to attack wood as a nutrient resource (Levy, 1967; Greaves, 1968), with Bacillus and Pseudomonas being especially important in these events. Greaves (1971) grouped bacteria which are isolated from wood into four categories determined by their functions once invasion of the resource had occurred. Those of group 1 functioned to increase the permeability of the wood to liquid penetration; those of group 2 had the capability to utilise cell wall, thereby reducing the strength of the wood. Bacteria

of group 3 were those which in association with other wood decay microorganisms could cause total wood decomposition, and those of group 4 were inactive in wood decay, but by their presence could influence other wood decay microorganisms by production of antagonistic chemicals inhibitory to microbial function and growth. Greaves (1971, op. cit.) also noted that bacteria and actinomycetes were probably the microorganisms most abundant in wood during decay, and Baecker & King (1981) showed that actinomycetes can occur in large numbers in decaying lime and pine. Importantly, both Greaves (1971, op. cit.) and Gray et. al. (1974) noted that for wood and leaf litter respectively, invasion and colonisation in soil by bacteria was very rapid, and Gray noted that tenfold increase in bacterial numbers occurred in the first three days of ash litter decomposition.

Wood also contains a significant quantity of soluble organic nutrients, which are concentrated at surface during drying, and which have been shown to move to soil when wood is emplaced therein (King, 1975). Subsequent analyses of these soluble nutrients have demonstrated the presence of many amino acids and simple sugars as major components (Nayagam, pers. comm.). It was therefore hypothesised that soil at the wood - soil interface was similar nutritionally to soil adjacent to the rhizosphere and that the wood input to soil was comparable to plant root exudate at least in the short term.

Bacteria found in wood and decomposing litter are taxonomically similar to those found in the rhizosphere. In view of the tactic mechanisms demonstrated by some rhizosphere

organisms (Currier & Strobel, 1977), the tropic responses by fungi to wood described in Chapter 2, and the positive responses shown by common soil bacteria to amino acids and simple sugars, it was decided that the influence of soluble nutrients in wood on tactic behaviour of wood inhabiting bacteria should be investigated.

Tactic responses by wood inhabiting bacteria have not been investigated to date, nor have their implications for the role of bacteria in wood decay been established. It was therefore decided that individual components of soluble nutrients in wood, as well as undifferentiated water soluble wood extract, should be tested to determine their effect on directional responses of motile bacteria representative of those found in wood during decomposition.

3.2. Materials and methods

3.2.1. Organisms

Bacteria used in this study were Bacillus subtilis (NCIB 11034), Bacillus polymyxa (NCIB 4747), Pseudomonas aeruginosa (NCIB 10545) and Agrobacterium radiobacter (NCIB 9043). Stock cultures of each bacterium were maintained on tryptone agar (Oxoid), except A. radiobacter which was cultured on nutrient agar (Oxoid), and subcultured every 7 days.

3.2.2. Growth and preparation of cells

Two methods of cell preparation were used; for *B. subtilis* and *B. polymyxa* the method was essentially as described by Ordal & Goldman (1975), and for *P. aeruginosa* and *A. radiobacter* the method was as employed by Moulton & Montie (1979). All media, buffers, solutions etc. were prepared with double glass distilled water.

3.2.3. Methods

1. (*B. subtilis*, *B. polymyxa*)
   $50cm^3$ of growth medium, consisting of 1% tryptone 0·5% NaCl containing 0·14mM $CaCl_2$, 0·20mM $MgCl_2$ and 0·01mM $MnCl_2$, in $200cm^3$ Erlenmeyer flask was inoculated with bacteria and incubated overnight on a rotary shaker at 150 r.p.m. and 30°C. $1cm^3$ of the bacterial suspension was transferred to $50cm^3$ of mineral salts medium, at pH7, consisting of 0·025M KH_2PO_4, 0·025M K_2HPO_4, 0·12mM $MgCl_2$, 1·0mM $(NH_4)_2SO_4$, 0·14mM $CaCl_2$, 0·01mM $MnCl_2$ and 0·02M sorbitol† in a $200cm^3$ Erlenmeyer flask. The flask was incubated at 37°C for 3 hours with rotary shaking (150 r.p.m.); 15 minutes prior to the removal of the flask the solution was made 5mM in sodium lactate and 0·05% in glycerol.
   The suspension of bacteria in mineral salts medium was centrifuged at x3400g for 10 minutes at room temperature. The pellet of bacteria was resuspended in $10cm^3$ of chemotaxis buffer,

† plus $1\mu g/cm^3$ biotin for *B. polymyxa*.

consisting of 0·005M K_2HPO_4, 0·005M KH_2PO_4, 0·12mM $CaCl_2$, 0·3mM $(NH_4)_2SO_4$, 0·1mM EDTA, 5mM sodium lactate and 0·05% glycerol and centrifuged at x3400g for 10 minutes at room temperature. The bacteria were washed twice as described before finally being resuspended in chemotaxis buffer, at a concentration of 1 x 10^6 cells/cm^3. Microscopic observation indicated that approximately 80-90% of cells were motile after this procedure and the final diluted suspension (1 x 10^6 cells/cm^3) used for the chemotaxis assay.

2. (P. aeruginosa, A. radiobacter)
$50cm^3$ of growth medium, consisting of 1% NaCl, 1% tryptone and 0·5% yeast extract, in $200cm^3$ Erlenmeyer flask was inoculated with bacteria and incubated overnight at 35°C on a rotary shaker at 150 r.p.m. $1cm^3$ of the bacterial suspension was transferred to $50cm^3$ of mineral salts medium, consisting of per litre, 7·0 K_2HPO_4, 3·0 KH_2PO_4, 1·0g $(NH_4)_2SO_4$, 0·05g $MgSO_4\ 7H_2O$, 2·5mg $FeCl_3 \cdot 6H_2O$ and 4·0g sodium succinate. This was incubated at 35°C on a rotary shaker at 150 r.p.m. for 3 hours. The bacterial suspension in mineral salts medium was centrifuged at x8000g for 10 minutes at 4°C and washed twice with chemotaxis buffer, pH7, 4°C, consisting of 0·025M K_2HPO_4, 0·005M $MgCl_2 \cdot 6H_2O$ and 0·1mM disodium ethylenediaminetetraacetic acid

(EDTA). Bacteria were finally resuspended in chemotaxis buffer at 30°C to a concentration of 1×10^6 cells/cm^3.

3.2.4. Preparation of wood extracts

Sapwood blocks of lime (Tilia vulgaris Hayne), pine (Pinus sylvestris L.), beech (Fagus sylvatica L.) and eucalyptus (Eucalyptus regnans Muell.), previously dried at 40°C and stored in ambient room conditions for periods of not less than three months, had outer surfaces removed to a depth of 5mm. 10g samples of each were cut into approximately 100mg pieces and placed in 1 litre glass beakers with 450cm^3 of cold sterile glass distilled water on magnetic stirrers for 12 hours. Wood pieces were removed and extracts filtered twice through prewashed Whatmans No.1 filter paper and twice through prewashed membrane filters (Whatmans 0·45μ) to remove wood debris. Extracts were divided into halves, each half made up to 250cm^3 with either constituents of chemotaxis buffer for method 1 or constituents of chemotaxis buffer for method 2. Wood extract solutions in chemotaxis buffer were sterilised by membrane filtration (Whatmans 0·45μ) and stored at 2°C in sterile, sealed glass flasks until use.

3.2.5. Preparation of glucose, amino acid and ammonium solutions

10^{-1}M solutions of D-glucose, L-arginine monohydroxychloride and the monosodium salt of L-glutamic acid

in appropriate chemotaxis buffer were sterilised by membrane filtration (Whatmans 0·45), and stored at $2^{o}C$ in sterile, sealed glass flasks until use. A $10^{-1}M$ solution of NH_4Cl was adjusted to pH7 by addition of 2M NH_4OH solution, and the solution sterilised by membrane filtration prior to use.

3.2.6. Chemotaxis assay

Capillary tube assays were performed essentially as described by Adler (1973). Each bacterial chamber was constructed by sealing the end of a 5cm long melting point capillary tube, prior to bending into a U shape; the U tube was then layed onto the surface of a glass slide, and a glass cover slip placed on top (Fig. 3.1.). Such chambers were filled with $0·3cm^3$ of bacterial suspension in chemotaxis buffer at a concentration of 1×10^6 cells/cm^3.

The capillary tubes for containing the attractant (1 µl disposable micropipettes. Intra END Balubrand) were soaked in sterile double glass distilled water overnight and rinsed prior to use. Small volumes of wood extract in chemotaxis buffer, individual attractant in chemotaxis buffer and chemotaxis buffer alone were placed in $50cm^3$ sterile beakers. Washed tubes, heat sealed at one end and handled with forceps at all times, were placed with their open ends immersed in the sterile solutions in the beakers. These were then placed in a vacuum chamber which was evacuated for approximately 5 seconds, so that on release of the vacuum the tubes were about two thirds full.

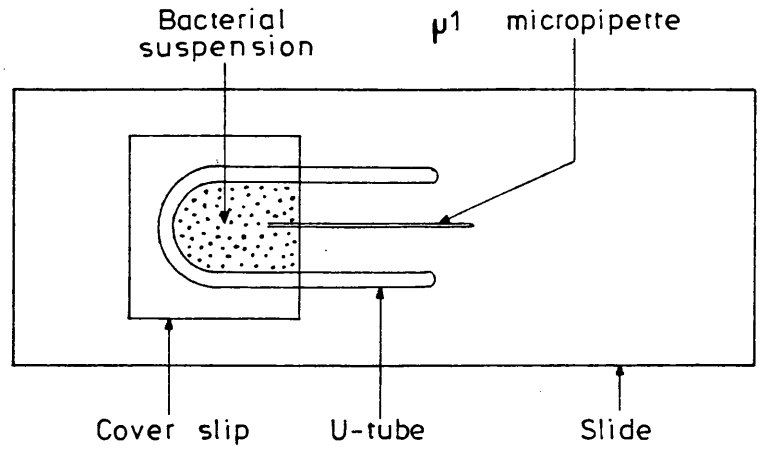


Fig. 3.1. Construction of bacterial chamber used in chemotaxis assays showing position of micropipette containing attractant.

Capillary tubes were removed from the beakers and their exteriors rinsed in a stream of double glass distilled water. A single capillary tube was placed on each bacterial chamber with its open end just inserted into the bacterial suspension. Chambers were placed on a preheated slide warmer at $30^{\circ} \pm 2^{\circ}C$ and incubated for 30 minutes; the warmer was then enclosed in a box to exclude light.

Triplicate assays for each wood extract, individual attractant and control were carried out with each bacterial species. Capillaries were removed, rinsed externally in a stream of sterile distilled water, the sealed end broken off and the contents ejected into $10cm^3$ tryptone broth at $2^{\circ}C$. Duplicate serial dilutions were carried out for each capillary in tryptone broth at $2^{\circ}C$, down to a 10^{-3} dilution. $1cm^3$ of each dilution was mixed with $2cm^3$ soft tryptone agar at $47^{\circ}C$ and poured over plates of solid growth media. Plates were

incubated at 30°C, and growth monitored. Counts were undertaken after 24-48 hours on plates containing between 30-300 colonies, except in the case of a small number of control plates, in which so few bacterial cells entered the capillary that only 3-4 colonies developed, even at the 10^{-1} dilution.

3.3. Results

Numbers of bacteria which entered capillaries with and without attractants are shown in Table 3.1. Values are means of six counts derived from duplicate serial dilutions of triplicate determinations, and are shown with their standard deviations, which indicate that the data are statistically meaningful. The table also shows that for each bacterial species variations occur in numbers of cells which accumulate in control capillaries between experiments. Such variations due to minor day to day differences in bacterial motility and physiological status makes comparisons between treatments and tests undertaken on different days difficult to assess. To overcome this problem, the results are expressed as ratios of numbers of bacteria which accumulate in capillaries containing attractants to those numbers which accumulate in controls (Table 3.2.).

Results presented in Table 3.2. clearly indicate that with the exception of B. subtilis, which did not demonstrate tactic behaviour to glucose at 10^{-1}M concentration, all bacteria responded chemotactically to all four wood extracts and individual nutrients in solution. Differences existed, however,

both in magnitude of responses demonstrated by the same organism to different test substances, and between different bacteria to the same substance. Maximum responses were shown to wood extracts by B. subtilis, B. polymyxa and A. radiobacter, whereas P. aeruginosa showed greatest tactic responses to amino acids and ammonium ions.

B. subtilis demonstrated a variable response to the different wood extracts, ranging from 5·9:1 for beech extract, 11·0:1 for eucalypt extract, 15·8:1 for pine extract to 20·3:1 for lime extract, the response to lime extract by B. subtilis being the highest response ratio recorded during the investigation. B. polymyxa showed similar response ratios of approximately 10·5:1 for all the wood extracts. Both bacilli responded more or less equally to different amino acids; B. subtilis had a response ratio of 6·6:1 for glutamic acid, and B. polymyxa a ratio of 6·5:1 for arginine.

A. radiobacter was tested only against pine and eucalypt extracts. In the presence of eucalypt extract the response ratio was 7·7:1, whereas only a limited response of 3·0:1 was shown towards pine extract. A. radiobacter, however, showed a response ratio of 6·5:1 to arginine, which was comparable to that for B. polymyxa to the same amino acid.

P. aeruginosa showed relatively consistent response ratios of approximately 6:1 to wood extracts, whereas responses to amino acids were as high as 17·9:1 for arginine, and 9·8:1 for glutamic acid. P. aeruginosa also demonstrated positive tactic responses to ammonium ions, the response ratio of 9·8:1 being comparable with that for glutamic acid.

Table 3.1. Numbers of bacteria in capillaries containing test solutions and controls. Figures represent means of three replicates and their standard deviations.

Test solution	Numbers of bacteria in capillaries ($\times 10^3$)							
	B subtilis		B polymyxa		P aeruginosa		A radiobacter	
	Solution	Control	Solution	Control	Solution	Control	Solution	Control
Lime extract	130·0± 41	6·5± 1·0	3·3±0·2	0·3±0·15	132·0±24	24·5±9·3	N.D.	
Pine extract	20·5±4·0	1·3±0·8	15·6±1·6	1·3±0·3	138·0±20	24·5±9·3	4·0±0·1	1·4±0·05
Beech extract	16·0±4·7	2·7±1·7	2·7±0·7	0·28±0·15	179·0±18·5	29·7±3·8	N.D.	
Eucalypt extract	3·5±0·9	0·3±0·13	11·7±1·6	1·3±0·3	180·3±53·7	29·7±3·8	9·5±0·48	1·4±0·05
Arginine	N.D.		27·6±4·6	4·3±0·2	71·3±26·9	4·0±0·71	10·5±0·5	1·6±0·28
Glutamic acid	11·8 2·6	1·8±1·2	N.D.		39·3 20·6	4·0 0·71	N.D.	
Glucose	1·7±0·4	1·8±1·2	13·9±0·8	4·3±0·2	14·5±0·2	5·2±1·1	5·5±0·48	1·6±0·28
NH_3/NH_4^+	N.D.		N.D.		39·3±18·2	4·0±0·71	N.D.	

N.D. not determined

Table 3.2. Ratios of numbers of bacteria in capillaries containing test solutions relative to numbers of bacteria in control capillaries.

Test solution	B subtilis	B polymyxa	P aeruginosa	A radiobacter
Lime extract	20·3:1	11·6:1	5·5:1	N.D.
Pine extract	15·8:1	11·8:1	5·7:1	3·0:1
Beech extract	5·9:1	9·5:1	6·0:1	N.D.
Eucalypt extract	11·0:1	8·8:1	6·1:1	7·1:1
Arginine	N.D.	6·5:1	17·9:1	6·5:1
Glutamic acid	6·6:1	N.D.	9·8:1	N.D.
Glucose	0·9:1	3·3:1	3·6:1	3·4:1
NH_3/NH_4^+	N.D.	N.D.	9·8:1	N.D.

N.D. not determined

Table 3.3. Ratio of numbers of bacteria which accumulated in capillaries containing amino acid and ammonium attractants to numbers in capillaries containing glucose

	B. subtilis	B. polymyxa	P. aeruginosa	A. radiobacter
Arginine	—	2·0 : 1	5·0 : 1	1·9 : 1
Glutamic acid	7·3 : 1†	—	2·7 : 1	—
Ammonium	—	—	2·7 : 1	—

† no response by this bacteria to glucose

Apart from B. subtilis, which did not respond to the presence of glucose, the three other bacteria demonstrated consistent, if somewhat limited, responses to the carbohydrate. The responses were above 2:1 in each case, and can therefore be considered significant with B. polymyxa, P. aeruginosa and A. radiobacter demonstrating responses of 3·3:1, 3·6:1 and 3·4:1 respectively.

3.4. Discussion

Prior to experiments described in this chapter, extensive preliminary work was undertaken to determine if bacteria which had been previously tested for chemotactic responses to both nutrient and toxic materials by other workers could be shown to respond to wood extract. For this purpose Escherichia coli (NCIB 9527) was chosen as its chemotactic responses have been studied in great detail, and its motility and nutritional requirements were well understood (Adler, 1966; Adler & Dahl, 1967; Adler & Templeton, 1967; Adler, 1969; Adler, 1973; Adler, Hazelbauer & Dahl, 1973; Tso & Adler, 1974).

This preliminary work was undertaken with water soluble extracts of lime (Tilia vulgaris Hayne), as this wood had been shown to be highly decay susceptible in soil during previous work at Dundee College of Technology, especially when containing soluble surface nutrients. Such surface nutrients when extracted from lime have been shown to contain a wide range of water soluble nutrients, e.g. glucose and arginine (Nayagam, pers. comm.). Initially E. coli was also tested

with the amino acid serine and a simple sugar, galactose, to determine the degree of its chemotactic response. These compounds had been shown by Adler (1969) to elicit marked responses from this organism. It was obvious that if the organism failed to respond to water soluble wood extracts, but was attracted to serine and galactose, it could be assumed that such wood extracts did not act as attractants. The results of this initial investigation showed that E. coli was strongly attracted to both water soluble extracts of lime and to solutions of both serine and galactose, and that further work on bacterial directional responses to wood extracts should be undertaken.

E. coli is not normally associated with wood or soil habitats unless as a contaminant, and media developed for E. coli are not necessarily suitable for classes of bacteria more usually found in such environments. It was established from the literature that Bacillus subtilis, Bacillus polymyxa, Pseudomonas aeruginosa and Agrobacterium spp. are commonly found in wood (DeGroot & Johnson, 1976), and it was considered that these representatives of Gram positive and Gram negative motile organisms might be useful candidates for chemotactic studies. Further, a strain of Bacillus subtilis (NCIB 11034 strain B) was available from the National Collection of Industrial Bacteria, which had been isolated from water stored Sitka spruce (Ward & Fogarty, 1972). Aquatic environments are essential for eliciting chemotactic responses, and wood stored in such environments might well be considered to be, at least partially, selective for chemotactic bacteria.

The composition of media for bacterial growth and

maintenance prior to chemotaxis assays are defined by the need to induce and sustain very high levels of motility, and as such they may be highly specific to individual organisms, or even strains. Such media may be complex, and should not contain materials likely to elicit chemotactic responses. Whereas B. subtilis and P. aeruginosa had been studied in relation to chemotaxis, no information was available for B. polymyxa or A. radiobacter. It was decided, therefore, because of the similarities between cell walls of these organisms, to apply the methods developed for the Gram positive B. subtilis to B. polymyxa, and that for the Gram negative P. aeruginosa to A. radiobacter. B. polymyxa, however, is auxotrophic for biotin, and it was necessary to amend minimal growth media with this vitamin at a concentration of $1\mu g/cm^3$. Apart from this, the media and buffers developed by Ordal & Goldman (1975) for B. subtilis was used for B. polymyxa. Similarly, the media described by Moulton & Montie (1979) for P. aeruginosa were also used for A. radiobacter.

Microscopic evaluation showed that strict adherence to the methods in the literature worked well for the bacilli. Both showed over 90% of cells were motile in chemotaxis buffer, such motility levels being maintained for at least 60 minutes, a duration twice as long as required for the chemotaxis assay. The method developed for the P. aeruginosa strain used by Moulton & Montie (1979 op. cit.) also worked well on the P. aeruginosa strain used in this investigation, with 90% of the cells being motile for at least 60 minutes. The method did not transfer quite as successfully to A. radiobacter, and

the maximum motility achieved was 70% of the cells for 60 minutes. It was considered, however, that this degree of motility was adequate for the chemotaxis assay.

As already stated, interpretation of results is difficult, due to day to day variations in numbers of bacteria in control tubes. Such fluctuations are a normal feature in chemotaxis assays, and Adler (1973) obtained a standard deviation of 17% when testing *E. coli* against 10^{-2}M L-glutamate over a period of 25 days, with absolute numbers ranging from $2{\cdot}5 \times 10^5$ to $5{\cdot}5 \times 10^5$ per capillary. To overcome this problem, Gotz *et. al.* (1982) introduced a 'standard cell count' in order to correct values obtained on a day to day basis. The standard was determined as the mean number of cells of *Rhizobium* which entered capillaries containing 10^{-3}M proline and determined as 3×10^4 bacteria. During each test a proline standard was also undertaken and cell numbers in other capillaries normalised on the basis of this result. Moulton & Montie (1979) normalised day to day variations in counts by expressing the results as a ratio of the numbers accumulated in attractant capillaries to those in the controls. This relative response was considered to be significant at a value of 2·0 or greater, and was the method adopted in this investigation. Such response ratios remained relatively constant in preliminary work prior to this investigation, using both *E. coli* and *B. subtilis*, responses in both cases being greater than 2·0:1. When results of experiments described in this chapter are examined, it is apparent that there are clear cut differences in the magnitude of responses demonstrated by different

bacteria to the same wood extract, e.g. B. subtilis showed a response of 20·3:1 to lime extract, whereas the response of P. aeruginosa was 5·5:1, or individual nutrient, and by the same bacterium to different test substances. Generally, however, maximum responses were shown to wood extracts with the exception of P. aeruginosa, which demonstrated greatest responses to individual amino acids.

The differences in magnitude of responses between wood extracts and individual nutrients is difficult to assess because of the undefined nature, in terms of content and concentration, of the wood extracts. Nayagam op. cit. showed that 1% of wood mass may exist as water soluble carbohydrates, with smaller proportions of amino acids and other undefined materials. Assuming a mean molecular mass of carbohydrates and other non-specific nutrients to be 200, then the solutions of wood extracts used in these investigations would have had a concentration of approximately 10^{-3}M of such soluble nutrients which would have stimulated very significant chemotactic responses.

The concentration chosen for the individual nutrients (10^{-1}M) was one commonly found by other workers to elicit maximum responses from bacteria to a wide variety of amino acids and sugars. This is, however, a general statement, and variation exists between bacterial species and their responses to differences of concentration in individual components. Although peak responses occur at concentrations of individual compounds between 10^{-3}M - 10^{-1}M, bacteria are very sensitive to even very dilute concentrations, and chemotaxis is demonstrated by some bacteria at 10^{-7}M concentrations of

some amino acids. Decreasing the concentration of the attractant decreases the response, until levels of nutrient concentration are so low that no chemotactic responses are demonstrated.

The greater response by all four bacteria to amino acids rather than the glucose is similar to that found in E. coli, which also responded more positively to amino acids rather than the sugars (Mesibov & Adler, 1972). Gitte et. al. (1978) demonstrated that Rhizobium strains tested also showed markedly greater responses to amino acids rather than sugars. Table 3.3. shows responses by P. aeruginosa and B. polymyxa to amino compounds at 10^{-1}M to be significantly greater than the response to glucose, also at 10^{-1}M. The response by A. radiobacter is also greater to the amino acid rather than glucose, but is just below the significance level of 2:1. The ratio of 7·3:1 for B. subtilis is not meaningful, however, because of the lack of a response by this organism to glucose.

No work described in the literature to date has investigated responses of bacteria to mixed solutions, and such mixed solutions might elicit greater chemotactic responses than single substances, however, much more work is necessary in this area.

The results presented in this chapter show conclusively that the bacteria used, representative of wood inhabiting genera, demonstrate very positive responses to water soluble extracts of wood. Even those wood species very low in soluble nutrients, e.g. beech and eucalypt elicit very significant responses. It should be noted, however, that in

the pure culture systems developed for bacterial chemotaxis assays, conditions are optimised for maximum motility and response, and that the chemotaxis assays were carried out in buffered solutions at pH7, whereas the wood extracts had pH values of approximately 5·3. Bowra & Dilworth (1981) demonstrated that motility in Rhizobium leguminosarum was optimal between pH5·5 - 8·0, and rapidly decreased below pH5·5, becoming absent at pH4·5. Adler & Templeton (1967) and Adler (1973) showed optimal pH values of between 6 and 7·5 for motility in E. coli; below pH6 motility decreased, and the organism was non-motile at pH4. Further work is necessary to determine the effects of decreasing pH on the degree to which chemotaxis takes place to wood extracts. It is unlikely, however, that on leaching from wood to soil the pH of such leachates would remain unaffected by the complex buffering system present in soil. Ion exchange between leachate and clays and colloidal organic matter would occur, affecting its pH value. The amount of insoluble carbohydrates and the presence of hydroxides would also significantly affect pH values, and the pH of leachates from wood would be as much dependent on the soils buffering capacity as on the wood itself.

Bacteria have been shown to be primary colonisers of both untreated and copper chrome arsenic treated wood in soil (Clubbe & Levy, 1982). Chemotactically mediated movement of bacteria to wood from soil may explain their early isolation in such colonisation studies. Baecker (1981) showed that actinomycete species alone may be present in wood at population levels greater than $1 \times 10^{8} gm^{-1}$, and these may

represent only a small part of the total numbers of bacteria in wood during decomposition; King et. al. (1980) have demonstrated that at these numbers bacteria can contribute significant amounts of nitrogen to wood, thereby increasing its nutrient status.

Of notable interest is the requirement by bacteria of environments free from heavy metals if they are to be significantly motile. Heavy metals inhibit bacterial motility by complexing with flagella, sometimes to such a degree that motility is lost (Bowra & Dilworth, 1981), and in this investigation a chelating agent, EDTA, was added to media to effectively remove such heavy metal ions from solution. Bowra & Dilworth (1981 op. cit.) demonstrated that omission of EDTA from solutions previously tested with Chelax-100 resin to remove heavy metal ions, subsequently followed by additions of Cu^{2+} at concentrations as low as 10μM, inhibited motility completely, but did not kill the organism.

The importance of heavy metal flagella complexes may be considerable in decomposition of copper chrome arsenic treated wood in soil. Bacteria demonstrating positive chemotaxis to nutrients leaching from wood may be rendered non-motile by copper chrome arsenic components in the wood, resulting in bacterial accumulation. If the copper chrome arsenic leaches to soil from treated wood, as recently demonstrated at this laboratory (Briscoe pers. comm.), then bacteria would be immobilised in that region of the soil with increased heavy metal concentrations. Such concentrations of heavy metals,

especially copper, may not be toxic, but due to inhibition of motility biomass build up may occur in these areas.

If copper is present at toxic levels in the wood, then bacterial products from lysed cells may chelate or complex with copper chrome arsenic components, making such components unavailable to act as toxicants. Copper chelated by cell products and therefore retained in solution would be easily leached from wood. Furthermore, most metal ions chelate readily with carboxylic and amino groups which may also be present in wood surfaces in large amounts, due to redistribution of nutrients during drying. Beveridge & Murray (1976) have shown that cell wall fragments of B. subtilis selectively adsorb and retain metal ions, amongst which copper is held particularly tenaciously and in substantial amounts (190μg Cu/mg dry cell wall - approx. 20% W/W). If wood is preserved, bacterial products, amongst which cell walls may be preeminent, may act to detoxify the wood by complexing and chelating copper chrome arsenic components, the chelated forms of which are very soluble and could be removed by leaching, leaving the wood unprotected.

The combined effects of chemotropic and chemotactic behaviour of microorganisms towards wood and water soluble wood extracts may be of fundamental importance in determining invasion sequences of wood in soil prior to colonisation. Such behavioural patterns could be maintained by continuous stimulation of the soil microflora, leading to considerable nitrogen transfer in the form of microbial biomass, from soil to wood and soil adjacent to wood. Increased microbial presence in the wood, especially by bacteria early on in the

invasion process, would supply substantial amounts of nutrients, vitamins etc., thereby satisfying the more fastidious of soft rot fungi.

General Conclusions

1) Bacteria, both Gram positive and Gram negative, and representative of wood and soil inhabiting genera have been shown to demonstrate positive chemotactic responses to cold aqueous wood extracts.
2) Individual amino acids and glucose, known to occur in such water soluble wood extracts, also elicit positive chemotactic responses, although at lower levels than wood extract itself.
3) Responses of bacteria to amino acids are significantly greater than the responses towards glucose.

Chapter 4.

Nitrogen contents of soil in relation to wood decay.

4.1. Introduction

Nitrogen is an important element in all biological systems, being an essential constituent necessary for the synthesis of many diverse groups of compounds, e.g. proteins, nucleotide bases etc. As enzymes are protein molecules and extracellular enzymes moderate all wood polymer breakdown, for active decay of wood to take place microorganisms must acquire nitrogen for enzyme synthesis.

Because of low levels of nitrogen in wood its availability to wood decay fungi may become a major limiting factor to rates at which decay can proceed. Wood typically has nitrogen contents of between 0·03%-0·10%(W/W) with C:N ratios varying between 350-500:1 (Cowling & Merrill, 1966). Even at these low levels many basidiomycete fungi can decay wood, and it has been postulated that such fungi can utilise their own autolytic products to conserve nitrogen (Levi *et al.*, 1968) and can adapt their physiologies by preferential allocation of the limited amounts of nitrogen to the production of enzymes necessary for substrate utilisation (Levi & Cowling, 1969). Soft rot fungi have been shown, however, to require larger amounts of nitrogen before becoming actively cellulolytic (Levi & Cowling, 1966).

Findlay (1934) demonstrated that increasing wood nitrogen content by additions of inorganic salts increased decay rates of Sitka spruce by *Trametes serialis*. Lundstrom (1972) also showed increased decay in wood veneers impregnated with soluble nitrogen. Butcher & Drysdale (1974) noted that

decay rates were accelerated only in those veneers which were dosed with lowest nitrogen concentrations. The C:N ratio of the veneers used by Butcher & Drysdale were approximately 120:1 and these authors considered such ratios to be of more importance in determining decay than absolute amounts of nitrogen. Later, Butcher (1976) confirmed that higher values of nitrogen, impregnated as soluble salts, caused decrease in the decay process, and estimated a C:N ratio of 250:1 to be optimal for soft rot decay.

Soil studies undertaken by King et al. (1981) also indicate that nitrogen contents are important as determinants of decay. Using buried blocks of different wood species and different initial nitrogen contents these authors demonstrated increased nitrogen ocurred in blocks without a corresponding weight loss. Weight loss became significant only when nitrogen contents had increased to an estimated C:N ratio of 200:1, i.e. approximately 0•2%W/W of the wood. Decay of wood in soil has been shown to be greater when nitrogen fertilizer has been used to amend the nutrient level of the soil (Allison & Murphy, 1962). Friis-Hansen (1976) showed increased decay in copper chrome arsenic treated transmission poles situated in cultivated, fertilised fields, compared to those poles in poorer forest soils.

Generally, as woods have very low nitrogen contents and high C:N ratios, decay fungi may have difficulty in assimilating adequate amounts of nitrogen to be metabolically active. The monitoring of nitrogen contents of wood in soil during decomposition has demonstrated that such contents

increase as decay proceeds (Hungate, 1940; Henningsson & Nilsson, 1976; King *et al.*, 1981 *op. cit.*). Three mechanisms have been described which facilitate nitrogen increases in wood in soil.

Wick movement

Baines & Levy (1979) demonstrated that when wooden stakes were placed in water with a portion above the surface and with the grain parallel to the longitudinal axis, water movement into the above ground portion would take place provided there was a gradient in water concentration between the two ends. This 'wick action' depended on evaporation of water from the wood above water surfaces, and they considered that for wood in soil any material in soil solution would be deposited in the wood at or above the groundline. Uju *et al.* (1981), using sterile Scots pine stakes half inserted into sterile soil, the latter having been amended with inorganic nitrogen salts, showed that nitrogen accumulation occurred not only in that portion of the stake below the groundline but also in wood above the soil surface. Major deposition of soluble salts took place at the groundline portion of the stake, that region at which most extensive decay is usually observed. Levy & Dickinson (1981) suggested that at the groundline, conditions of moisture and oxygen availability favour fungal activity, and it is here that organisms received a continuous supply of nutrients by wick action. Thus

with such favourable conditions, growth and decay potential of fungi are maximised, resulting in normal decay patterns.

Nitrogen fixation

A second process which can contribute to increased nitrogen in wood is mediated by bacterial nitrogen fixation. Sharp & Millbank (1973) and Levy et al. (1974) have demonstrated that nitrogen fixation can occur in wood. Aho et al. (1974) and Larsen et al. (1978) have shown asscciated nitrogenase activity with decay of wood. Aho et al. also determined that of 130 gram-negative bacteria isolated from decay area of white fir trees, 68 were capable of fixing nitrogen and were recovered in numbers varying from 10^5 - 10^6 cells/cm^3 of expressed sap. However, non-symbiotic nitrogen fixation by free living bacteria is a high energy demanding process, and Campbell & Lees (1967) noted that Azotobacter, in ideal conditions, require to metabolise 50g of carbohydrates to fix 1g of nitrogen. For nitrogen fixation to occur in wood in soil therefore solubilisation of substantial amounts of wood substrate would be required to permit any significant increases in nitrogen content of wood.

Microbial transfer

King et al. (1981, op. cit.) proposed that increases

in nitrogen noted by them during the decay of wood in soil is correlated with microbial transfer from soil to wood. During experiments in which buried blocks were used as test material, thereby eliminating the possibility of nitrogen accumulation due to wick action, large increases in wood nitrogen contents were observed. Such increases in nitrogen were significantly correlated with mass loss of wood as decay proceeded. Interestingly, in blocks which had been buried and wetted by soil solution but remained undecayed, no increases in nitrogen were noted. Furthermore, treatment of wood with copper chrome arsenic not only decreased decay rates but also nitrogen input. In blocks treated with copper chrome arsenic at concentrations which gave full protection nitrogen increases were not seen. The presence of redistributed soluble nutrients at wood surfaces stimulated and accelerated nitrogen input and rates of decay in both untreated and copper chrome arsenic treated wood.

The variable performance demonstrated by wood in soil may thus be due to complex interactions between many biological, physical and chemical parameters.

It was considered by Gersonde and Kerner-Gang (1976) that non-sterilised soils gave poor reproducibility when used in preservative evaluations. Savory & Bravery (1971), suggested that use of non-sterile soil was important because

it exposed treated wood to a wide spectrum of naturally occurring soil microflora. Such variability between soils on decay rates of wood was confirmed by Amburgey (1978) who noted, however, that such differences in decay rates between soils was not a result of variations in pH value or water holding capacity.

The importance of nitrogen, however, to soft rot decay of wood in soil and pure culture systems suggests it may be of exceptional importance to decomposition processes. The implied importance of site variability and perhaps of nitrogen dynamics in the study of wood decay in soil resulted in the International Research Group on Wood Preservation (I.R.G.) to canvas members of the world-wide co-operative field experiment to determine the performance of copper chrome arsenic treated timber, with especial interest in soft rot (Dickinson, 1976) and to submit soil samples for nitrogen analysis to Dundee College of Technology (Leightley, 1980). The aim of this investigation was to determine whether any correlation existed between several physical and chemical parameters, amongst which total nitrogen was to be considered, and the reported decay status of various reference wood species at different sites and treated to various loadings of copper chrome arsenic.

The chemotactic and chemotropic responses demonstrated by bacteria and fungi to water soluble wood extracts and wood volatiles and described in chapters 2 and 3, if occurring in soil could have considerable influence on microbial nitrogen transfer to wood in terrestrial systems. Qualitative assessment of sapwood stakes of lime and pine in soil, used in

investigations by other workers and examined as a preliminary to the work described in this chapter, indicated gross changes in soil structure in that soil adjacent to the wood. On gentle removal of the stakes quantities of soil remained adhering to the wood; such soil had well developed crumb structure generally indicative of high microbial biomass presence. It was also noted that many crumbs were held to the wood surface by hyphal strands, and microscopic examination demonstrated the presence of large numbers of bacteria and protozoa. Soil 5mm distant from the wood surface had no such extensive crumb development. These observations suggested that microbial biomass accumulation occurred at wood-soil interfaces during wood decay processes.

It was concluded from the above observations that any large biomass accumulations might be measurable in terms of nitrogen content of the soil and prior to controlled laboratory investigations it was considered that an examination of soil surrounding wood already in service would be of value. It was considered that soil surrounding fence posts of known wood type, treatment and date of insertion should be investigated, and that such soils should be collected either at the wood-soil interface or at some distance from the interface to determine any differences in nitrogen contents which might exist.

Micro-Kjeldahl methods are normally employed to determine nitrogen contents of wood and soil. Such methods use either a mixture of copper sulphate/selenium/potassium sulphate (Bremner, 1965) or mercury (II) oxide/potassium sulphate (Hesse, 1971) in the digest as catalysts. A method had been

developed for wood at this laboratory which could be used to determine both nitrogen and copper chrome arsenic contents in wood from a single digest; this entailed using 100 volume hydrogen peroxide as the oxidant in a concentrated sulphuric acid digest. After neutralisation of the acid digest and alkaline steam distillation to drive off the ammonia for nitrogen determination, the residual solution was retained and carefully collected from a modified Markham still. This solution was then reacidified ($2{\cdot}5 M H_2SO_4$) and the copper, chrome and arsenic concentrations determined by atomic absorption spectrophotometry using a standard additions technique.

Methods incorporating heavy metals as catalysts are not applicable where nitrogen and copper, chrome and arsenic determinations are performed on the same digest due to loss of sensitivity because of excess heavy metal presence. Therefore use of heavy metals in soil nitrogen analyses necessitates analysis of separate samples for copper chrome and arsenic. As the amounts of recovered soil from small wood stakes is necessarily small due to the restricted area contiguous with the wood which may be sampled the ability to perform both determinations on one sample was considered advantageous. Since it was considered that it would be useful at a later date to study copper, chrome and arsenic losses from treated wood to soil, it was decided, as a preliminary experiment, to compare the hydrogen peroxide method with the copper sulphate/selenium and mercury (II) oxide methods to determine its effectiveness in nitrogen determinations in soil with a view to using such a method as the standard procedure at the laboratory.

The aims of the work described in this chapter were therefore as follows:

1. To evaluate the suitability of the hydrogen peroxide method as an appropriate method to determine nitrogen in soil.
2. To determine nitrogen contents in soil adjacent to wood in service at wood-soil interfaces and at some distance from the wood-soil interface thus to evaluate soil nitrogen content as a possible parameter in monitoring microbial biomass accumulations in soil adjacent to decomposing wood.
3. To determine the soil nitrogen contents of soils obtained from the International Research Group on Wood Preservation co-operative field test sites and to investigate the correlation, if any, between such values and the published decay status of timbers at such sites (Levy & Dickinson, 1980).

4.2. Materials and methods

4.2.1. Comparative study of methods for soil nitrogen determination

4.2.1.1. Introduction

Three methods described below were compared for nitrogen determination,

a. using hydrogen peroxide (H_2O_2.100vol.) in the digest,

b. using a mixture of copper sulphate/selenium/potassium sulphate ($CuSO_4 \cdot 5H_2O/Se/K_2SO_4$) (Bremner, 1965 op. cit.),

c. mercury (II) oxide/potassium sulphate (HgO/K_2SO_4) (Hesse, 1971 op. cit.) in the digest.

These methods were used to determine the nitrogen contents of:

1. Standard solutions of glycine in aqueous solution ($1 mgN/cm^3$:0•1%W/V).
2. Alluvial loamy soil from the Scottish Crop Research Institute, Invergowrie, Tayside and used in all soil burial programmes at this laboratory. Such soil remained fallow and unfertilised for one year before sampling.
3. Unmodified coal, used as a high nitrogen content, recalcitrant, organic material.

4.2.1.2. Preparation of samples

Standard nitrogen solutions

Glycine (B.D.H. chromatographically homogenous) was dissolved in double glass distilled water to give a Standard solution of concentration 1mgN/cm^3. 1·00cm^3 aliquots of this solution, measured using 1cm^3 grade A pipettes, were used for nitrogen determination. Controls consisted of 1·00cm^3 aliquots of double glass distilled water.

Coal

200g of coal was ground in an agate pestle and mortar to a fine powder and stored without prior drying (to prevent loss of volatiles) in a resealable plastic bag. 50mg of fine powdered coal were used rather than 200mg samples because of high nitrogen content determined from preliminary experiments.

Soil

Soil, collected from a site previously hand cleared of plant material, was removed down to a depth of 10cm and passed through a 2mm screen into large bins. Approximately 250g of this soil was spread out on glass sheets and dried at 40°C in a fan oven for six hours. The soil was examined and any obvious remaining plant material was removed. 20g portions of the dried soil were ground to a fine powder in an agate pestle and mortar, the portions remixed and the 250g of the powdered soil stored in a resealable plastic bag at 4°C until use. 500mg samples were dried to constant mass in an oven at 102°C±2°C to determine residual moisture content. The residual

moisture value was used as a correction factor to give % nitrogen of each sample on a dry mass basis. Approximately 200mg of ground soil was accurately weighed for each nitrogen determination.

4.2.1.3. Methods of analysis

Samples for analysis were either 200mg Invergowrie soil, 50mg coal, 1·00cm^3 standard glycine solution or 1·00cm^3 double glass distilled water.

A. Digestion stage

Method 1.

100g potassium sulphate (K_2SO_4), 10g copper sulphate ($CuSO_4 \cdot 5H_2O$) and 1g selenium (Se) giving a mass ratio of 100:10:1 respectively were weighed and ground separately in an agate pestle and mortar, mixed and then reground to give an intimate mixture of 111g of these reagents. The samples were measured into 50cm^3 Kjeldahl digestion flasks and 1g of the above misture and 3cm^3 18·4M sulphuric acid (B.D.H. nitrogen free) were added. The flasks were heated gently in a fume hood over gas so that the sulphuric acid condensed not more than halfway up the neck of the flask, for five hours (Bremner, 1965 op. cit.).

Method 2.

100g potassium sulphate (K_2SO_4) and 5g mercury (II) oxide (HgO) giving a mass ratio of 20:1 respectively were weighed and ground separately, mixed and reground in an agate

pestle and mortar to give an intimate mixture of 105g of these reagents. The samples were measured into 50cm^3 Kjeldahl digestion flasks and 2g of the above mixture and 3cm^3 of nitrogen free 18.4M sulphuric acid were added. The flasks were heated gently as above for 2-3 hours until clear (Hesse, 1971 op.cit.).

Method 3.

Samples were measured into 50cm^3 Kjeldahl digestion flasks and 3cm^3 of nitrogen free 18.4M sulphuric acid added. 2cm^3 of 100 volume hydrogen peroxide were added dropwise and the flasks gently heated as above to reconcentrate the acid. The digests were allowed to cool prior to each further addition of 2cm^3 of 100 vol. H_2O_2. This process was repeated until the digest was clear, usually after addition of between 6-8cm^3 100 vol. H_2O_2 for soil.

B. Distillation stage.

After complete digestion flasks were allowed to cool and contents transferred to the distillation chamber of a Markham apparatus. The digestion flasks were rinsed three times with approximately 3cm^3 of glass distilled water each time and washings transferred to the chamber. Ammonia was steam distilled from the chamber after the addition of excess ($>$11.0cm^3) 40% W/V sodium hydroxide solution for methods 1 and 3 (or with 50% W/V sodium hydroxide and 2.5% W/V sodium thiosulphate ($Na_2S_2O_3$) for method 2) and collected in 5 cm^3 2% W/V boric acid (H_3BO_4) solution, containing

5 drops methyl red/ ethanolic bromocresol green indicator in 100 cm^3 Erlenmeyer flasks marked to indicate a volume of 25 cm^3. The distillation continued in all cases until the volume in the receiver flask reached the 25cm^3 mark.

Ammonia - N in the distillate was determined by titration with standard 0.01M hydrochloric acid (1cm^3 0.01M HCL ≡ 0.14 mg N) and the nitrogen content of the wood calculated as a % of preburial mass.

4.2.2. Nitrogen content of soils adjacent to and distant from wood in service

Fence posts of known wood type, preservative treatment and date of insertion were located by Mr. R. Robinson of R & D Chemicals Ltd., Glasgow. Selected fence posts were either 'Scots pine rounds' (Pinus sylvestris L.) of 3 inch diameters, situated on arable, agricultural land, or 6x6 inch beech (Fagus sylvatica L.) barrier supports on a motorway slip road. All posts had been treated to BS 4072, 1974 with copper chrome arsenic preservative, except for one post of pine which had been creosote treated. Pine posts were chosen at random from fence lines, and soil removed to a depth of 8cm, using a 8mm diameter cork borer, either within 10mm of the wood-soil interface, or at a distance of 100mm from the wood-soil interface. Two beech posts were chosen at random from a length of crash barrier and soil sampled as for pine, but in addition soil was removed carefully, using a spatula, within 1mm of the wood-soil interface. Also, the outer 2mm of beech post wood that had been in ground contact just below the groundline was sampled.

All soil and wood samples were placed in resealable plastic bags and stored at $4^{o}C$ within 8 hours of sampling. All posts were examined for decay and surface softening.

Soils were prepared for nitrogen analysis as described in section 4.2.1.2. and such analyses were carried out using the hydrogen peroxide method.

Beech wood was dried to constant mass at $102^{o}C \pm 2^{o}C$

and 100mg samples weighed accurately for wood nitrogen determinations. All soil nitrogen determinations were performed in triplicate for soil around pine and four replicates for both wood and soil around beech.

4.2.3. Nitrogen contents of soils at I.R.G. field sites

Soils were received from eleven collaborators in the field experiments (Dickinson, 1976 op. cit.). No standardisation of sampling technique had been reported, thus soils arrived in a variety of conditions, i.e. wet, dry, unsterile, sterile etc., and had sometimes spent considerable time in transit. Soils varied in amounts of obvious plant material and some were assumed to have been sieved prior to dispatch because of uniform maximum particle size.

Soils were prepared for nitrogen analyses as described in section 4.2.1.2. which entailed the removal of organic matter in the form of twigs, leaves etc. which may have contained nitrogen. Nitrogen was determined using the hydrogen peroxide method described in section 4.2.1.3.

4.3. Results

4.3.1. Comparison of methods for soil nitrogen determination

Nitrogen recoveries from standard glycine solution, Invergowrie soil and coal are presented for all three methods in Table 4.1. Figures represent means of six replicate determinations for standard glycine solution and soil, and of twelve replicate determinations for coal using methods $CuSO_4/Se/K_2SO_4$ and H_2O_2. The HgO method produced problems throughout the whole series of experiments, resulting in very low nitrogen values compared to other methods, and therefore replicates were reduced to three for standard glycine solution and soil, and four replicates for coal.

Nitrogen recoveries from standard glycine solution using $CuSO_4/Se/K_2SO_4$ and H_2O_2 were 92% and 94% respectively, recovery using HgO was only 67%. Nitrogen concentration of Invergowrie soil as determined by H_2O_2 and $CuSO_4/Se/K_2SO_4$ were again similar, with mean values of 0·206% dry mass soil and 0·192% dry mass soil respectively. Nitrogen concentration as determined by HgO was 0·087% dry mass soil, comparing very unfavourably with the other two methods.

A higher nitrogen concentration for coal was found using H_2O_2 compared to $CuSO_4/Se/K_2SO_4$ with mean values of 1·70% and 1·57% respectively. A very low value was obtained using HgO with a mean nitrogen concentration of 1·15%.

The H_2O_2 method consistently recovered the highest nitrogen values in Standard glycine solution, coal and soil

and was therefore adopted for all further nitrogen determinations in soil in further investigations.

4.3.2. Nitrogen concentrations of soil adjacent to and distant from wood posts in service

Results of nitrogen concentrations of soil within 10mm and 100mm from wood-soil interfaces for three inch diameter copper chrome arsenic treated pine fence posts are shown in Table 4.2. Results for soil samples within 10mm of wood-soil interfaces for different posts vary a great deal, ranging from 0·203% (Post 3) to 0·555% (Post 2). A similar spread of values is seen for soil 100mm from wood-soil interfaces, 0·215% (Post 4) to 0·539% (Post 1). Nitrogen concentrations of soil at the two sample positions about the same post indicate that for poles 3,4 and 5 little variation in such concentrations occurred. Soils at the two sample positions at posts 1 and 2 do, however, show significant differences. Soil nitrogen concentration is greater 100mm from the wood-soil interface (0·539%) of post 1, compared to soil within 10mm of the interface (0·462%). The reverse is seen in post 2 where soil nitrogen concentration is greater within 10mm of the post (0·555%), compared to the nitrogen value 100mm from the interface (0·470%). Interestingly the creosoted post (Post 6) had the lowest soil nitrogen concentration of any post for soil within 10mm of the wood-soil interface, a value of only 0·147% dry mass, whereas the nitrogen concentration 100mm distant increased to 0·240% dry mass.

Results for the two 6x6 inch beech posts, both of which showed extensive signs of soft-rot decay, indicated soil nitrogen concentrations increased with proximity to the wood-soil interface, especially within 1mm of the wood surface (Table 4.3.). For both posts there were approximate 100% increases in soil nitrogen concentrations between soil at 100mm distant from the wood surface to soil within 1mm of the wood surface. The nitrogen concentration of the surface 2mm of wood sampled is based on the dry mass of wood as sampled rather than the undecayed preinsertion masses, and hence such values may be inflated by material loss from the wood. Allowing for this, however, the figures of 1•428% and 1•21% are high and indicate that substantial nitrogen increases have occurred in the wood.

4.3.3. Nitrogen concentrations of soils at I.R.G. field sites

Soil nitrogen concentrations for eleven sites submitted to Dundee College of Technology are shown in Table 4.4. Values range from 0•032% (Site 29) to 0•555% (Site 02) representing considerable variation between test sites. Nitrogen values may well be elevated in some soils if obvious plant material had not been removed, as such organic matter acts as reservoirs of mobilising nitrogen. When these values are compared with decay estimates at field sites (Levy & Dickinson, 1980 op. cit.) little relationship is observed between soil nitrogen values and decay status of the wood. Comparison is difficult, however, due to small numbers of soil

samples submitted and incomplete reporting of field performance results to the coordinators.

Table 4.1.

Nitrogen concentrations of: 1. Standard glycine solution (0·1%N W/V), 2. Coal (% W/W) and 3. Soil (% W/W dry soil) as determined using a) H_2O_2, b) $CuSo_4/Se/K_2SO_4$ and c) HgO. Figures represent means† with standard deviations.

		Method		
		(a) H_2O_2	(b) $CuSo_4/Se/K_2SO_4$	(c) HgO/K_2SO_4
Sample	(1) Standard (0·1%N W/V)	0·094±0·003	0·092±0·004	0·067±0·006
	(2) Coal	1·70 ±0·087	1·57 ±0·127	1·15 ±0·248
	(3) Soil	0·206±0·023	0·192±0·015	0·087±0·016

† for replication see text

Table 4.2.

Nitrogen contents of soil at 10mm and 100mm distances from Scots pine fence posts (75mm diameter). Posts 1-5 were treated with copper chrome arsenic preservative and inserted in soil in year indicated. Post N°. 6 was impregnated with creosote and had been inserted in soil for more than 25 years. Nitrogen values (% dry mass soil) are means of three replicate determinations and their standard deviations.

Post	Sample distance from post (mm)	Date of insertion in soil	Preservative	Decay status	Soil N (%W/W dry soil)
1.	10 100	1964	CCA	Sound	0·462±0·025 0·539±0·024
2.	10 100	1966	CCA	Soft rot	0·555±0·018 0·470±0·033
3.	10 100	1973	CCA	Sound	0·203±0·010 0·234±0·005
4.	10 100	1973	CCA	Sound	0·216±0·011 0·215±0·043
5.	10 100	1975	CCA	Sound	0·505±0·024 0·512±0·026
6.	10 100	> 25 years	Creosote	Sound	0·147±0·018 0·240±0·004

Table 4.3.

Nitrogen contents of surface 2mm of beech wood and soil 1mm, 10mm and 100mm from wood-soil interface. Posts were treated with copper chrome arsenic and had been inserted in soil for approximately 6 years. Figures represent means of four replicate determinations and their standard deviations.

Post	Distance (mm)	Decay status	% Nitrogen W/W dry basis
1	Wood		1·428 ± 0·230
	1	Soft rot	0·320 ± 0·008
	10		0·281 ± 0·023
	100		0·189 ± 0·011
2	Wood		1·121 ± 0·150
	1	Soft rot	0·335 ± 0·009
	10		0·218 ± 0·021
	100		0·155 ± 0·027

Table 4.4.

Nitrogen contents (% W/W dry soil) of soils submitted for analysis by collaborative members of the cooperative field experiment. Figures are means of four replicate determinations and their standard deviations.

Site N°.	Country	Nitrogen (% W/W)
02	Australia	0·555 ± 0·038
13	India	0·128 ± 0·005
15	Italy	0·144 ± 0·026
20	Portugal	0·101 ± 0·011
23	Switzerland	0·262 ± 0·007
24	U.K.	0·421 ± 0·029
26	U.K.	0·303 ± 0·014
27	U.S.A.	0·306 ± 0·004
28	U.S.A.	0·097 ± 0·016
29	U.S.A.	0·032 ± 0·007
33	U.K.	0·148 ± 0·013

Table 4.5.

Sources of soil nitrogen

1.	Plants, plant litter and fauna (if surface vegetation present)	
2.	Partially degraded organic matter	Light fraction or cellular fraction
3.	Microorganisms	
4.	Humus and bound nitrogen	
5.	Inorganic forms of nitrogen	

4.4. Discussion

Extensive preliminary experimental investigations were undertaken to develop the hydrogen peroxide method and it is very clear from the results that the use of 100 vol. hydrogen peroxide as an oxidant in the digest is at least as efficient as the more traditional copper sulphate/selenium catalytic method for determination of nitrogen in soil. Furthermore, the hydrogen peroxide method has the very useful added advantage that heavy metal catalysts such as copper are not required, which, if used, would negate any further use of the digest for copper chrome arsenic analysis. It is possible, therefore, by the use of this method in conjunction with a modified Markham steam distillation unit to determine both nitrogen and copper chrome arsenic concentrations from a small single soil sample.

The use of mercury as the catalyst in the digest proved very unsatisfactory, not only in low recoveries of nitrogen, but also in lost results with all materials tested. It was obvious from colour changes of the indicator in the bromic acid that during neutralisation of the acid digest in the Markham apparatus an acid volatile was evolved. Such volatiles crucially altered subsequent titration values, leading to low aberrant results for nitrogen concentrations.

Hydrogen peroxide had been evaluated by McKenzie & Wallace (1954; in Hesse, 1971); they found the method gave incomplete nitrogen recoveries. Since 1954, however, hydrogen peroxide of increased strength (100 volumes) and of consistently

high quality has become available. Grimshaw (pers. comm.) suggested reevaluating the method using improved 100 vol. H_2O_2, such evaluations should include analysis of nitrogen concentrations in coal, a recalcitrant material known to be resistant to digestion, and compared with catalytic methods normally employed. The results of such comparisons showed determined nitrogen concentrations of coal to be slightly higher (1·70%W/W) when using 100 vol. H_2O_2, than when $CuSO_4$/Se was employed (1·57%W/W). All further soil nitrogen determinations were undertaken using 100 vol. hydrogen peroxide.

Analysis of nitrogen concentrations of soil proximal to and at some distance from copper chrome arsenic treated posts in service proved very interesting. The beech posts examined were situated within 3 metres of each other and both were decayed due to soft rot, which was verified by subsequent microscopic examination of the wood in which widespread soft rot cavities were evident. The results for nitrogen concentrations in soil about the beech posts are similar to each other and indicate very significant nitrogen increases at the wood-soil interfaces. Soil samples about the posts were taken at distances of 1mm, 10mm and 100mm from the wood-soil interface, and it was within the 1mm depth of soil that largest nitrogen accumulations were observed (0·320% and 0·335%W/W), compared to nitrogen values of 0·281% and 0·218% at 10mm and 0·189% and 0·155% at 100mm from the wood surface.

Samples of beech surface wood, to a depth of 2mm, were removed from the posts just below the groundline and it

was seen that such wood had very elevated nitrogen contents (1·43% and 1·12%W/W). Even allowing for normal increases expected in wood nitrogen content due to loss of carbohydrate material during decay such high nitrogen values indicate significant nitrogen accumulation within the wood.

There was considerable variation in soil nitrogen between the locations of different pine posts at both 10mm and 100mm distances from wood-soil interfaces. At 10mm from wood-soil interfaces nitrogen values varied between 0·203% and 0·555%, and at 100mm values varied between 0·215% and 0·539%. Such wide variations in values may reflect the extended area over which sampling was undertaken necessitated by the need to encompass posts of varying periods of insertion into soil.

Results indicated that at only one of the pine posts had nitrogen accumulation occurred at the wood-soil interface and it was this post which was undergoing decay. Of the remaining four posts, none of which showed signs of decay, three showed no differentiation in soil nitrogen concentrations between the two sampling positions, and at the fourth post soil nitrogen was depleted at its surface compared to soil nitrogen 100mm from the wood-soil interface.

The greater nitrogen accumulation in soil adjacent to beech rather than pine may be due to the restricted volume of soil sampled at beech posts, one sample of which was within 1mm of the wood-soil interface, and the greater extent to which decay was occurring in beech. The similar soil nitrogen profiles for both beech posts suggest, because of their proximity to each other, that soil nitrogen concentrations

prior to insertion of the posts were similar and events which have occurred since insertion were likewise similar.

Interestingly, the pine post treated with creosote had the lowest soil nitrogen concentration 10mm from the wood-soil interface of any soil tested. The nitrogen content of soil at this site was 0•147%, whereas 100mm from the wood surface soil nitrogen concentration was 0•240%.. Qualitative assessment of plant growth around bases of posts indicated no differences in amounts of vegetation present around the base of the creosoted post compared to copper chrome arsenic treated posts, and presumably organic matter input to soil would be similar. It may be postulated that volatile emissions from creosoted posts to soil inhibit microbial activity to such a degree that organic matter accumulates. Such organic matter would be removed during soil processing and preparation prior to nitrogen determinations with consequent loss of nitrogen from the soil.

Although the investigations undertaken were preliminary in nature and restricted in extent the results for beech indicated significant increases in nitrogen in soil adjacent to wood undergoing decay, and as such were considered to warrant further investigation. It was also considered that the form of nitrogen and processes which mediated such increases might be of fundamental importance in wood decomposition in soil. If, as King et. al. (1981) argued that such nitrogen increases were the result of increased microbial presence then significant movement and growth of soil microorganisms towards wood in soil must have taken place.

Agricultural soils generally have total nitrogen concentrations of between 0·020%-0·500%W/W dry soil (Campbell, 1977). Total soil nitrogen concentrations at the eleven International Research Group's sites distributed worldwide ranged between 0·032%-0·555%W/W dry soil, indicating comparable values within the range of values for average soils.

The collaborative field experiment (Levy & Dickinson, 1980) was undertaken to determine the performance of copper chrome arsenic treated stakes of selected wood species at various field sites around the world, with particular reference to soft rot. Four reference wood species were chosen, Alstonia scholaris, Betula pendula, Fagus sylvatica and Pinus sylvestris; stakes were either untreated or treated to refusal with either 0·66%, 1·53%, 3·01% or 5·60% (W/W) copper chrome arsenic. Decay estimates were undertaken at 6, 12, 18, 24 and 30 month burial periods, and the condition of the stakes ranked 0, 1, 2, 3 or 4 depending on the severity of attack, 0 equivalent to no attack and 4 complete failure.

Results of the test (Levy & Dickinson, 1980 op. cit.) were incomplete with inconsistent reporting of decay estimates to the coordinators. Correlation between total soil nitrogen at field sites with decay estimates was therefore difficult to assess and limited to those individuals who submitted both decay estimates and soil for analysis. Evaluation of the relationship between the two parameters was necessarily qualitative and untested statistically; however, available data indicated that no correlation existed between total soil

nitrogen and decay.

Nitrogen availability is generally considered to be an important determinant for soft rot and is an essential resource determining rates of all forms of decomposition of organic matter in terrestrial ecosystems (Swift, Heal and Anderson, 1979) yet little critical consideration has been given to the mobilisation of this nutrient in the decomposition of preserved wood in soil. The lack of correlation of the results presented in this document with decay estimates does not mean that the former are of no value, as they can act as a starting point from which an evaluation of the role of soil nitrogen on intersite variation can be made. However, if this is to be done there is a need for standardisation of sampling methodology on site and agreement on soil preparation and analytical procedures.

The small size of the specimens submitted for analysis, unless these were representative samples, may mean that these do not reflect the nutritional status of the individual sites. Similarly, while it is well known that the greater proportion of nitrogen in soil is in the form of organic matter, microorganisms, or adsorbed to soil colloids (Table 4.5.), standard analytical methods exclude bulked organic matter, e.g. plant detritus, which may be the primary resource of mobilisible nitrogen.

Estimation of total nitrogen availability in soils should therefore include reference to those nitrogen components outlined in Table 4.5. as all are implicated in soft rot decomposition of preserved and unpreserved wood. If

immobilised nitrogen in the form of microorganisms is the major means of nitrogen transfer to wood in soil as postulated by King et al. (1983), then further studies should include determination of cellular fraction soil organic matter and total organic matter contents along with a microbiological analysis as important considerations in the total nitrogen economy during decomposition of preserved wood.

Chapter 5.

Changes in microbial populations in soil adjacent to wood.

5.1. Introduction

Preliminary investigations of total nitrogen contents of soil adjacent to decaying wood described in chapter 4 indicated that nitrogen accumulation took place in soil contiguous with wood surfaces and that increases in nitrogen concentrations of the order of 100% occurred in soil within 1mm of the wood soil interfaces of the beech posts examined. Qualitative visual assessment of soil crumb development of soil surrounding small stakes (150 x 10 x 5mm), used in decay studies by other workers at this laboratory, appeared to indicate that microbial population densities increased in soil adjacent to decaying wood. Microbial accumulation was not evident however in soil surrounding preserved undecayed blocks which were completely buried in soil for similar time periods. Blocks removed from soil for nitrogen and mass loss determinations in these experiments showed that microbial accumulation, as indicated by soil crumb development and soil adhesion to blocks, only occurred in those blocks undergoing decay. It was considered therefore that a relationship existed between events occurring during wood decay and the observed development of microbial accumulations taking place in soil at wood soil interfaces.

Chapters 2 and 3 of this thesis have demonstrated the important influences that wood components have on behaviour patterns of both fungi and bacteria. Some wood decay fungi showed hyphal extension towards wood during growth and such responses were demonstrated by fungal

colonies developing up to 35mm from wood baits. Bacteria commonly isolated from wood and soil habitats showed marked tactic responses to cold water sapwood extracts of several wood species and such bacteria accumulated in area of high nutrient concentration especially responding to those nutrients with a nitrogen component. It was postulated therefore that chemostimulation of soil microflora by wood emplaced in soil could initiate increased microbial populations at wood soil interfaces, and that the microbiology and microbiological activity in soil about wood would be markedly different for unammended soil.

In all wood decay studies undertaken at this laboratory the nitrogen content of wood samples buried in soil increased during the decay process and for reasons considered in chapter 4 it was postulated that such increases must be due to microbial populations migrating from soil to wood. Microbial propagules at wood-soil interfaces may directly colonise freshly emplaced wood in soil. This however would not involve significant nitrogen increases in wood because of the limited numbers of such propagules coincident with wood surfaces. Chemostimulation of the soil microflora by wood volatiles and leachates could attract microorganisms at some distance into the soil and thus stimulate growth and movement of microflora to wood. By such a process numbers of microorganisms in soil surrounding wood and not only those located at the wood surface might be stimulated to invade wood in large numbers thereby significantly increasing its nitrogen content. It was

considered therefore that an investigation into microbial population dynamics in soil adjacent to wood during wood decomposition would be of fundamental importance to an understanding of wood decay.

A major problem in quantifying microbial populations in soil is that widely varying values are obtained depending on methods of evaluation employed. Direct counts i.e. by microscopic examination generally give estimates of cell numbers and hyphal lengths several hundred times greater than other methods used e.g. viable counts. Direct microscopic observations of soil microflora is difficult because of the opaque nature of soil particles. To overcome this problem, microscopic estimation of numbers of bacterial cells and fungal hyphal lengths are generally undertaken on small amounts of soil dispersed in water of known volume. These are then mixed with molten agar and thin agar films prepared and mounted on microscope slide which can then be stained (Jones & Mollison, 1947, Thomas et al, 1965). Bacterial numbers and hyphal lengths thus determined can then be used to determine biomass (Olson, 1950; Berg & Soderstrom, 1979; Baath & Soderstrom, 1979 a.b.).

The disadvantage of direct observation of stained thin soil films is the inability to distinguish between viable and non-viable organisms. This disadvantage has been partially overcome for fungi by use of phase contrast microscopy (Frankland, 1975) and special vital staining techniques (Soderstrom, 1977). Counting of viable bacterial cells by serial dilutions from dispersed soil in water has the

drawback of media selectivity with growth of disparate taxonomic groups of bacteria either promoted or restricted. Thus complex procedures and numerous selective media are required in order not to omit major bacterial groupings. Furthermore such methods do not provide useful quantitative data on fungal populations because of the filamentous nature of fungal growth and the inability to distinguish between colonies arising from hyphal fragments as opposed to spores.

Chemical determination of soil microbial biomass can be undertaken by a number of methods. Hexosamine assays measure amounts of fungal cell wall but is only effective for pure culture determinations with individual fungi due to variation in hexosamine content of cell walls between different fungal species (Swift, 1973). Adenosine triphosphate (A.T.P.) content of the microflora in soil is presently undergoing extensive investigation as a possible effective and reliable evaluator of soil microbial biomass (Jenkinson & Oades 1979). A.T.P. is present in cells of all organisms, it is however strongly absorbed into clay particles in soil and is only stable outside cell walls for limited time periods dependent on the chemical and physical environment. To overcome these problems soil is flooded, prior to A.T.P. extraction from cells, with a compound which occupies the sites on the clay particles which would absorb the A.T.P. and the A.T.P. molecule stabilised by addition of buffers to the soil at low temperatures. Once A.T.P. has been extracted its soil concentration is determined by measuring the relative quantity of light produced when the A.T.P. is mixed with luciferin - luciferase in a luminometer

and light output values compared to a standard curve. (Paul & Johnson, 1977; Eiland, 1979). The A.T.P. method was evaluated for use in this investigation but it was shown that amounts of soil available from about blocks was below the amount required for an A.T.P. assay.

Respirometric and calorimetric methods generally require large soil samples ($>5g$) for useful measurements to be made although microcalorimetry which assesses activity in small samples of soil might prove useful however such a method utilises special apparatus unavailable during this investigation (Sparling, 1981). It was considered therefore that the agar film method was most appropriate to obtain accurate data on both fungal hyphal lengths and bacterial numbers in soil.

Measurement of soil microbial activity can also be undertaken by enzyme assays which include cellulases, amylases, invertases and pectinases however such methods are limited because of substrate specificity. Non substrate specific enzymes such as dehydrogenases, which are enzymes present in all organisms, give a more useful indication of overall activity as they are a direct measure of the metabolic respiratory apparatus. After preliminary investigations it was considered that dehydrogenase assays were the most useful method to measure relative activity in the soil microbial population as the enzyme is not substrate specific and only required small soil samples for measurements to be made. Dehydrogenase assays are straight forward colormetric procedures which allow a sufficient number of samples to be

processed to permit the necessary replication to be undertaken in the available time.

As soft rot decay of wood in soil is largely considered to be a problem of hardwoods especially when treated with copper chrome arsenic preservatives, it was decided that a comparative study using both an untreated and copper chrome arsenic treated hardwood and softwood would be carried out to determine whether the postulated changes in microbial population densities in soil differed between wood type and treatment. Few studies have been undertaken to examine microbial population dynamics in soil adjacent to wood during wood decomposition processes, it was considered therefore that a simultaneous study of wood and soil surrounding wood during wood decay would be of value to an understanding of microbial behavious prior to and during wood decomposition. Such a study would be of especial interest in examing possible relationships between microbial behaviour in soil during wood decomposition and the induction and decay phases of wood decay described by Smith (1980).

Results presented earlier in this thesis have shown that wood acts as a chemostimulant to both fungi and bacteria. It was postulated that such stimulation of soil microflora would result in increased microbial populations in soil in proximity to wood - soil interfaces. In view of previous results for soil nitrogen presented in chapter 4, nitrogen contents of soil were also assessed to determine whether this parameter could be used as an indicator of microbial presence.

The aims of the investigation described in this

chapter were therefore:

1. To monitor microbial populations in soil adjacent to wood during decay by direct measurement of bacterial numbers and fungal hyphal lengths.
2. To measure metabolic activity of such populations by dehydrogenase assay.
3. To determine whether there was a correlation between changes in soil nitrogen content and decay status of wood as measured by mass loss and nitrogen input.
4. To determine if soil microbial population density and dehydrogenase activity were in any way related to events taking place in wood during the decomposition process.

5.2. Materials and Methods

5.2.1. Preparation of wood blocks.

Quartersawn planks of lime (Tilia vulgaris Hayne.) and pine (Pinus sylvestris L.), previously dried in a fan oven at 40°C and stored in a ventilated cupboard at ambient laboratory temperatures for six months, had radial surfaces removed to a depth of 2mm. Blocks measuring 30 x 20 x 5mm were cut from the sapwood of such planks so that the 20 x 5mm faces were in transverse section, the 30 x 5mm faces in tangential section and the 30 x 20mm faces in radial section. Blocks were dried in an oven at 102° ± 2°C to constant mass and weighed. Half the lime and half the pine blocks were then impregnated with 0.5% W/V and 0.25% W/V copper chrome arsenic solutions (BS 4072:1974) respectively.

Impregnation was carried out by weighting down blocks with glass slides in glass containers placed in a vacuum dessicator. A vacuum was drawn for 15 minutes followed by the introduction of the treating solution and the release of any residual vacuum (BS 6009:1982). Blocks were left submerged for 30 minutes, removed from solution blotted dry and placed on 30 x 5mm faces on glass sheets in a large glass tank. A small amount of xylene in a glass vessel was placed at the bottom of the tank and the tank sealed with a glass sheet cover. Blocks were turned onto alternate 30 x 5mm faces twice weekly. The tank was kept sealed for two weeks, partially open for a third week and fully open for a

further week. Blocks were stored in closed glass petri dishes for a period of six weeks prior to leaching.

Blocks were leached prior to burial by soaking for 24 hours in glass distilled water (21 blocks/5 litres). They were then removed and placed on a plastic mesh in a vacuum dessicator and a vacuum drawn for 10 minutes, any extracted liquid running off blocks through the mesh. Blocks were blotted dry and impregnated with fresh glass distilled water as described for copper chrome arsenic solutions. They were then soaked for a further 72 hours in glass distilled water with a change of water every 24 hours. Blocks were removed from the water, blotted dry and allowed to air dry at ambient laboratory temperatures to approximately 100% moisture.

5.2.2. Burial

The moisture content and water holding capacity were determined (Savory, 1973) for Invergowrie soil previously sieved through a 2mm screen. Preweighed plastic containers measuring 280 x 200mm and 95mm deep were filled to a depth of 40mm with soil. Blocks were inserted to a depth of 10mm with 30 x 5mm tangential faces in the horizontal plane and were positioned using a template later used to locate blocks for recovery. Pine and lime blocks were buried in separate boxes with three replicate treated and three replicate untreated blocks, randomly located at template positions, per box. Controls consisted of two similarly buried, prewashed perspex blocks of the same dimensions as the wood. Boxes were then

filled with soil to a depth of 80mm giving 30mm of soil below the lower horizontal 30 x 5mm face and 30mm of soil above the upper 30 x 5mm face. Boxes were weighed and wetted evenly over the surface with enough distilled water to bring the soil to either 80% or 100% of its water holding capacity for boxes of lime or pine blocks respectively.

Boxes of soil were incubated in the dark at 25°C and weighed twice weekly and where necessary distilled water added over the surface to maintain soils at required water holding capacity. Seven boxes were prepared for each wood type and one box of each wood type sacrificed at 1:2:3:4:6: 12 & 24 week time periods.

After burial the positions of blocks were determined using the template and the four upper corners of each block located and marked with 50mm fine steel pins. Blocks were recovered together with adjacent soil to within 3mm of wood-soil interfaces. This was achieved using a piece of thin, tin-plated, sheet steel folded into an open ended rectangular mould the cross section of which measured 36 x 11mm i.e. greater by 6mm than the dimensions of the horizontal 30 x 5mm tangential faces of the block. The positioning of the mould was guided by the four steel pins located at the upper four corners of the block. Soil above and below the upper and lower surfaces of the block was removed to within 3mm of the wood-soil interface using a narrow spatula marked at 3mm and both block and soil placed in sterile glass petri dishes.

Soil was removed from blocks by gentle brushing and collected in the sterile glass petri dishes, blocks were

weighed prior to drying to constant mass in an oven at $102^{o} \pm 2^{o}C$ before reweighing. From these weighings block moisture contents were determined on post burial weight and mass loss calculated on preburial weight. Soil was mixed thoroughly by stirring with a glass rod to homogenise samples prior to analyses.

5.2.3. Wood analysis - determination of nitrogen content of wood

Reagents used were all of Amalar grade and volumetric glass ware grade A. Dried wood blocks were finely divided with a scalpel and approximately one third of the mass randomly collected weighed accurately then placed in a $50cm^3$ dry micro-kjeldahl digestion flask. $3cm^3$ concentrated, nitrogen free sulphuric acid (18M) was added followed dropwise by $2cm^3$ hydrogen peroxide (100 vol.) Flasks were gently heated on digestion stands and when the sulphuric acid began to fume flasks were allowed to cool prior to the addition of a further $2cm^3$ hydrogen peroxide. Flasks were reheated and the above procedure repeated with a third $2cm^3$ volume of hydrogen peroxide. In each case a total volume of $6cm^3$ hydrogen peroxide was sufficient for complete digestion indicated by the solution remaining clear on continual heating. The time for the digestion process varied between 60 - 90 minutes. Distillation was carried out as described in section 4.2.1.3. B using 40% W/V sodium hydroxide solution.

5.2.4. Soil analysis

5.2.4.1. Determination of nitrogen content of soil

Nitrogen contents of soils were determined using hydrogen peroxide in the digest as described in section 4.2.1.3.

5.2.4.2. Dehydrogenase assay

Dehydrogenase assays were undertaken after the manner of Casida et al (1964) and Burns (1978) and modified as part of this project for use with small soil samples. Approximately 1.5g of soil was weighed accurately and mixed thoroughly with 15mg calcium carbonate. The intimate mixture was transfered to a 120 x 15mm screw top test tube and saturated with $2cm^3$ 0.75% W/V 2,3,5, - triphenyltetrazolium chloride (TTC) aqueous solution and mixed thoroughly on a vortex shaker. Tubes were sealed and incubated in the dark for 24 hours at $30^{\circ}C$.

After incubation $5cm^3$ ethanol was added to each tube and mixed for 5 minutes. Heavier soil particles were allowed to settle and the liquid decanted into centrifuge tubes. Remaining solids were rinsed with a further $3.0cm^3$ ethanol and the final $10cm^3$ centrifuged at x 7000g for 10 minutes to remove lighter soil particles. About $5cm^3$ of the clear liquid was transfered by pipette to a quartz cuvette and the absorbance of the red coloured 2,3,5, - triphenyl tetrazolium formazan (TTF) produced determined spectrophotometrically (Cecil CE 202)

at 485mm. The concentration was calculated by reference to a standard curve of TTF in ethanol.

5.2.4.3. Determination of soil microbial populations

5.2.4.3.1.Preparation of agar films

The method of agar film preparation was as described by Thomas et al (1965), a modification of the original method of Jones & Mollison (1947). Approximately 1.5g of soil was weighed accurately and transferred to a mortar and ground for 5 minutes with $5cm^3$ sterile distilled water. Soil was allowed to settle and the liquid decanted into a sterile $50cm^3$ Erlenmeyer flask. This procedure was repeated three times with further $5cm^3$ aliquots of sterile distilled water but with grindings limited to 2 minutes. The $20cm^3$ soil water suspension so prepared was transferred to a $100cm^3$ Erlenmeyer flask containing $30cm^3$ of 2.5% agar agar (Oxoid L.28) kept molten in a water bath at 50^oC giving a final $50cm^3$ volume of soil suspension in 1.5% agar agar.

The soil suspension was thoroughly mixed by shaking and allowed to settle for 10 secs and small amounts removed from 10mm below the surface using sterile Pasteur pipettes. One drop was placed on the platform of a haemocytometer (Weber BS 748), depth 0.1mm and a coverslip immediately placed over the platform. The agar was allowed to solidify and the coverslip removed gently. Excess agar was removed from the moats at the sides of the platform using a scalpel and the agar

film floated off the haemocytometer below water either by gentle agitation or with a small, fine, soft bristle brush. Agar films were transferred, whilst still in water, onto glass slides. Excess water was removed by gentle blotting. Films were allowed to air dry at ambient laboratory temperatures as even minor heating causes excessive cracking of the agar film due to rapid shrinkage. Dried films were stained for 1 hour with phenolic aniline blue (Jones & Mollison, 1947) and washed 4 times in 98% V/V ethanol solution.

5.2.4.3.2. Estimation of fungal hyphal length and bacterial numbers.

Length of fungal hyphae was estimated using the method of counting numbers of intersections hyphae make with grid lines etched on an eyepiece graticule inserted in the eyepiece objective of a microscope. The method was originally developed by Olson (1950) for measuring lengths of filamentous algae.

Agar films were examined at x 1000 magnification and viewed through a 10 x 10 square grid previously calibrated to determine distance between grid lines at x 1000 magnification. Twenty fields of view, randomly chosen, per agar film were examined and three films per wood block prepared for each wood type and treatment for 1, 12 and 24 week time periods.

Numbers of intersects that hyphae make with both vertical and horizontal grid lines were counted excluding

those intersects made with the two outermost grid lines, vertically on the extreme right and the basal horizontal line. Similarly all bacteria enclosed by the outer perimeter of the grid were counted. Mean numbers of hyphal intersects and bacterial numbers were calculated for the twenty random fields of view per agar film.

Hyphal length was calculated using the equation (Olson 1950).

$$\text{Hyphal length} = \text{number of intersections} \times \frac{\pi}{4} \times \text{unit distance of grid}$$

The unit distance was the distance between one gridline and the next, 0.01mm at x 1000 magnification for the grid used in this investigation.

From these data a mean value for fungal hyphal length and bacterial numbers was calculated for the area beneath the grid. The agar film had a depth of 0.1mm, the platform depth of the haemocytometer, and the area enclosed by the grid measured 0.1 x 0.1mm. The volume of agar film examined per field of view was therefore $0.1 \times 0.1 \times 0.1\text{mm}^3 = 0.001\text{mm}^3$.

As a known mass of soil had been dispersed in the final volume of soil suspension of 50cm^3 ($50{,}000\text{mm}^3$), each field of view represented $50{,}000/0.001 = 5 \times 10^{-7}$ of the original mass of the soil. Hyphal lengths and bacterial numbers per field of view were multiplied by 5×10^7 to determine hyphal length and bacterial numbers in the original soil mass. Values so calculated were then expressed as meters of hyphae or numbers of bacteria per g dry soil.

5.3. Results

5.3.1. Wood: nitrogen content and mass loss

Results for nitrogen contents and mass losses for both copper chrome arsenic treated and untreated lime and pine blocks are presented in figs 5.1 - 5.4. During the first week of burial total nitrogen contents of all blocks increased by approximately 40% regardless of wood type or treatment. After this period rates at which nitrogen contents of blocks increased differed between wood type and presence or absence of preservative. Mass loss did not become significant ($>3\%$) in any block before week 4 and rates of mass loss differed between wood type and whether there was a preservative presence.

Nitrogen contents of untreated lime blocks (fig 5.1.) remained constant throughout the period weeks 2 to 3 after an initial increase of 0.042% during the first week. During the same period mass also remained constant at approximately 2%. In the period week 3 to 4 there was a marked increase in nitrogen contents of blocks from 0.170% to 0.220% with a concomitant increase in mass loss from 2.2% to 6.9%. During weeks 4 to 24 nitrogen content of blocks continued to increase but with a gradual diminution in the rate of increase with time. Rates at which mass loss occurred decreased between weeks 4 to 6 and thereafter remained constant with an approximate 1.8% mass loss occurring per week for the period week 6 to 24.

Results for nitrogen contents and mass loss for lime blocks treated with 0.5% copper chrome arsenic solution are presented in fig 5.2. Nitrogen content of blocks increased during the first two weeks of burial from a preburial value of 0.125% to 0.204%, thereafter until week 6, nitrogen contents remained relatively constant at approximately 0.205%. During the same period, weeks 0 - 6, mass loss remained insignificant at below 3%. Nitrogen contents of blocks increased during the period weeks 6 - 12 and reached a value of 0.234% by week 12, also during the 6 - 12 week period as nitrogen content of blocks increased there was a simultaneous increase in mass loss from 2.7% to 6.3%. After week 12 rates of increase for both nitrogen content of blocks and mass loss accelerate reaching values of 0.410% and 24.6% respectively. Such values represent mean weekly incremental increases in nitrogen content of blocks of 0.004% and 0.015% for the 6 - 12 week and 12 - 24 week periods respectively. For the same periods mean weekly rates of mass loss increase from 0.6% during the 6 - 12 week period to 1.5% for the 12 - 24 week period.

Values for nitrogen contents and mass loss of untreated pine blocks are shown in fig 5.3. During the first week of burial nitrogen contents of blocks showed a large increase of 0.049% thereafter until week 6, with the exception of week 3, there was a mean nitrogen increase of 0.004% per week reaching a value of 0.143% by week 6. In the period up to week 6 no significant loss of mass of blocks occurred. From week 6 to week 12 nitrogen contents of

blocks increased at a mean weekly rate of 0.007%, in the same period mass loss becomes significant (>3%) reaching a value of 3.8% by week 12 which represented a rate of loss of 0.5% per week. During the period week 12 - 24 nitrogen contents of blocks increase from 0.187% to 0.242% at a mean weekly rate of 0.005% and mass loss reached 9.2% representing an incremental loss of 0.5% per week.

Results for nitrogen contents and mass loss of pine blocks treated with 0.25% copper chrome arsenic solution are presented in fig 5.4. Nitrogen contents of blocks increased by 0.055% during the first week of nurial followed by a further 0.020% increase in the weeks 1 to 2 period. Nitrogen contents then increased gradually but erratically until week 12 reaching a value of 0.187%. In the period weeks 1 - 12 mass loss was not significant and only became significant (>3%) by week 24 by which time nitrogen contents of blocks were increased to 0.205% a rate of increase of 0.0016% per week.

The relationship between nitrogen contents of blocks and mass loss over the decay period of 24 weeks is shown in fig 5.5. A significant correlation exists ($r = 0.95$) between nitrogen content of blocks and the decay status of the wood regardless of wood type or treatment. Nitrogen contents of wood increased as mass loss increased however a level of nitrogen of approximately 0.200% was necessary before significant mass loss occurred.

5.3.2. Soil measurements

5.3.2.1. Dehydrogenase assays.

Dehydrogenase activity in soil within 3mm of untreated lime blocks and inert control blocks is presented in fig 5.6. The results show that during the first week of burial dehydrogenase activity showed a minor increase in soil about wood blocks but remained unchanged in soil about perspex blocks. By week 2 activity had increased in soil about both wood and control blocks but during the period weeks 2 - 3 dehydrogenase activity declined rapidly in soil about the inert controls but remained unaltered in soil within 3mm of wood blocks. During the period weeks 3 - 24 dehydrogenase activity about inert control blocks showed a gradual decline and by week 24 was at a level below that at 0 week. In soil about untreated lime blocks there was a very significant increase in dehydrogenase activity during the period weeks 3 - 4 and this corresponded with the accelerated rate of mass loss and nitrogen content of these blocks during the same period (fig 5.1). After week 4 dehydrogenase activity continued to increase but the rate of increase declined both at periods weeks 4 - 6 and 6 - 12.

The relationship between dehydrogenase activity in soil about untreated lime blocks and the nitrogen contents and decay status of such blocks is presented in fig 5.7. A significant correlation exists between soil dehydrogenase activity and nitrogen contents and mass loss of blocks. As

nitrogen content and mass loss in the wood increased dehydrogenase activity in soil increased significantly also. The significant correlation of dehydrogenase activity in soil to both mass loss and nitrogen content of blocks is expected because of the very significant correlation these two parameters showed during wood decay (fig 5.5) in this investigation.

Dehydrogenase activity in soil within 3mm of lime blocks impregnated with 0.5% copper chrome arsenic and inert controls is presented in fig 5.8. Results show that dehydrogenase activity in soils around both wood blocks and controls was broadly similar until the 4 - 6 week period at which time dehydrogenase activity began to differ. After week 6 dehydrogenase activity in soil surrounding wood blocks began to increase gradually, at the corresponding time period mass loss and nitrogen content of blocks began to increase significantly (fig 5.2).

The relationship between dehydrogenase activity in soil about the 0.5% copper chrome arsenic treated lime blocks and nitrogen contents and mass loss is shown in fig 5.9. As for untreated lime blocks there is a significant correlation between dehydrogenase activity in soil and both nitrogen content of wood and its mass loss, as the values of mass loss and nitrogen content in wood increase there is a concomitant increase in dehydrogenase activity in soil.

Dehydrogenase activity in soil within 3mm of untreated pine blocks is shown in fig 5.10. Initially increased activity in soil surrounding both wood and control blocks took place however after week 2, activity in soil

about control blocks decreased gradually. Dehydrogenase activity in soil about pine blocks was somewhat erratic during the first 6 weeks of burial after which time enzyme activity maintained a gradual increase. As for both treated and untreated lime blocks there was a positive correlation between dehydrogenase activity in soil and nitrogen contents and mass loss in blocks (fig 5.11). The graph indicates that dehydrogenase activity in soil increased contemporaneously with mass loss and nitrogen increases in wood; where no significant mass loss had taken place enzyme activity remained unchanged.

Dehydrogenase activity in soil within 3mm of pine blocks treated with 0.25% copper chrome arsenic solution and inert controls are presented in fig 5.12. The results show that after initial increased activity in the period weeks 1 - 2 enzyme activity in soil about both wood and controls decreased throughout the 2 - 24 week period. Interestingly it would appear from fig 5.13 that there is a negative correlation between enzyme activity in soil about preserved pine and mass loss and nitrogen content in wood, however mass loss had only increased to 3.6% by week 24 indicating that decay was only just significant ($>3\%$).

Dehydrogenase activity in soils about control perspex blocks in all boxes showed similar patterns of change. After increased activity during the first two weeks of burial activity declined during the remaining 22 week period. Enzyme activity in soils about different wood types and treatments showed marked variation (fig 5.14.) Untreated

lime blocks underwent the greatest degree of decay and enzyme activity in soil about these blocks demonstrated the largest increases. Presence of copper chrome arsenic preservative in lime blocks slowed rates of mass loss and inhibited dehydrogenase activity in soil about such blocks, at similar mass losses enzyme activity was much greater in soil about the untreated blocks. Dehydrogenase activity in soil about untreated pine blocks also increased with increase in mass loss from blocks, but such increases in activity are significantly less than activity in soils about untreated lime blocks at similar mass losses. In soil about treated pine blocks dehydrogenase activity decreased throughout the 24 week period giving rise to a negative correlation between enzyme activity and mass loss; activity was thus lowest in soil about such blocks. It is therefore possible to rank dehydrogenase activity in soil in decreasing order of magnitude according to the blocks from where soil was sampled: lime > lime + CCA > pine > pine + CCA; interestingly mass loss and nitrogen content of blocks demonstrate similar ranking.

5.3.2.2. Soil nitrogen.

Results for soil nitrogen values are presented in Table 5.1. The results show that only minor changes occurred in nitrogen content of soil within 3mm of blocks. Values for soil nitrogen content remained relatively constant for soil surrounding inert control blocks, whereas nitrogen

values for soils about wood blocks showed a greater degree of variation. Nitrogen contents of soils increased from week 1 to week 12 around all blocks and then remained relatively unchanged for the remaining 12 week period. For weeks 12 and 24, nitrogen values are higher for soils around wood blocks compared to control values; however, variation in nitrogen values for all soils including the control values indicate that soil nitrogen values are not significantly different.

5.3.2.3. Estimates of fungal hyphal length and bacterial numbers in soil.

5.3.2.3.1. Fungal hyphal lengths.

Estimates of lengths of fungal hyphae in soil within 3mm of buried untreated lime blocks and inert controls blocks at weeks 1, 12 and 24 are presented in Fig. 5.15 (a). The results show an increased fungal presence in soil about the lime blocks, increasing from 335m g^{-1} dry soil at week 1 to 756m g^{-1} dry soil by the 24th week of burial, an increase by a factor of x2·3. Fungal hyphal length in soil within 3mm of inert control blocks decreased slightly during weeks 1 - 12, followed by a rapid deplation of fungal hyphae down to only 18m g^{-1} dry soil by week 24. Fungal hyphal length in soil within 3mm of 0·5% copper chrome arsenic treated blocks (Fig. 5.16.) showed little alteration over the 24 week burial period. Compared to control blocks, which showed significant

decreases, these results indicate that a marked difference of fungal hyphal lengths occurred in soil about lime wood rather than inert control.

Fungal hyphal lengths in soil within 3mm of pine blocks (Fig. 5.17.) decreased in both periods, weeks 1 - 12 and weeks 12 - 24, decreasing from 219m g^{-1} dry soil at week 1 to 144m g^{-1} dry soil at week 24. In soil about 0·25% copper chrome arsenic treated pine blocks (Fig. 5.18) fungal hyphal lengths decreased from 208m g^{-1} dry soil to only 11m g^{-1} dry soil at week 24. Values for fungal hyphal lengths in soil about inert control blocks showed a similar pattern of decrease as those for treated pine blocks, fungal presence decreased from 262m g^{-1} dry soil to 27m g^{-1} dry soil at week 24.

Results for fungal hyphal lengths in soil within 3mm of all wood blocks and inert controls are presented in Table 5.2 (a). The results presented here show that lengths of fungal hyphae decreased significantly in soil around control blocks and in soil around treated pine blocks. Fungal hyphal lengths also decreased in soil within 3mm of untreated pine blocks, but the decrease was not as great as for the treated pine or inert control blocks. Lengths of fungal hyphae in soil about treated lime blocks remained unchanged during the 24 week period of burial, whereas in soil about untreated lime blocks fungal hyphal lengths showed significant increases.

Relationship between mass loss from blocks and fungal hyphal lengths in soil within 3mm of such blocks is

shown in Fig. 5.19 (a). The results show that there is a significant correlation between these two variables at both the 12 and 24 week periods. As decay increased the fungal hyphal lengths in soil about decaying blocks also increased, and where little or no decay occurred the fungal presence in soil about such blocks decreased.

5.3.2.3.2. Bacterial numbers.

Bacterial numbers in soil within 3mm of untreated lime blocks are shown in Fig. 5.15 (b). The results show that bacterial numbers increased by 330% over the 24 week burial period. Bacterial numbers in soil about treated lime blocks showed an increase of 200% over the same time period (Fig. 5.16 (b).). Numbers of bacteria in soil about inert controls showed a small decrease over the 24 week period.

Bacterial numbers in soil within 3mm of pine blocks (Fig. 5.17 (b).) showed a small increase during the 24 week burial period, from $3{\cdot}0 \times 10^7$ cells g^{-1} dry soil to $4{\cdot}6 \times 10^7$ cells g^{-1} dry soil. Numbers of bacterial cells in soil about copper chrome arsenic treated pine blocks (Fig. 5.18 (b).) remained unchanged during the 1 - 12 week period. During the 12 - 24 week period, however, bacterial numbers decreased to only $1{\cdot}8 \times 10^7$ cells g^{-1} dry soil. Numbers of bacteria in soil about inert controls remained unchanged over the 24 weeks of burial.

Relationship between bacterial numbers in soil and mass loss in blocks is shown in Fig. 5.19 (b). The results

indicate, especially at week 24, that a strong correlation exists between bacterial numbers in soil within 3mm of wood blocks and the decay status of blocks. As decay proceeded in blocks bacterial numbers in soil about blocks increased in all cases except treated pine blocks. In copper chrome arsenic treated pine blocks decay did not become significant (3%) until week 24 at which point mass loss was 3.6%. Between weeks 1 - 12 mass loss from treated pine blocks remained below 1.5% and bacterial numbers remained constant at 3.0×10^7 cells g^{-1} dry soil; however, once mass loss increased to a level greater than 3% bacterial numbers declined to 1.8×10^7 cells g^{-1} dry soil.

Relationship between fungal hyphal length - bacterial numbers and dehydrogenase activity in soil within 3mm of wood-soil interfaces is shown in Figs. 5.20 (a) (b). The measure of activity does not differentiate between the contribution each group of organisms makes to the total activity. In terms of biomass fungal mycelium, because its continuous filamentous nature results in large biovolume, dominates over bacteria with their discrete minute units which requires very large numbers (10^9) to contribute significantly to biomass. Both fungal hyphal lengths and bacterial numbers have been compared with total dehydrogenase activity; however, although such a value does not reflect the true value of enzyme activity for each group of organisms. It is likely, however, that since fungal biomass contributes the greatest proportion to total biomass the enzyme activity was probably due in the main to fungal metabolic processes.

Fig. 5.20 (a) indicates that a significant correlation exists between fungal hyphal length and enzyme activity in soil. The graph differentiates between enzyme activity and fungal hyphal lengths at both 12 and 24 weeks; each of these periods have correlations of $r = 0{\cdot}99$ (24 weeks) and $r = 0{\cdot}94$ (12 weeks) between the variables. Similarly bacterial numbers correlate significantly with enzyme activity in soils, at 24 weeks $r = 0{\cdot}97$ and at 12 weeks $r = 0{\cdot}98$.

Fig. 5.1. % Nitrogen (------) and % Mass Loss (——) in lime sapwood blocks buried for time periods indicated. Points are means of three replicate blocks and standard errors.

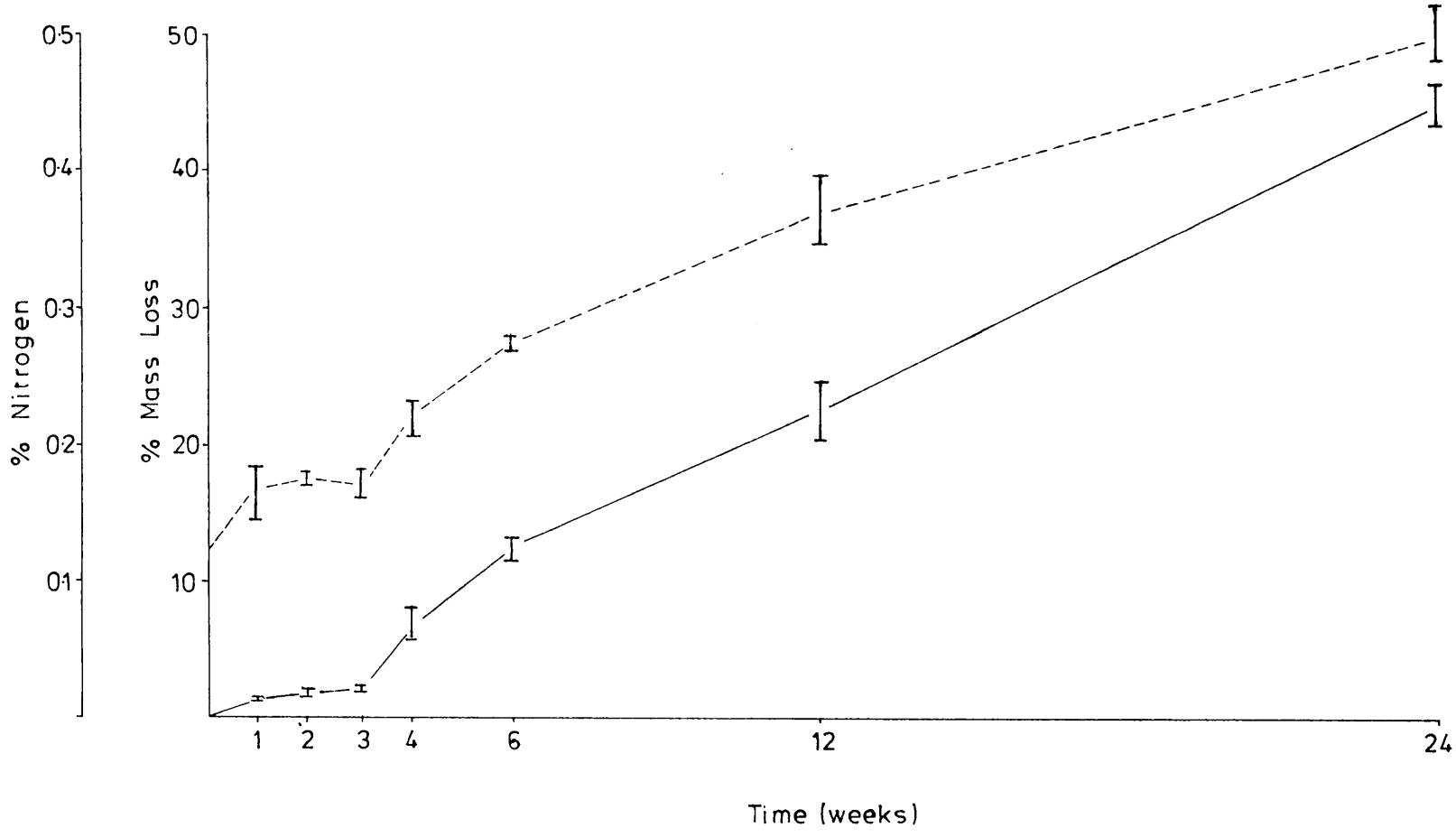


Fig. 5.2. % Nitrogen (-------) and % Mass Loss (———) in 0·5% CCA treated lime sapwood blocks buried for time periods indicated. Points are means of three replicate blocks and standard errors.

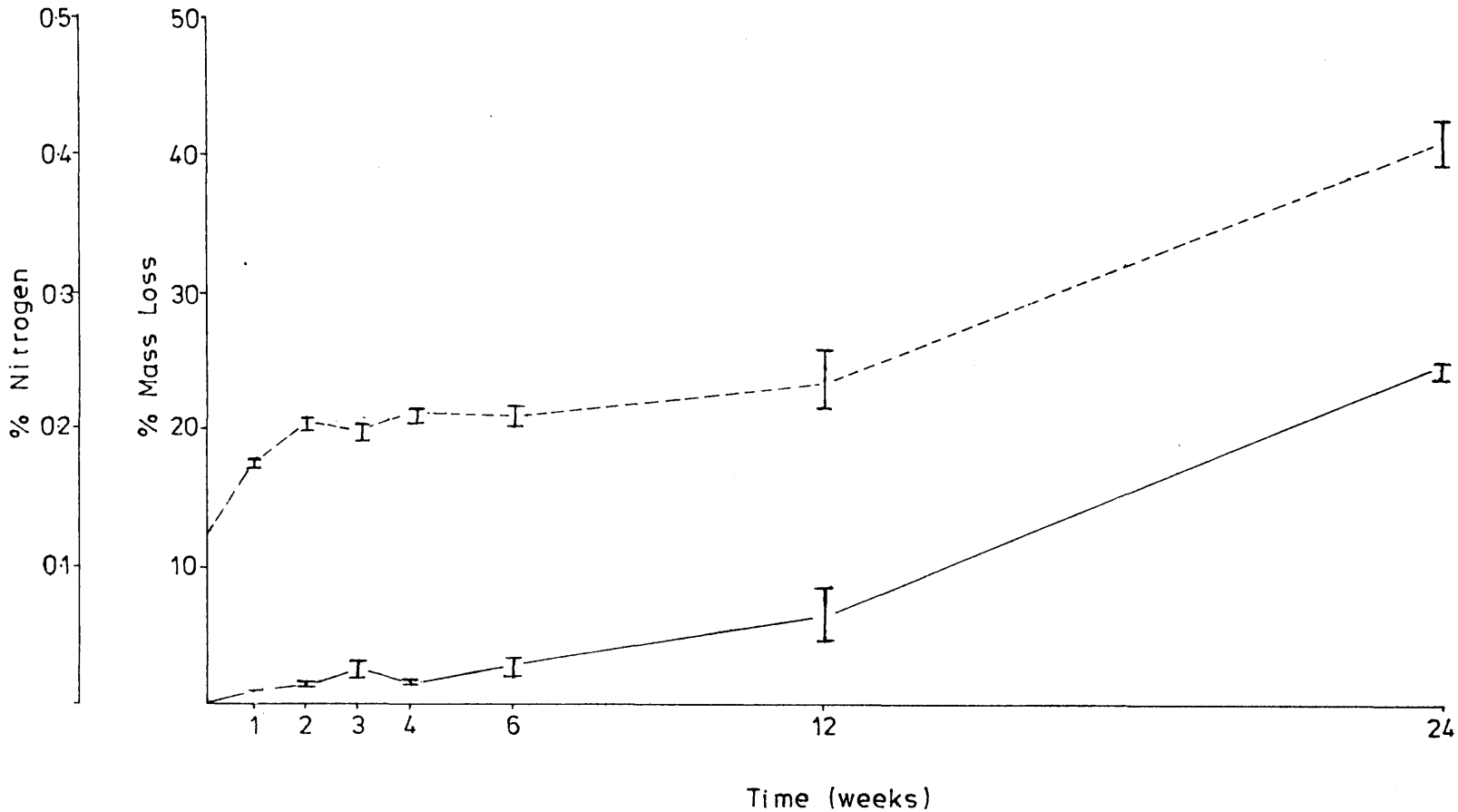


Fig. 5.3. % Nitrogen (-------) and % Mass Loss (——→) in pine sapwood blocks buried for time periods indicated. Points are means of three replicate blocks and standard errors.

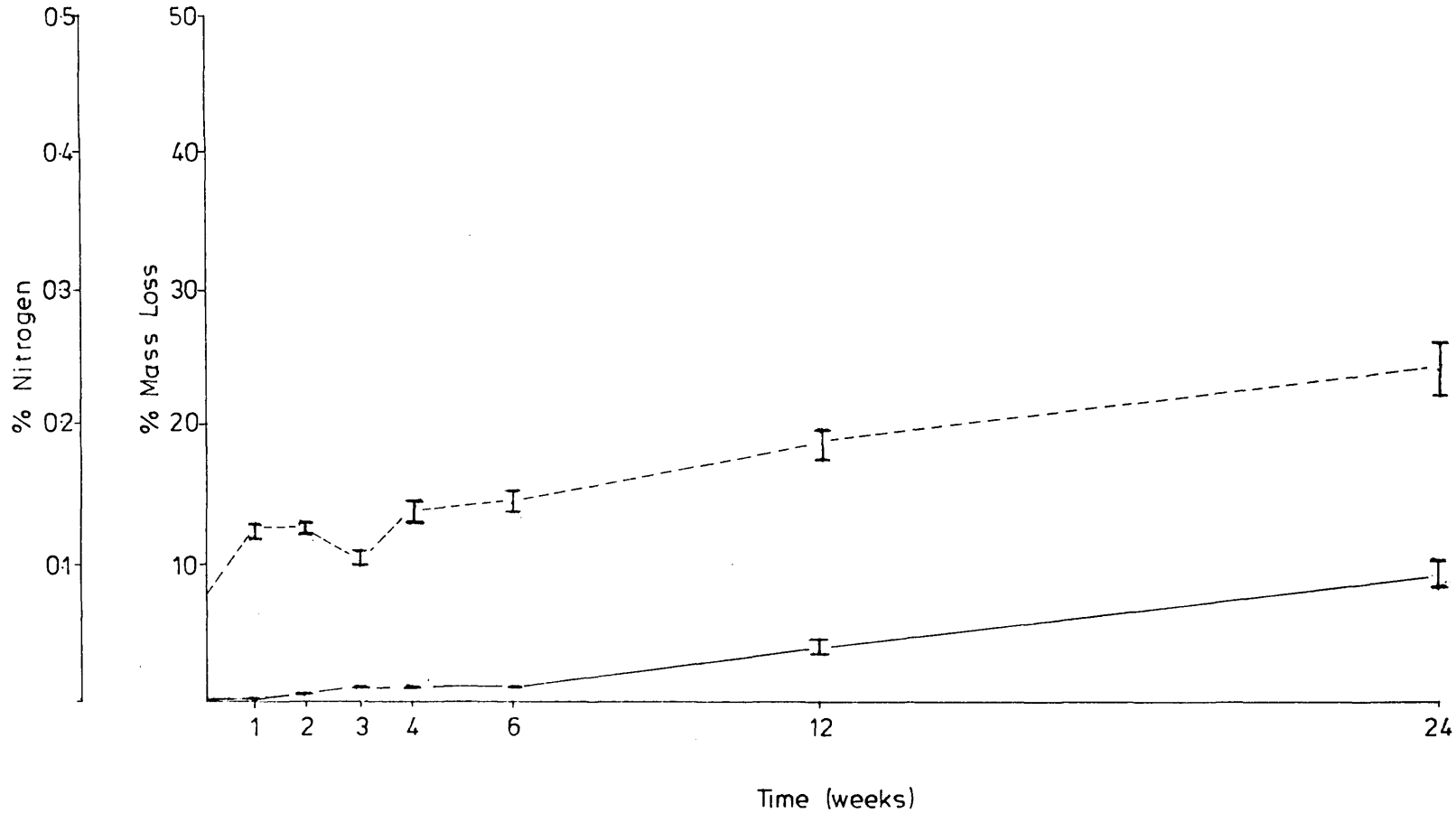


Fig. 5.4. % Nitrogen (------) and % Mass Loss (——) in 0·5% CCA treated pine sapwood blocks buried for time periods indicated. Points are means of three replicate blocks and standard errors.

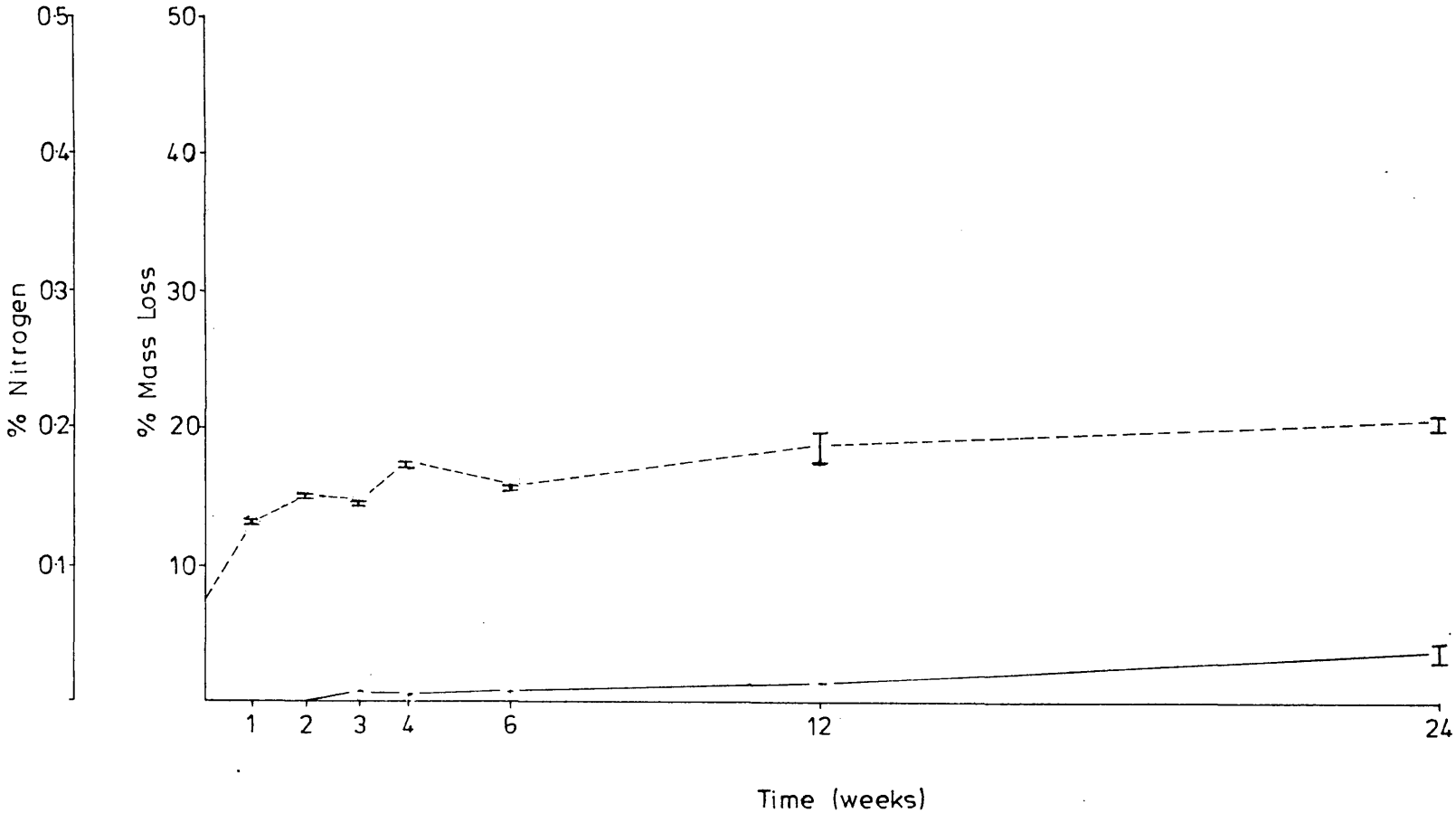


Fig. 5.5. Relationship between % mass loss and % nitrogen for both untreated and 0·5 CCA treated lime and pine sapwood blocks. Untreated lime (●); 0·5% CCA treated lime (O); untreated pine (■); 0·5% CCA treated pine (□). Points are means of three replicates.

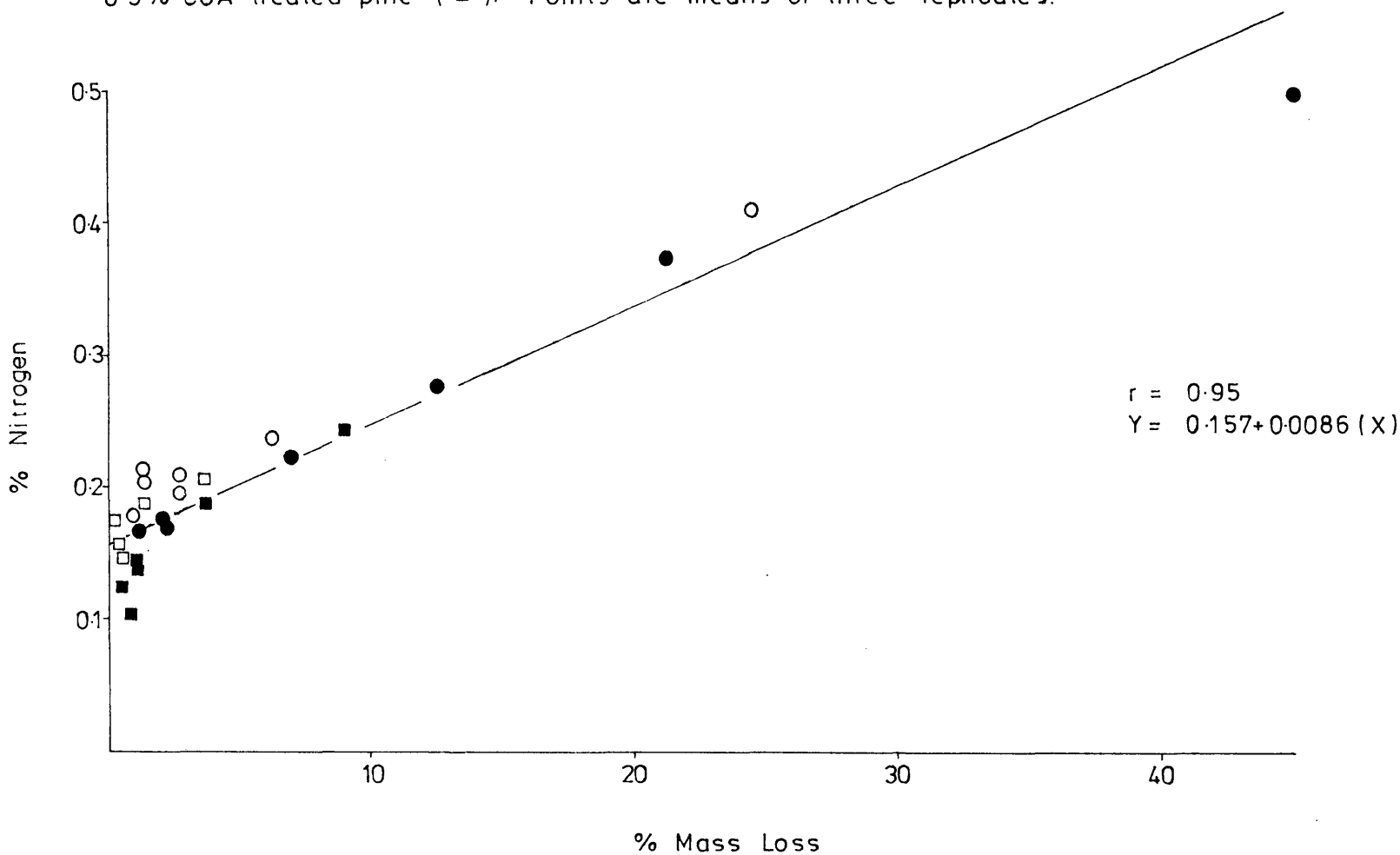


Fig. 5.6. Dehydrogenase activity in soil within 3mm of lime sapwood blocks (————) and inert plastic blocks (---------) at times indicated. Points are means of three replicate blocks and standard errors.

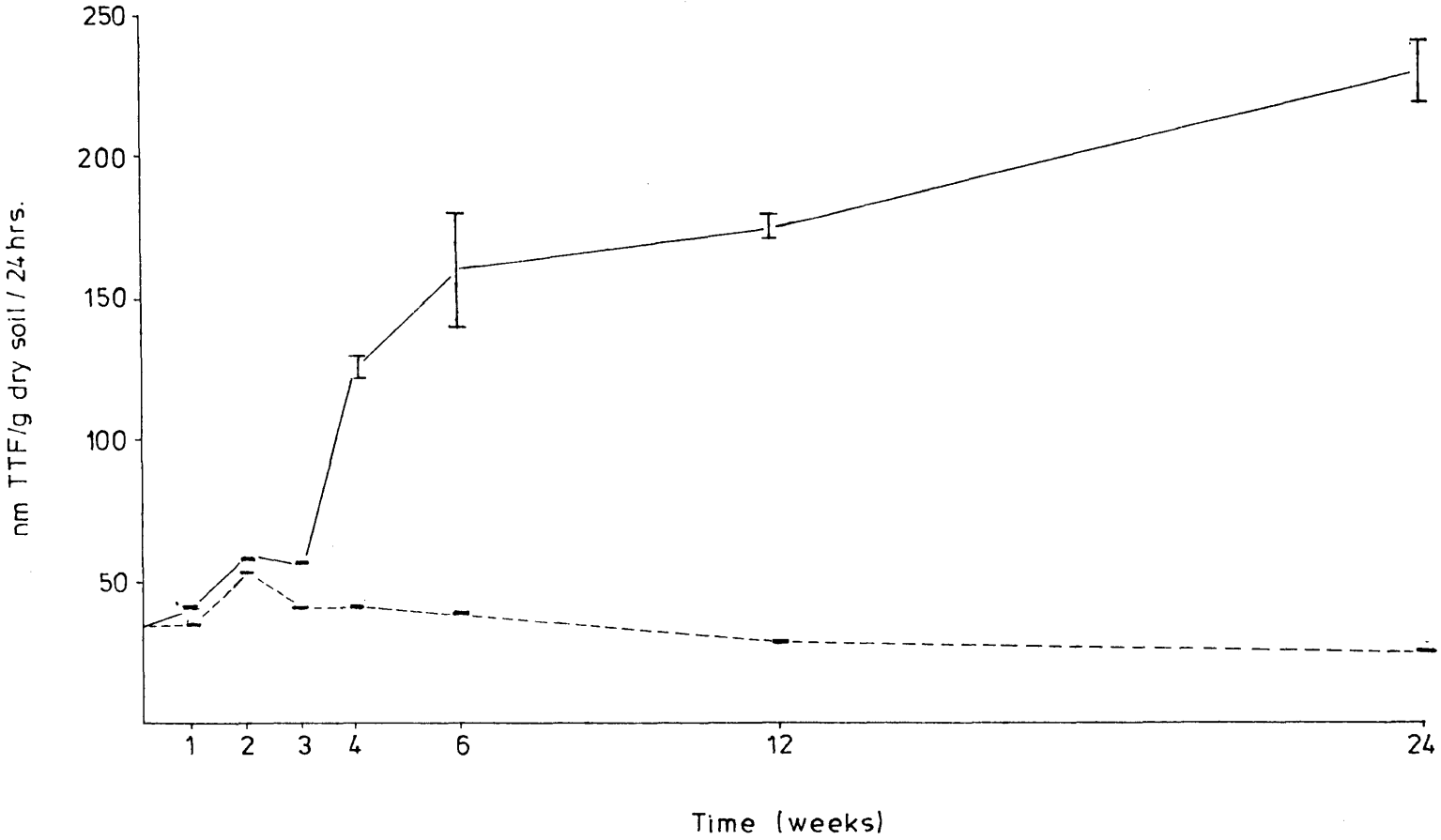


Fig. 5.7. Relationship between % mass loss (■), % nitrogen (●) in buried lime sapwood blocks and dehydrogenase activity in soil within 3mm of the blocks. Points are means of three replicates.

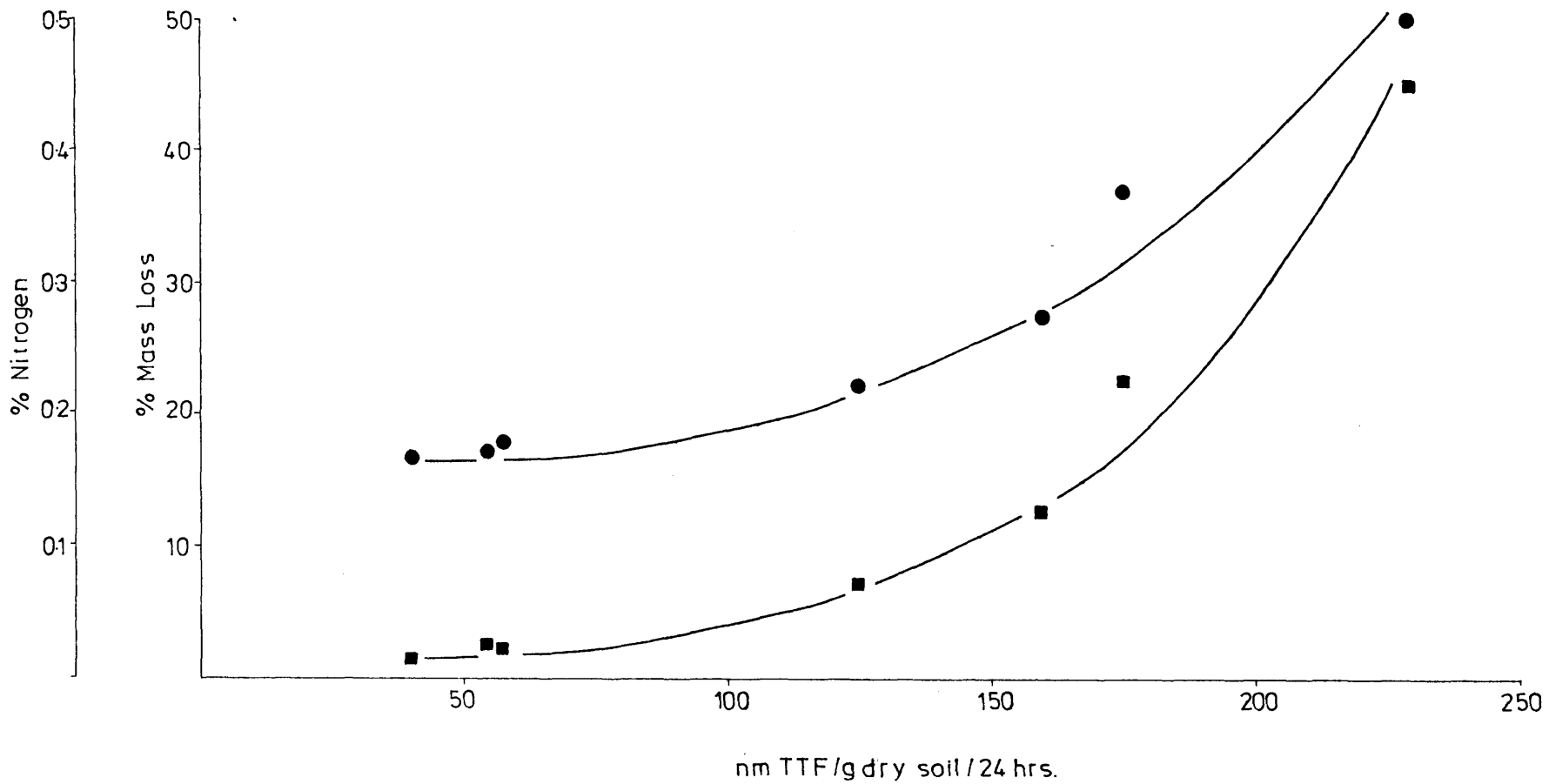


Fig. 5.8. Dehydrogenase activity in soil within 3mm of 0·5% CCA treated lime sapwood blocks (———) and inert plastic blocks (-------) at times indicated. Points are means of three replicate blocks and standard errors.

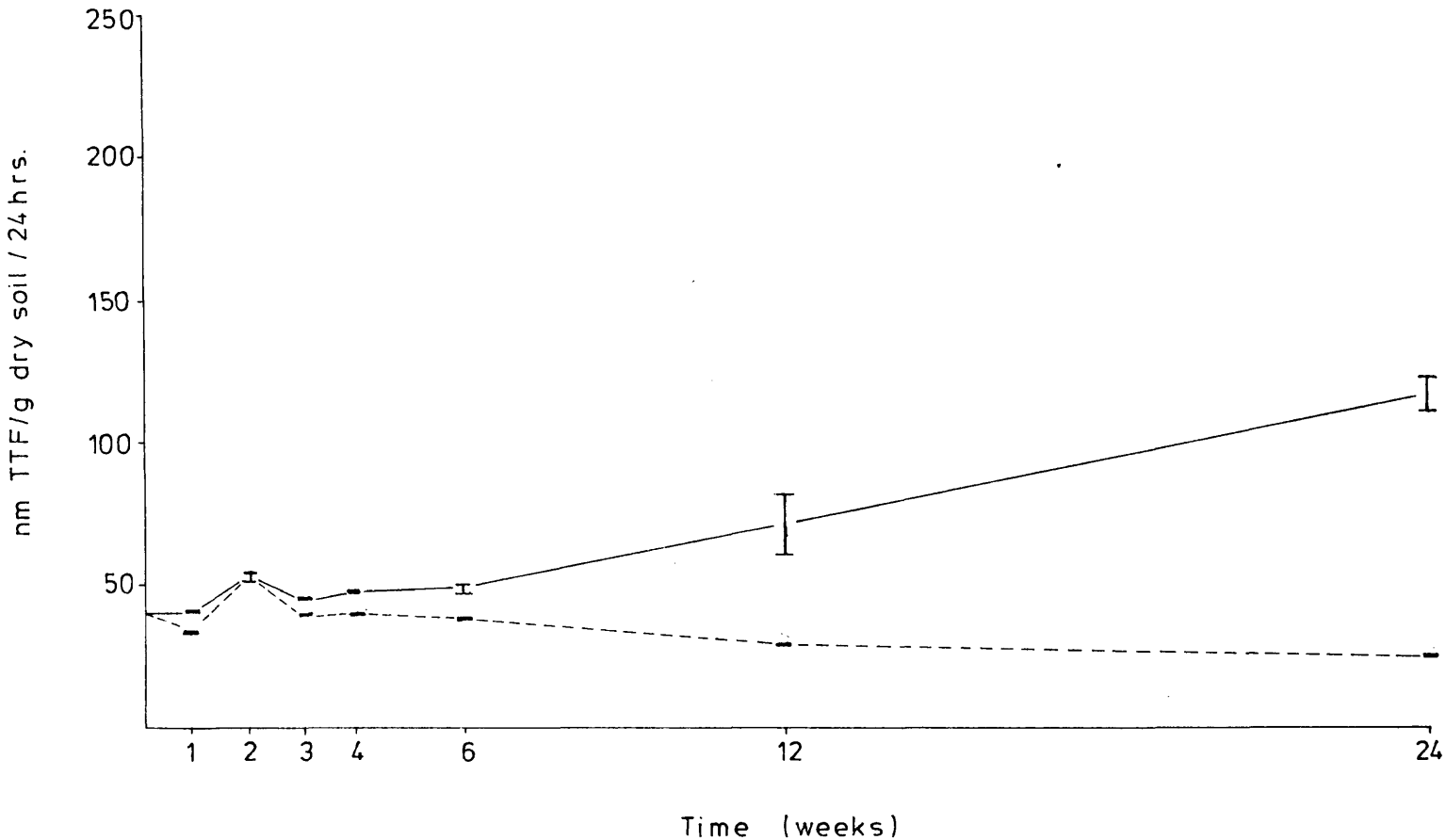


Fig. 5.9. Relationship between % mass loss (■), % nitrogen (●) in buried 0·5% CCA treated lime sapwood blocks and dehydrogenase activity in soil within 3mm of the blocks. Points are means of three replicates.

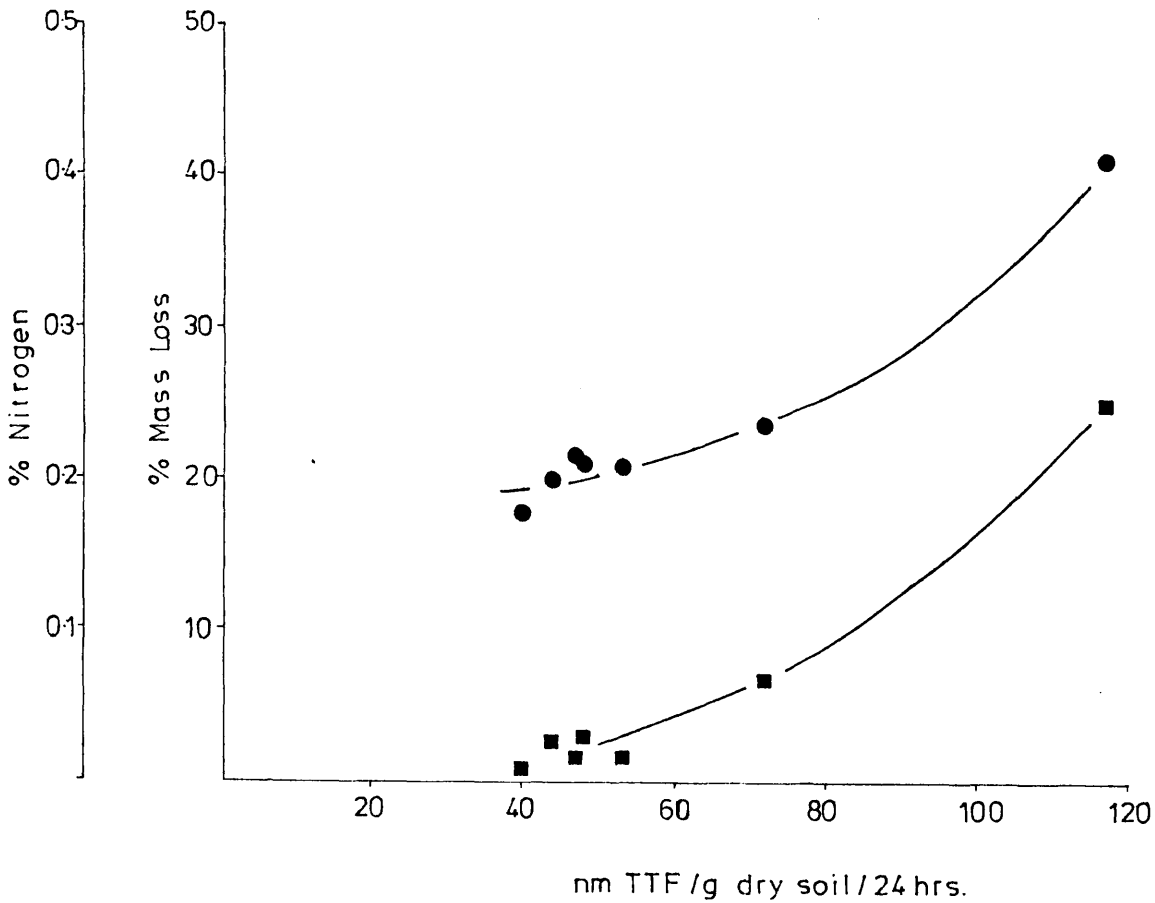


Fig. 5.10. Dehydrogenase activity in soil within 3mm of pine sapwood blocks (———) and inert plastic blocks (--------) at times indicated. Points are means of three replicate blocks and standard errors.

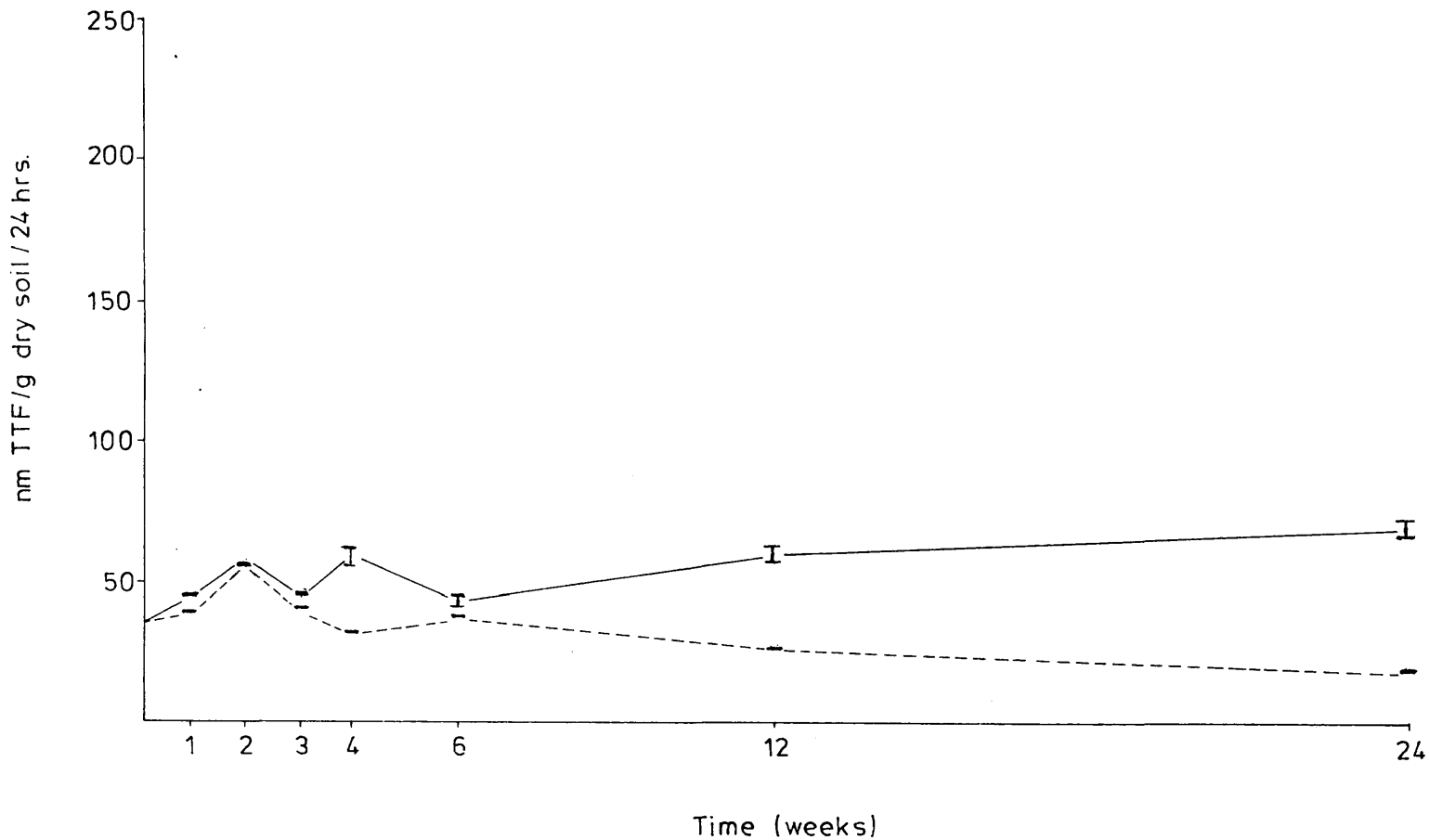


Fig. 5.11. Relationship between % mass loss (■), % nitrogen (●) in buried pine sapwood blocks and dehydrogenase activity in soil within 3mm of the blocks. Points are means of three replicates.

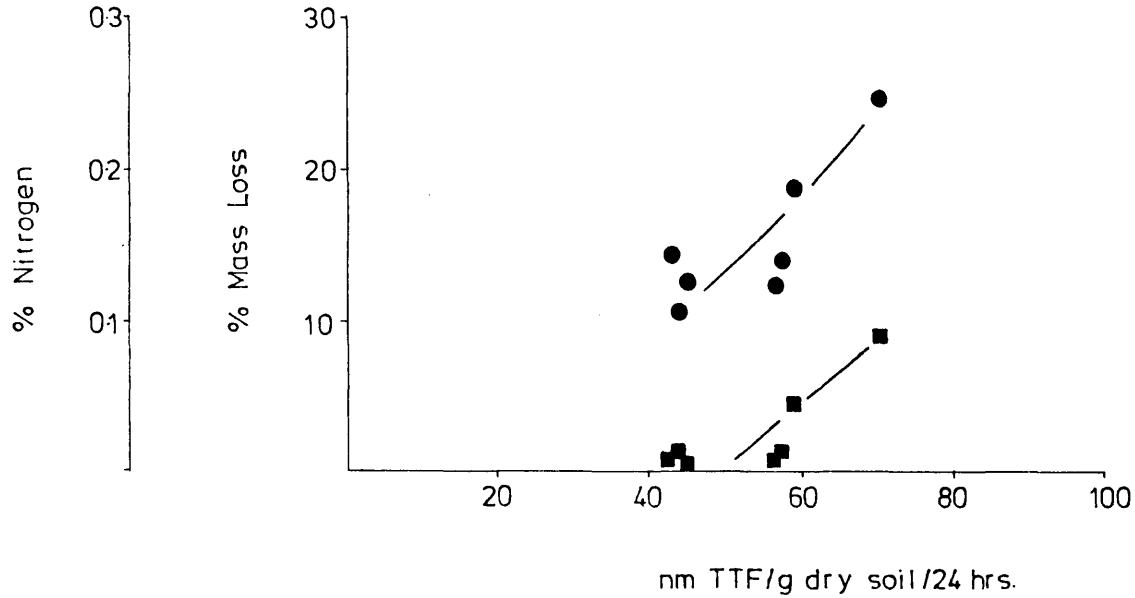


Fig. 5.12. Dehydrogenase activity in soil within 3mm of 0·5% CCA treated pine sapwood blocks (——) and inert plastic blocks (------) at times indicated. Points are means of three replicate blocks and standard errors.

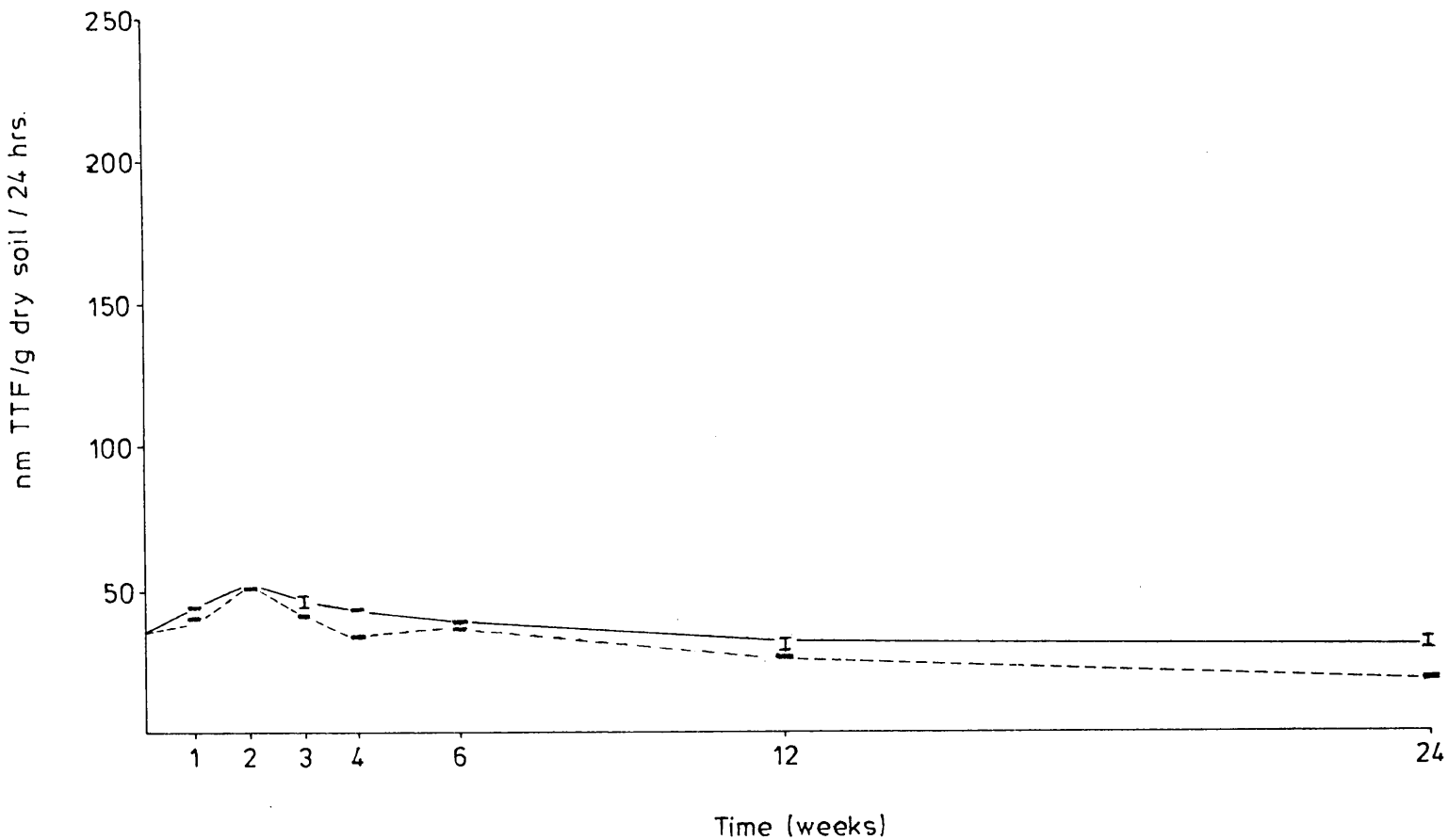


Fig. 5.13. Relationship between % mass loss (■), % nitrogen (●) in buried 0·5% CCA treated pine sapwood blocks and dehydrogenase activity in soil within 3mm of the blocks. Points are means of three replicates.

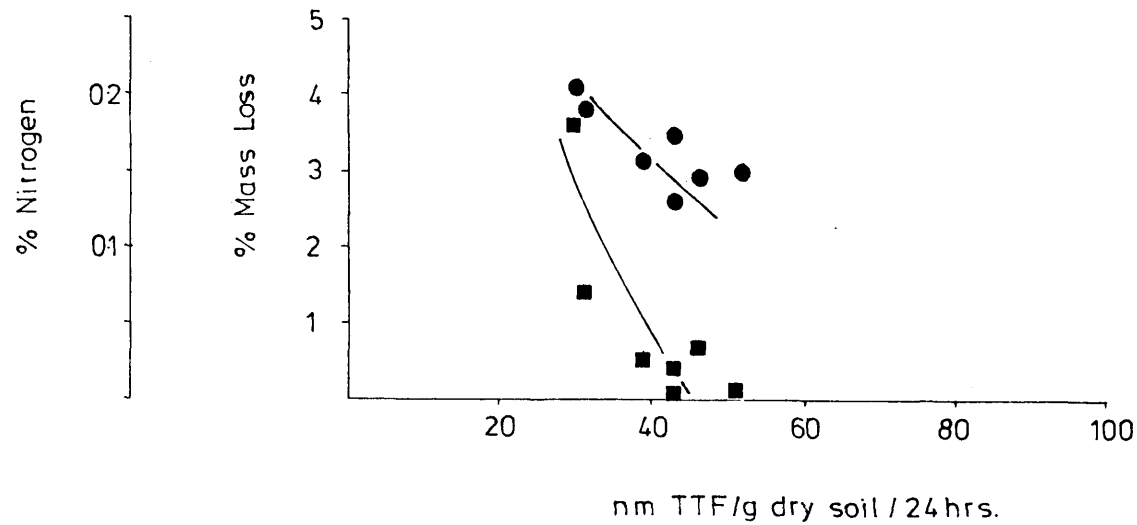


Fig. 5.14. Relationship between mass loss and dehydrogenase activity in soil within 3mm of wood blocks: lime untreated (□—□); lime treated (■----■); pine untreated (o—o) and pine treated (●····●).

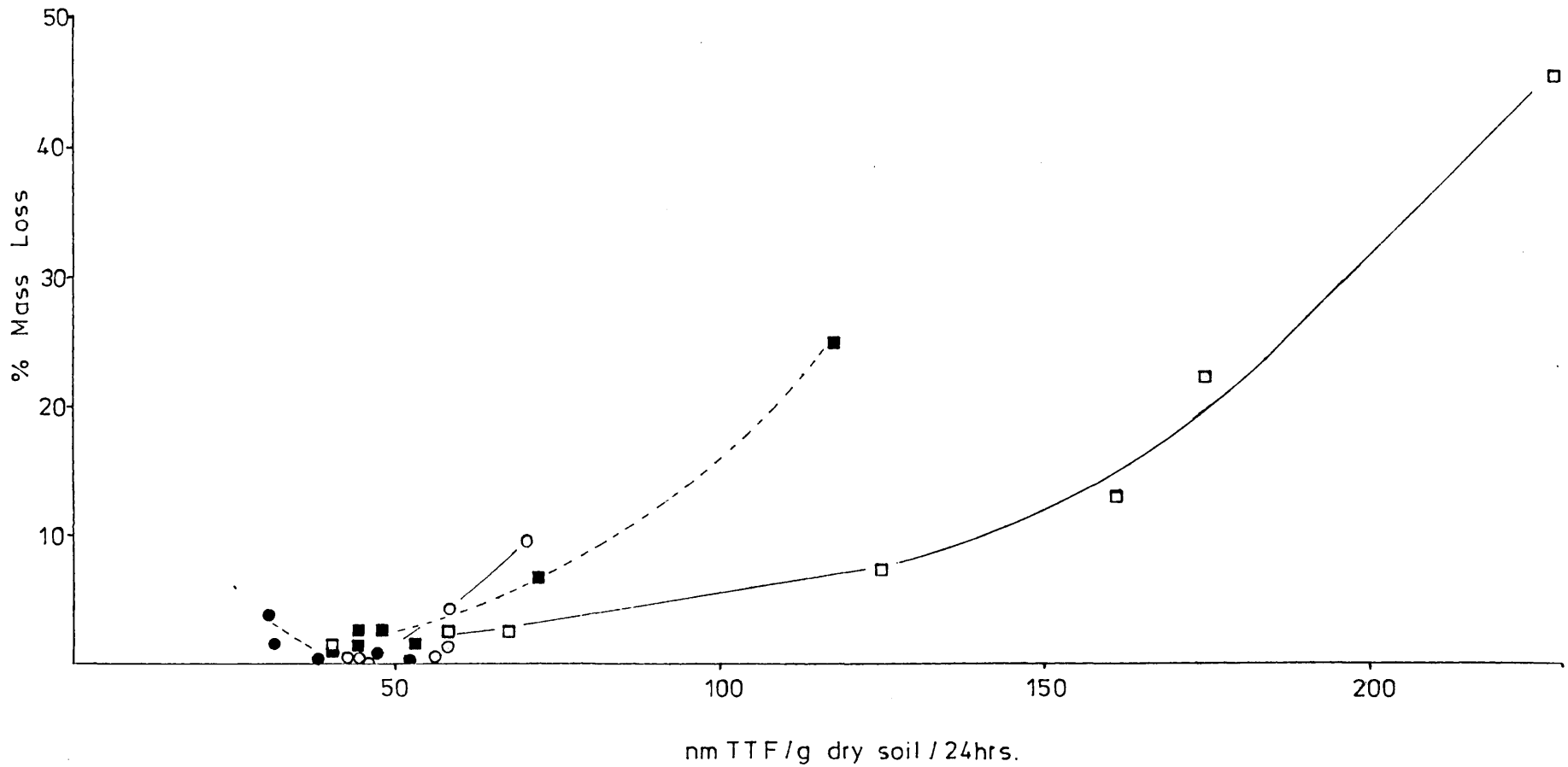


Fig. 5.15. Fungal hyphal lengths and bacterial numbers in soil within 3mm of buried untreated lime sapwood blocks and inert perspex blocks at times indicated. Points are means of three replicate blocks and standard errors. (——) wood; (– – –) perspex.

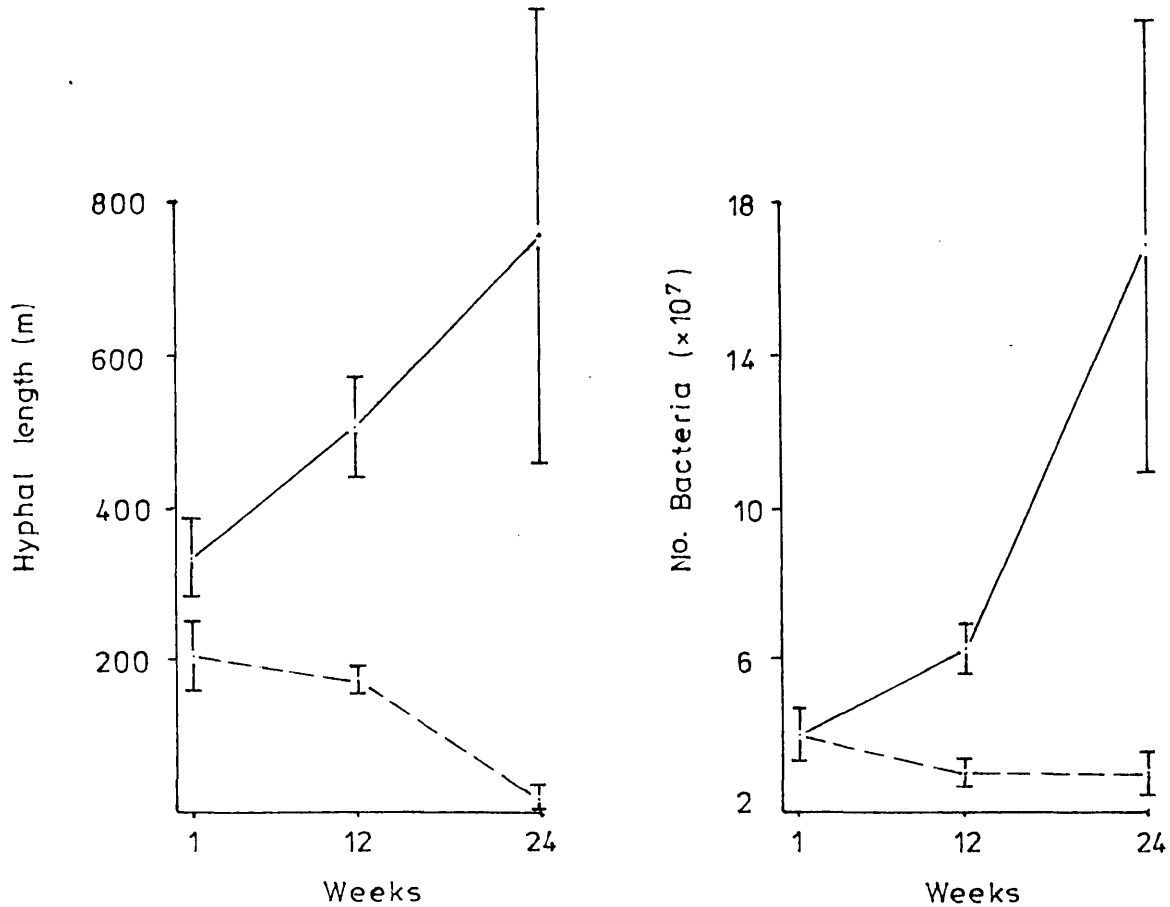


Fig. 5.16. Fungal hyphal lengths and bacterial numbers in soil within 3mm of buried 0·5% CCA treated lime sapwood blocks and inert perspex blocks at times indicated. Points are means of three replicate blocks and standard errors. (——) wood; (- - -) perspex.

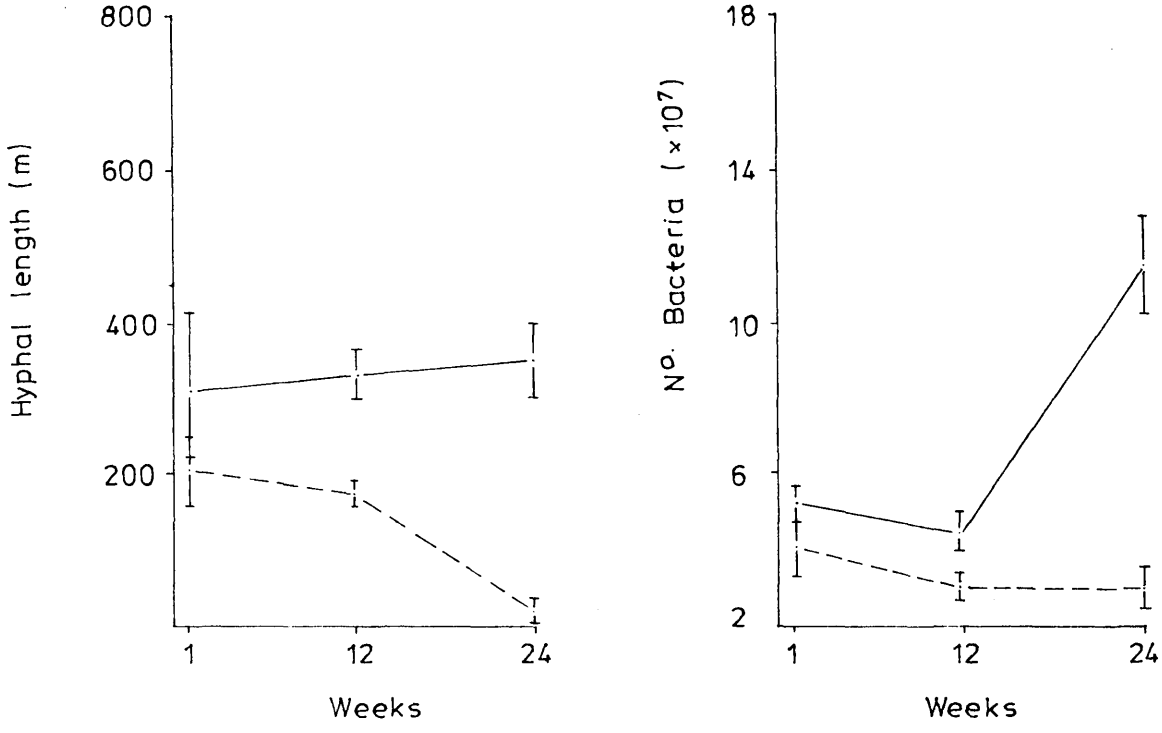


Fig. 5.17. Fungal hyphal lengths and bacterial numbers in soil within 3mm of buried untreated pine sapwood blocks and inert perspex blocks at times indicated. Points are means of three replicate blocks and standard errors. (——) wood; (----) perspex.

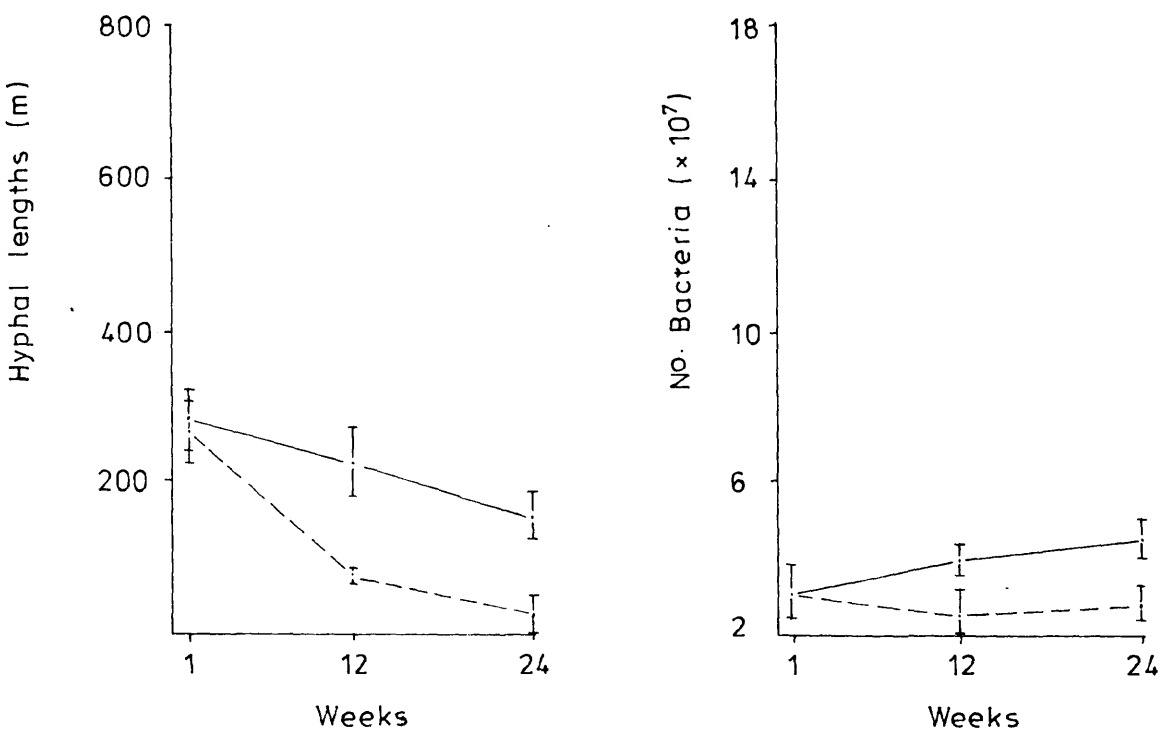


Fig. 5.18. Fungal hyphal lengths and bacterial numbers in soil within 3mm of buried 0·25% CCA treated pine sapwood blocks and inert perspex blocks at times indicated. Points are means of three replicate blocks and standard errors. (——) wood; (----) perspex.

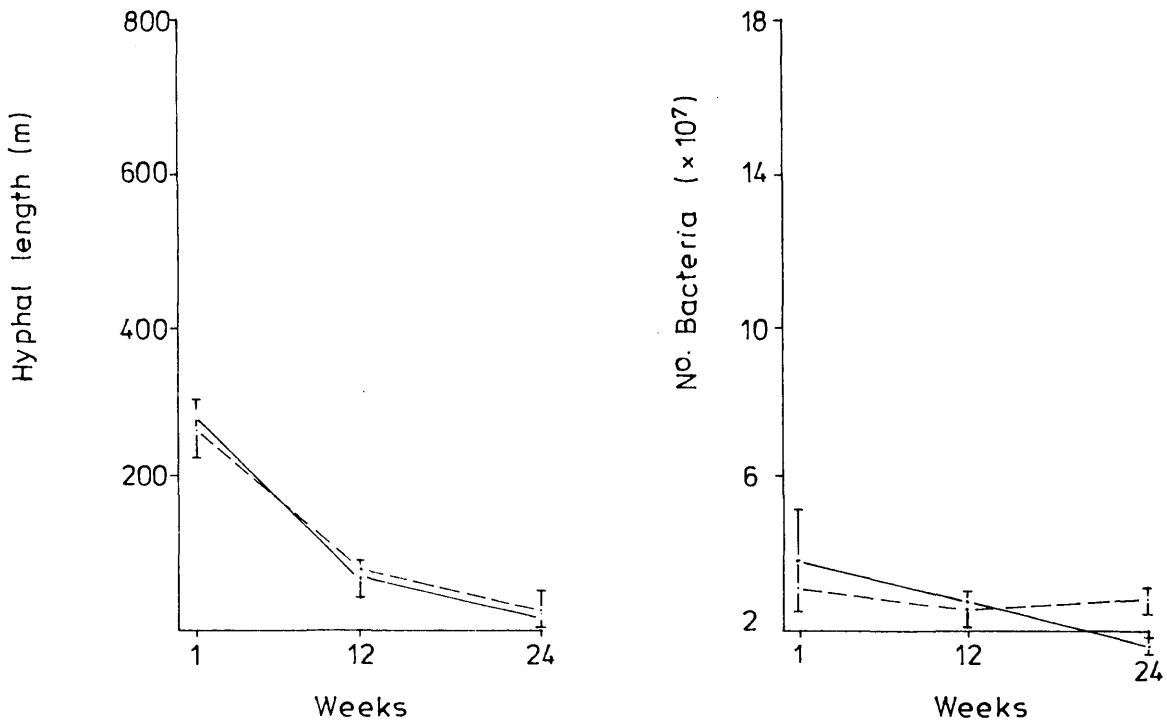


Fig. 5.19. Relationship between fungal hyphal lengths and bacterial numbers in soil within 3mm of blocks and mass loss from such blocks at 12 weeks (□---□) and 24 weeks (■—■).

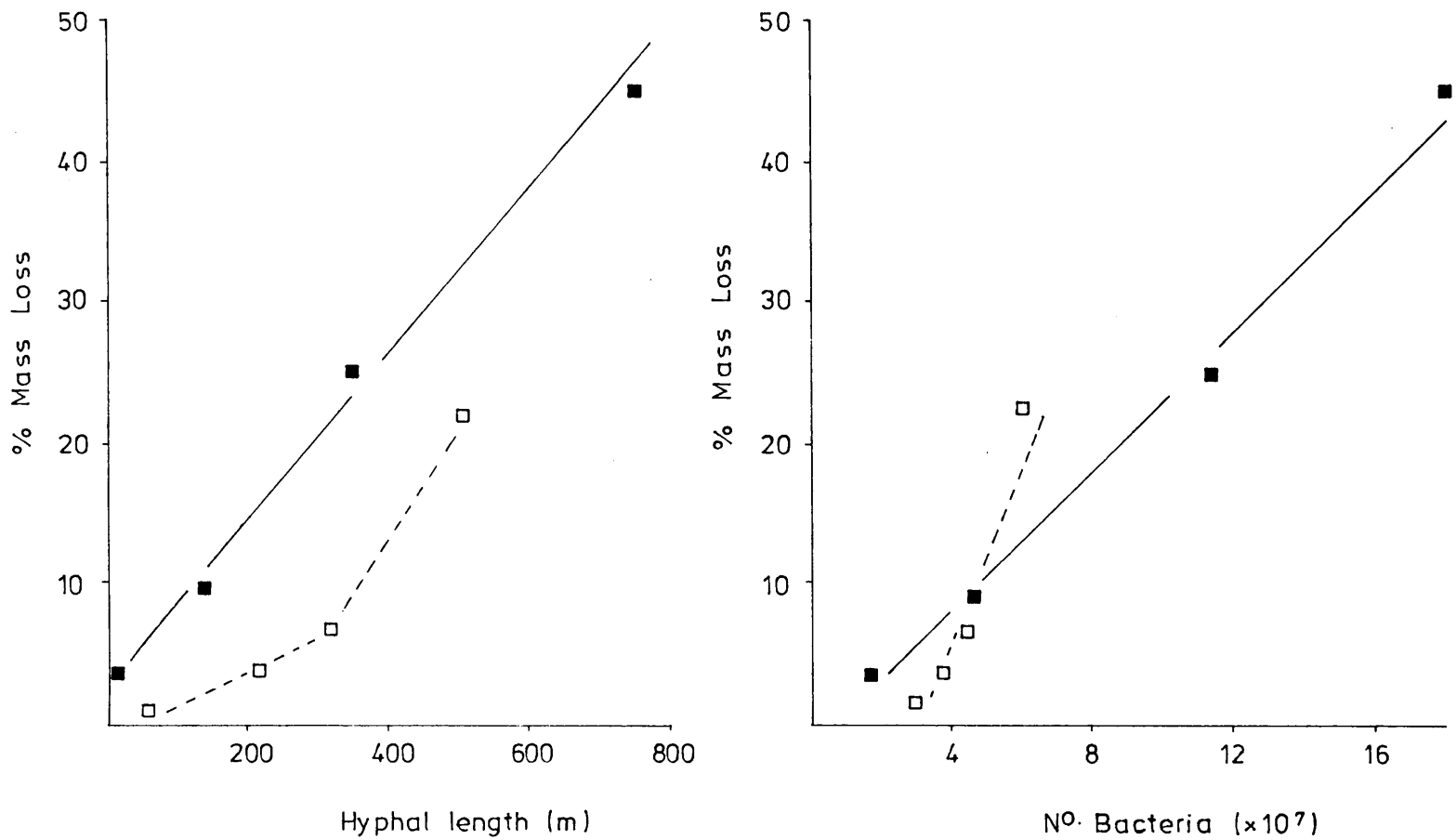


Fig. 5.20. Relationship between fungal lengths and bacterial numbers in soil within 3mm of blocks and dehydrogenase activity in same soils at 12 weeks (□---□) and 24 weeks (■—■).

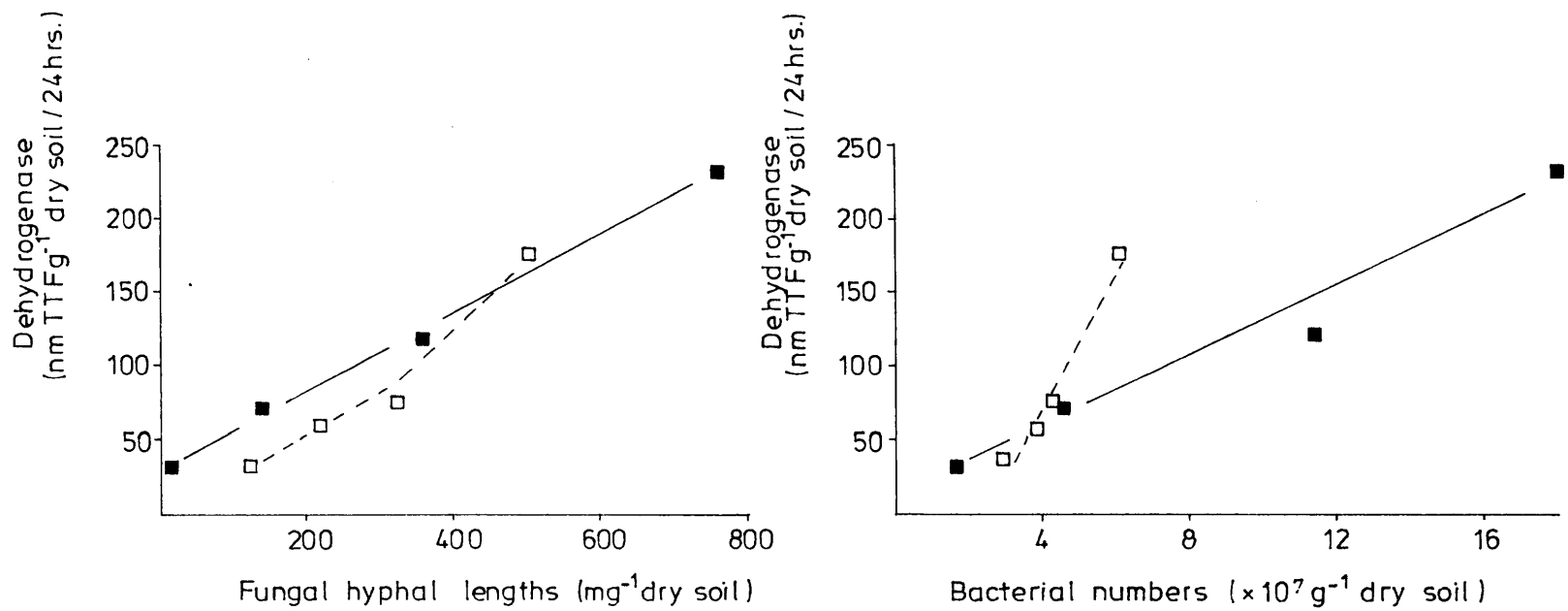


Table 5.1.

% Nitrogen contents of soil within 3mm of: 1) Lime untreated, 2) Lime treated with 0·5% W/V copper chrome arsenic, 3) Pine untreated, 4) Pine treated with 0·25% W/V copper chrome arsenic and inert controls. Figures represent means of three replicate determinations with standard deviations at time periods indicated.

		Week 1	Week 12	Week 24
Lime	Untreated	0·175±0·019	0·206±0·008	0·199±0·019
	Treated	0·164±0·002	0·209±0·008	0·219±0·038
	Control	0·191±0·018	0·191±0·005	0·188±0·011
Pine	Untreated	0·178±0·007	0·206±0·002	0·197±0·002
	Treated	0·172±0·008	0·196±0·008	0·205±0·008
	Control	0·190±0·012	0·179±0·009	0·184±0·015

Table 5.2.

Fungal hyphal lengths (m g^{-1} dry soil) and bacterial numbers ($\times 10^7$ g^{-1} dry soil) in soil within 3mm of wood blocks indicated or inert control blocks at time periods 1, 12 and 24 weeks. Figures represent means of three replicates and standard errors.

		Week 1		Week 12		Week 24	
		Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
Lime	Untreated	335±52	4·2±0·46	505± 63	6·2±0·63	756±296	17·0±6·10
	Treated	310±98	5·3±0·36	330± 33	4·5±0·51	356± 47	11·5±1·30
	Control	205±44	4·1±0·64	172 ± 15	3·0±0·33	18± 11	3·0±0·49
Pine	Untreated	283±41	3·1±0·60	218± 55	3·9±0·27	144± 25	4·6±0·50
	Treated	286±59	3·8±1·32	62± 22	3·0±0·48	11± 4	1·83±0·19
	Control	262±64	3·0±0·61	69±10	2·5±0·74	27± 18	3·1± 0·45

5.4. Discussion

One of the aims of this soil burial experiment was to monitor changes in microbial populations in soil contiguous with wood during decomposition processes. For this investigation the dimensions of blocks were selected to present to soil a large surface area resulting, on burial, in a greater wood-soil interface in contact with soil than normally achieved with the smaller 10 x 10 x 5mm blocks usually used for decay studies at this laboratory. The increased wood-soil contact was necessary to allow relatively large amounts of soil to be sampled for study and analysis. Stakes were avoided primarily to eliminate any possible wick action. This mechanism has been demonstrated to increase nitrogen contents of wood by deposition of soluble nitrogen salts from the soil solution at evaporative surfaces within the wood. Soil solution moves through stakes in response to water loss from that portion of the stake above the groundline. As water is lost it is replaced by further soil solution moving into that portion of the stake emplaced in the soil below the groundline (Uju et al, 1981).

Blocks were buried to a depth of between 40 - 60mm in well aerated soil, at between 80% - 100% water holding capacity. The presence of free oxygen is inhibitory to nitrogen fixation, nitrogenases being very sensitive to oxygen concentrations even at very low levels (Child, 1981). The well aerated soil used in this investigation would mitigate against the development of anaerobic conditions

and therefore would not encourage bacteria to fix nitrogen. Thus it was considered unlikely that significant nitrogen fixation processes would operate in wood in soils in this investigation.

Nitrogen increases were shown to occur in all blocks during the first week of burial regardless of wood type or treatment. Large increases in nitrogen content of blocks only occurred, however, in those blocks undergoing decay and a significant correlation was established between nitrogen content and mass loss of these blocks. Nitrogen contents of blocks were calculated on preburial weights and values were therefore not inflated by mass loss. It is generally considered that mass loss from wood blocks is not significant below 3%. In this study 3% mass loss occurred at a wood nitrogen value of 0·180% (Fig. 5.5.), a value in close agreement with that determined by King et.al. (1983) in other wood decomposition studies undertaken at this laboratory.

If, as earlier hypothesised that nitrogen contents of blocks would not be increased in this study by either a wick mechanism or nitrogen fixation, then another source of nitrogen is necessary. The strong correlation between nitrogen contents of blocks and mass loss plus the fact that nitrogen did not show further increases in wood after the first week unless decay occurred, strongly indicates a microbial involvement in such nitrogen increases.

Rates of mass loss and nitrogen input to blocks were closely related in both wood types whether untreated

or treated with copper chrome arsenic. However, such rates of increase in nitrogen content of wood and mass loss from wood were dependant on wood type and preservative presence. Untreated lime blocks demonstrated greater rates of mass loss and increases in nitrogen contents than untreated pine blocks. Similarly untreated wood blocks showed rates of mass loss and nitrogen input greater than copper chrome arsenic treated blocks of the same wood type. Notable is the fact that rates of nitrogen increase and mass loss were greater in 0•5% copper chrome arsenic treated lime than they were in untreated pine; such a result emphasises the observation that soft rot decay of copper chrome arsenic treated wood in soil may be primarily related to hardwoods.

Effects that wood has on soil microbial populations when wood is placed in soil were assessed by monitoring changes in microbial populations in soil adjacent to wood and evaluating changes in total microbial metabolic activity Dehydrogenase assays were used to determine metabolic activity in soil microflora. Dehydrogenase enzymes moderate transfer of hydrogen ions in redox reactions of the Krebs cycle (Smith & Pugh, 1979). 2, 3, 5, - triphenyl tetrazolium chloride (T.T.C.) is assumed to act as the terminal hydrogen ion acceptor in place of oxygen in the tricarboxylic acid cycle and be reduced to red 2, 3, 5, - triphenyl tetrazolium formazan (T.T.F.). Spectrophotometric measurement of the TTF formed thereby gives a direct indication of the rate of turnover of the tricarboxylic acid cycle. The method has, however, been criticised because TTC is considered to be

relatively inefficient in competing with oxygen for hydrogen ions and in being unsatisfactory in assessing differences in microbial activity between different soils (Benefield et. al., 1977). However, as noted by Benefield et. al. op. cit., conditions during the assay are made anaerobic, the aerobic dehydrogenases utilising the TTC as the final hydrogen ion acceptor. The advantage of using dehydrogenase assays as indicators of microbial metabolic activity in soil is that if used as a relative rather than an absolute measurement within the same soil, then preferential assessment of substrate specific organisms is avoided.

Results for dehydrogenase assays, fungal hyphal lengths and numbers of bacterial cells in soil adjacent to decayed and undecayed wood and inert perspex control blocks clearly demonstrate that decomposing wood in soil considerably modifies microbial population densities and total metabolic activity within such soils. In soil within 3mm of decaying wood, lengths of fungal hyphae, bacterial numbers and dehydrogenase activity all increased with the exception of soil about untreated pine blocks in which hyphal lengths showed a gradual decrease. In soil surrounding undecayed blocks or inert controls fungal hyphal lengths decrease and dehydrogenase activity declines, although bacterial numbers remain little altered.

Dehydrogenase activity, hyphal lengths and bacterial numbers in soil all show a strong correlation with mass loss from blocks. In untreated lime as decay proceeds with time the microbial population increases with

a corresponding increase in dehydrogenase activity. The effect of copper chrome arsenic preservative in lime blocks is to slow the rate of mass loss from blocks. At similar mass losses, however, e.g. 25% dehydrogenase activity is much greater in soil about untreated rather than copper chrome arsenic treated blocks. Untreated lime blocks lose 25% of mass loss by week 12, whereas treated blocks only reach 25% mass loss by week 24. A comparison of microbial populations in soil at week 12 for untreated lime and at week 24 for treated lime show a larger amount of fungal hyphae in soil around the untreated blocks, 505m compared to 356m in soil about the treated blocks. There is, however, significantly more bacteria in soil about the treated blocks as opposed to the untreated wood, $11{\cdot}5 \times 10^{7}$, compared to $6{\cdot}2 \times 10^{7}$ g^{-1} dry soil. Thus the increased dehydrogenase activity around untreated blocks may be due to the larger fungal population and may not be compensated for by the greater numbers of bacteria about the treated wood. From these data it can be seen that fungal populations, in terms of biovolume, predominate in the soil population examined; 1m fungal hyphae with a mean diameter of 3μmhas a volume equivalent to 7×10^{6} bacterial cells of mean volume 1μm^{3} per cell. At the 12 week period soil about untreated lime contains $35 \times 10^{8} \mu m^{3}$ fungal hyphae and only $0{\cdot}6 \times 10^{8} \mu m^{3}$ bacterial cells. At 24 weeks, soil about treated lime contains $25 \times 10^{8} \mu m^{3}$ fungal hyphae and $1{\cdot}2 \times 10^{8} \mu m^{3}$ bacterial cells. Totals of biovolumes in each soil are therefore $35{\cdot}6 \times 10^{8} \mu m^{3}$ and $26{\cdot}2 \times 10^{8} \mu m^{3}$ for soils

about untreated lime and treated lime respectively, a difference of $9.4 \times 10^{8} \mu m^{3}$. Soil about untreated lime therefore has 35·9% more microbial biovolume in soil within 3mm of the wood-soil interface, compared to similar soil about treated lime blocks, although both are at similar levels of decay, approximately 25% loss of mass. The higher level of dehydrogenase activity in soil about untreated lime compared to treated lime is therefore probably a measurement of the greater fungal presence.

The rate of mass loss from pine blocks is much slower than that for lime; untreated pine blocks had a mass loss of only 9·2% by week 24, such a level of decay had taken place in untreated lime blocks by week 5 and by week 24 mass loss was 45%. This comparative slowness of mass loss by pine compared to lime is reflected in microbial population densities and therefore dehydrogenase activity in soil about pine blocks. Both fungal hyphal lengths and enzyme activity in soil are significantly less at similar time periods than such values in soil about lime. Fungal hyphal lengths in soil about untreated pine decreased by 30% over the 24 week burial period; however, in soil about inert controls and copper chrome arsenic treated pine fungal hyphal lengths in soil decreased by approximately 92%. Treated pine blocks underwent only minimal decay with a mean mass loss of only 3·6% at week 24, a mass loss value which is generally considered to be only just significant. In soil about treated pine and inert controls, both fungal hyphal lengths and dehydrogenase activity show very

significant decreases.

The results for fungal hyphal length, bacterial numbers and dehydrogenase activity in soil about wood clearly demonstrate the pronounced effect decaying wood has on the soil microflora. As mass loss increases from wood blocks microbial population densities increase and enzyme assays reflect such increases. Interestingly, although lengths of fungal hyphae decrease in soil about untreated pine by 30%, enzyme assays indicate increased activity, which suggests that although there is a smaller fungal population, it is metabolically more active than the fungal population at the beginning of the burial.

Rates at which microbial biovolume or microbial population densities change vary between wood types and whether there is a preservative presence. Such differences in rates of population change in soil are directly correlated with mass loss from the wood, increased mass loss is reflected in increased population densities in soil. The reasons why rates of mass loss vary between wood types is not fully understood, although postulates are numerous. It is evident from the results of this investigation that significant mass loss does not occur from wood below a certain threshold level of nitrogen content in wood. In this study mass loss of 3% occurred at a mean nitrogen value in wood of 0·180% although the regression equation indicates that mass loss below 3% is initiated at 0·157%. Both lime and pine blocks used had nitrogen contents of 0·125% and 0·075% respectively,

and therefore, before mass loss could occur increases in nitrogen contents of both wood types was necessary. As pine initially has a lower nitrogen content, the time requirement to reach the nitrogen threshold value would of necessity be of a longer duration than that for lime, assuming rates of increase are similar for both wood types. The delay in initiation of decay caused whilst nitrogen contents of wood increase might explain why decay is not an immediate event, but does not explain the prolonged period of time it takes for nitrogen increases to occur in pine blocks and to some extent treated lime.

If, as postulated, that nitrogen increases are due to increased microbial presence in wood, then the delay in nitrogen increases in pine and treated lime must be attributable to a mechanism which hinders invasion processes occurring prior to and during wood decomposition.

Clearly, the results presented in this chapter indicate conclusively that decaying wood in soil has a significant effect on soil microbial populations and their metabolic activity. Soil testing may possibly be utilised as a method of predictive testing for wood decay in soil. Enzymatic and population studies on soil adjacent to wood could be evaluated to determine the degree to which decay has taken place. The results for soil nitrogen content indicated a raised level of nitrogen in soil about wood, but not at a sufficient level to be considered significant. It may perhaps be necessary to delimit the volume of soil sampled to within 1mm of wood-soil interfaces before the

nitrogen content of soil could be used as a parameter of measurable significance.

Further work is necessary to determine accurate methods for determining microbial populations and metabolic activity in wood itself. Without such methods it is not possible to conclusively demonstrate and quantify that increases in nitrogen contents of wood during decay are solely attributable to microbial biomass.

The investigation presented in this chapter does, however, clearly demonstrate that far from being inert and passive, wood has a profound influence on the microorganisms and the extent of microbial activity in soil adjacent to wood-soil interfaces, and that analyses of soil about wood may be useful indicators in themselves of the events taking place in wood during the decomposition process.

The results show specifically that:

1. The nitrogen content of wood totally buried in soil increases during the decay process, and that such increases are strongly correlated with mass loss from the blocks.
2. Dehydrogenase activity in soils within 3mm of wood-soil interfaces increase significantly only about wood undergoing decay, except for such activity in soil about 0·25% CCA treated pine, where enzyme activity decreases at least in the early stages of decay.
3. Fungal hyphal lengths increase in soil within

3mm of lime blocks undergoing decay, but decreases slightly about pine blocks which had also undergone significant mass losses. In soil about control blocks and 0·25% CCA treated pine blocks, fungal hyphal lengths decreased to low levels.

4. Bacterial numbers increase significantly in soil about wood undergoing decay, but decrease in soil about controls and 0·25% CCA treated pine blocks.

5. Both fungal hyphal lengths and bacterial numbers are significantly correlated with mass loss and therefore nitrogen content of the wood.

Chapter 6.

Effects of physical barriers on decay of wood in soil contact.

6.1. Introduction

The previous chapter described investigations of wood decay in soil and clearly demonstrated that during decomposition processes events occur in both wood and soil adjacent to wood which are inter-related. Microbial population densities and enzyme activity of microorganisms in soil showed very significant correlations with the decay status of wood as measured by mass loss. If, as proposed in the last chapter, nitrogen increases in wood during decay are a consequence of microbial biomass transfer from soil to wood, then physical barriers at wood - soil interfaces may inhibit such transfers and delay decomposition processes.

Chapters 2 and 3 demonstrated that wood acted as a chemostimulant towards both fungi and bacteria. Chemostimulation of the soil microflora by wood volatiles or soluble nutrients might be the initiator of such proposed microbial transfer to wood and hence increase nitrogen content of the wood. Barriers impervious to simple wood nutrients and wood volatiles might not only prevent microbial transfer across wood - soil interfaces simply by their physical presence but also by disrupting the chemostimulatory mechanism.

The aim therefore of this investigation was to determine the effects physical barriers closely applied to wood stakes had on microbial population changes in both wood and soil during prolonged exposure to soil. Stakes were chosen rather than completely buried blocks as soft rot decay of wood in soil often occurs with most severity at the ground line. The positioning of physical barriers was chosen to

protect the most vulnerable area of stakes and therefore such barriers extended above and below the groundline.

Lime was chosen as the wood type because a comparison was available for completely buried blocks from chapter 5. Stakes were treated with 0.5% W/V copper chrome arsenic, a concentration known to allow soft rot attack, from previous studies at this laboratory, but which also limited decay by potential basidiomycete colonisers.

As yet no satisfactory method has been developed to determine and quantify microbial presence in wood. The use of dehydrogenase assays as a relative indicator of microbial activity in soil was considered a useful technique as dehydrogenase activity in soil showed significant correlation with the density of microbial population present therein. In being non-substrate specific the assay also evaluates metabolic activity amongst a diverse spectrum of organisms and are not substrate specific and thus limited to specific flora as cellulase assays would be. Such assays would evaluate microorganisms capable of degrading cellulose as a substrate but omit those which might be scavenging for simpler nutrients. Using dehydrogenase assays on wood might therefore give an indication of microbial population densities in wood and be of use in evaluating the effectiveness of physical barriers.

The purpose of the experiments described in this chapter were to:

1. Determine effects of physical barriers on decay of wood in soil contact.

2. Evaluate effects of physical barriers on nitrogen transfer to wood from soil.
3. Determine effects of physical barriers on dehydrogenase activity in soil.
4. Compare dehydrogenase activities in wood and soil.

6.2. Materials and methods

6.2.1. Preparation of wood blocks

Quartersawn planks of lime (Tilia vulgaris Hayne) were dried in a fan oven at 40°C and stored at ambient laboratory temperatures in a ventilated cupboard for at least six months. Planks then had radial faces removed to a depth of 2mm to eliminate wood with nutrient rich surfaces. (King et.al.1975). Stakes measuring 80 x 20 x 5mm were accurately cut from the sapwood of such planks so that the 20 x 5mm faces were in transverse section, the 80 x 20mm faces in radial section and 80 x 5mm faces in tangential section. Stakes were dried to constant mass in an oven at 102° ± 2°C and weighed and then treated with 0.5% W/V copper chrome arsenic solution.

Impregnation of stakes was carried out by weighting down stakes with glass slides in glass containers placed in a vacuum dessicator. A vacuum was drawn for i5 minutes followed by the introduction of the copper chrome arsenic solution and releasing any residual vacuum (BS 6009:1982). Stakes were left submerged for 30 minutes, removed from

containers, blotted dry and placed on 80 x 5mm faces on glass sheets in a large chromatography tank. A small volume of xylene in a glass container was introduced into the tank and the tank sealed with a glass cover sheet. Stakes were turned onto alternate 80 x 5mm faces twice weekly. The tank was kept sealed for two weeks, partially open for the third week and fully open for a final week. Stakes were stored in ventilated sterile glass petri dishes for a period of eight weeks prior to leaching.

Stakes were leached by soaking in glass distilled water for 24 hours with 10 stakes per 6 litres of water in each container. Stakes were removed and placed on plastic mesh in a vacuum dessicator and a vacuum drawn for 10 minutes, extracted liquid running away through the mesh. Stakes were blotted dry and impregnated with fresh sterile glass distilled water as described for the copper chrome arsenic solution. Stakes were then soaked for a further 72 hours in glass distilled with a change of water every 24 hours. Stakes were removed from the water, blotted dry and air dried to approximately 100% moisture prior to burial.

Before burial half the stakes were fitted with heat shrinking polyethylene collars to act as barriers. The collars were formed from thermofit plastic tubing (Tuga Nr.12 Black Raychem Ltd. England) which contracts upon heating above 120°C to a minimum diameter of 9.3mm, such shrinkage taking place in approximately 2 seconds. One of the two 20 x 5mm transverse faces was labelled 0mm to aid orientation, such faces being the transverse face above the groundline on burial. 80mm

lengths of plastic tubing were fitted around each stake and the tubing gently heated, with continual movement in a bunsen flame made just non-luminous for approximately 8 seconds. Upon heating the diameter of the tubing contracts forming a tightly fitting collar around the stakes. Excess plastic was removed using a sharp scalpel so that plastic covered that length of the stake between 10 - 50mm from the 0mm labelled transverse face.

6.2.2. Burial

Moisture content and water holding capacity were determined for soil previously sieved through a 2mm screen (Savory,1973.). Preweighed plastic containers, measuring 300 x 210mm and 140mm deep, were filled to a depth of 100mm with soil and reweighed. 15 stakes fitted with plastic barriers and 15 uncovered stakes were randomly inserted, 10 to each container, to a depth of 60mm with 20 x 5mm faces in the horizontal plane leaving a 20mm section of the stake above the groundline and the 0mm labelled transverse face uppermost. In this orientation stakes fitted with plastic barriers were protected by such barriers to a distance of 10mm above soil surfaces and 30mm below soil surfaces.

Boxes were weighed and wetted evenly over surfaces with enough distilled water to bring the soil to 80% of its water holding capacity and incubated in the dark at 25°C for 24 weeks, a length of time shown to be necessary for adequate decay to take place in 0.5% copper chrome arsenic lime blocks from other work undertaken at this laboratory. Boxes were weighed twice weekly and where necessary distilled water

added evenly over the soil surface to maintain it at 80% of its water holding capacity.

Stakes were recovered together with soil within 3mm of wood or plastic covered surfaces for the whole 60mm buried portion of the stake. This was achieved using a thin, tin plated, steel sheet folded into an open ended rectangular mould similar to that used for the recovery of buried block in chapter 5. The mould was positioned on the soil surface by measurement and then inserted carefully so that the mould remained normal to the soil surface and fully enclosed the stake. In two cases the mould deviated by more than 1mm from the normal and in such cases these stakes were not included in the results.

Both plastic covered and uncovered stakes were seperated into two sets, one set used to determine nitrogen content of wood, mass loss and moisture content and the other set for dehydrogenase activity. Determination of wood nitrogen content and mass loss necessitated drying wood at above 100°C thereby destroying activity in most microorganisms present. A second set of stakes were therefore used to determine dehydrogenase activity of the microorganisms present in the wood and in the soil about those sections of wood chosen. In all analyses wood was sampled from three positions of the stake, taking 0mm to represent the top of the stake above the groundline then samples were removed from between 0-10mm, 35-45mm and 60-70mm, designated sections 1.2. & 3 respectively the 35-45mm section being beneath the protective plastic barrier in those stakes fitted with such barriers. Soil was

sampled for dehydrogenase assay with 3mm of the 35-45mm and 60-70mm sections. Five replicate stakes with barriers and five without barriers were used for dehydrogenase assay and seven replicate stakes with barriers and seven without barriers were used for nitrogen content, mass loss and moisture content determination.

The mould containing stake and surrounding soil was laid flat on a clean glass sheet and opened along its unfixed edge. For each of 5 uncovered stakes and 5 protected stakes chosen at random from the twelve of each type a 10mm wide band of adjacent soil was carefully removed at two positions (i) 15-25mm below groundline (35-45mm from the 0mm labelled transverse face) and (ii) 40-50mm below groundline (60-70mm from the 0mm labelled transverse face) which was sufficient soil for one dehydrogenase assay. Dehydrogenase activity was assessed on each of these samples as described in chapter 5.

For the 5 protected stakes the plastic barriers were removed by cutting along their lengths. Then together with the 5 stakes which had no barrier 10mm wide sections of wood were accurately cut out at positions matching those from which the soil had been sampled,35-45mm and 60-70mm sections from the 0mm labelled face. The wood sections were weighed and then finely divided into thin slivers using a scalpel and a clean glass sheet. Dehydrogenase measurements were carried out as for soil. Heat sterilised wood of the same dimensions, wetted with sterile water, were used as controls.

From the remaining seven plastic covered and seven unprotected replicate stakes 10mm wide sections of wood were

cut accurately from three positions. (1) 0 - 10mm, the topmost section of the stake and above the groundline (ii) 15 - 25mm below groundline (35 - 45mm from the 0mm labelled transverse face) (iii) 40 - 50mm below groundline (60 - 70mm from the 0mm labelled face). Such 10mm wide sections of wood were considered to have $\frac{1}{8}$th or 12.5% the mass of the original stake prior to any mass loss during burial.

The 10mm wood samples were weighed wet and then dried in an oven at 102°C ± 2°C to constant mass and reweighed. Mass loss was calculated on preburial weight which was calculated as 12.5% of the original mass of the stake. Moisture content was calculated on the post burial mass of the wood. Each sample was then divided into approximately two halves, each half weighed accurately and nitrogen contents determined for each half of the wood section by micro Kjeldahl procedures described previously in chapter 4 and calculated on the preburial mass of the wood.

6.3. Results

Results for mass loss from wood are presented in fig 6.1. The results show that mass loss occurred in all sections of wood below the groundline. Decay did not take place in those sections of wood above the groundline and therefore not in soil contact. Wood sampled from between 15 - 25mm below the groundline (section 2) had mean mass loss values of 5.0% for those stakes fitted with plastic barriers and 13.99% for matched stakes without barriers,

t - test for the significance of the difference indicates that the probability P = 99.9% (0.001). Wood sampled from between 30 - 40mm below the groundline (section 3) had the highest mass loss values, 25.65% for stakes fitted with barriers compared with 19.70% for stakes not fitted with barriers, significantly different at a probability P = 95% (0.05).

Results for nitrogen contents of wood are shown in fig 6.2. Prior to burial nitrogen content of wood was 0.125%, therefore in all sections nitrogen content had significantly increased. In wood sections above the groundline nitrogen contents had increased to 0.312% and 0.315% for stakes with and without barriers respectively, i.e. increases of 152% compared to initial nitrogen content of the wood, however the difference between nitrogen contents of wood is not significant between the matched stakes with and without barriers.

In section 2, 15 - 25mm below the groundline, wood nitrogen content beneath barriers had increased by 97% to a valueof 0.246% whereas in matched stakes without barriers nitrogen content had increased by 222% to 0.402% very significantly different at P = 0.001. In sections 3, nitrogen content of wood increased by 342% and 310% respectively for stakes with and without barriers respectively to values of 0.467% and 0.435%, such values not being significently different. Nitrogen content of wood above the groundline (section 1) is significantly lower than nitrogen content of wood sampled at section 3 for both sets of stakes regardless of whether barriers were fitted or absent.

Results for dehydrogenase activity in wood and soil are presented in figs 6.3 and 6.4. The results for wood demonstrate that in section 2 of stakes with plastic barriers dehydrogenase activity is sighificantly lower at P = 0.01 than for any wood section in soil contact. No 2,3,5, - triphenyltetrazolium formazan was extracted from heat sterilised wood indicating no dehydrogenase activity occurred and that wood itself did not reduce 2,3,5, - triphenyltetrazolium chloride.

Results for dehydrogenase activity in soil within 3mm of wood-soil or barrier-soil interfaces show that dehydrogenase activity was very limited in soil adjacent to plastic barriers surrounding wood. 39.0 nM TTF g^{-1} dry soil per 24 hours were recorded for these stakes whereas in matched positions of stakes not fitted with barriers enzyme activity was 68.3 nM TTF g^{-1} dry soil per 24 hours, significantly different at P = 0.025; background dehydrogenase activity in soil at least 50mm from wood stakes was 31 nM TTF g^{-1} dry per 24 hours. In section 3 of stakes fitted with barriers enzyme activity is significantly higher than in section 2 of the same stakes, dehydrogenase activity being the highest recorded in soils within 3mm of section 3 of stakes fitted with barriers. The values recorded in soil about section 3 of stakes fitted with barriers was 133.1 nM TTF g^{-1} dry soil per 24 hours compared with a mean value of 71.5 nM TTF g^{-1} dry soil per 24 hours for soil adjacent to section 3 of stakes not fitted with barriers significant at P = 0.025.

The relationship between mass loss and nitrogen contents of wood are shown in fig 6.5 for stakes not fitted with plastic barriers and in fig 6.6 for stakes fitted with barriers. A strong correlation exists between degree of mass loss and nitrogen content of wood in all stakes. For stakes not fitted with barriers $r = 0.81$ significant at $P = 0.01$ and for stake fitted with barriers $r = 0.94$ significant at $p = 0.001$.

The relationship between dehydrogenase activity in wood and % mass loss and % nitrogen content in matched stakes is shown in fig 6.7 In both cases dehydrogenase activity correlates with both parameters. The significance of the correlation coefficient is low for mass loss results $r = 0.81$ ($P = 0.1$) but significant for nitrogen values $r = 0.93$ ($P = 0.05$). As matched blocks were employed it was not possible to plot individual mass loss or nitrogen content data against individual assays of dehydrogenase activity in the wood. Out of necessity therefore means were plotted against means thus reducing the numbers of observations to 4. Such a low number of observations lowers the value of significance and statistical significance may in fact be higher than that recorded in the results.

The relationship between dehydrogenase activity in soil and % mass loss and % nitrogen content in stakes is shown in fig 6.8. There is a significant correlation between enzyme activity and % mass loss $r = 0.92$ ($P = 0.05$) but only a low level of significance for nitrogen values $r = 0.82$, ($P = 0.1$) Again only 4 observations are plotted because of

need to use matched sets of stakes making it necessary to plot only mean values.

The relationship between dehydrogenase activity in wood and dehydrogenase activity in soil shown in fig 6.9. The results show that little correlation exists between the two sets of values with a great deal of variability in corresponding values of enzyme activity in wood and soil. The graph clearly illustrates however that where plastic barriers are present activity in both soil and wood are limited.

Fig. 6.1. Weight loss (%) in lime sapwood stakes determined at three indicated positions: 1. 10mm above ground line. 2. 15mm below ground line. 3. 40mm below ground line. Stakes were either uncovered or covered at the ground line with inert thermofit plastic (blocks were treated with 0·5% C.C.A. solution and figures represent means of seven replicates and their standard deviations).

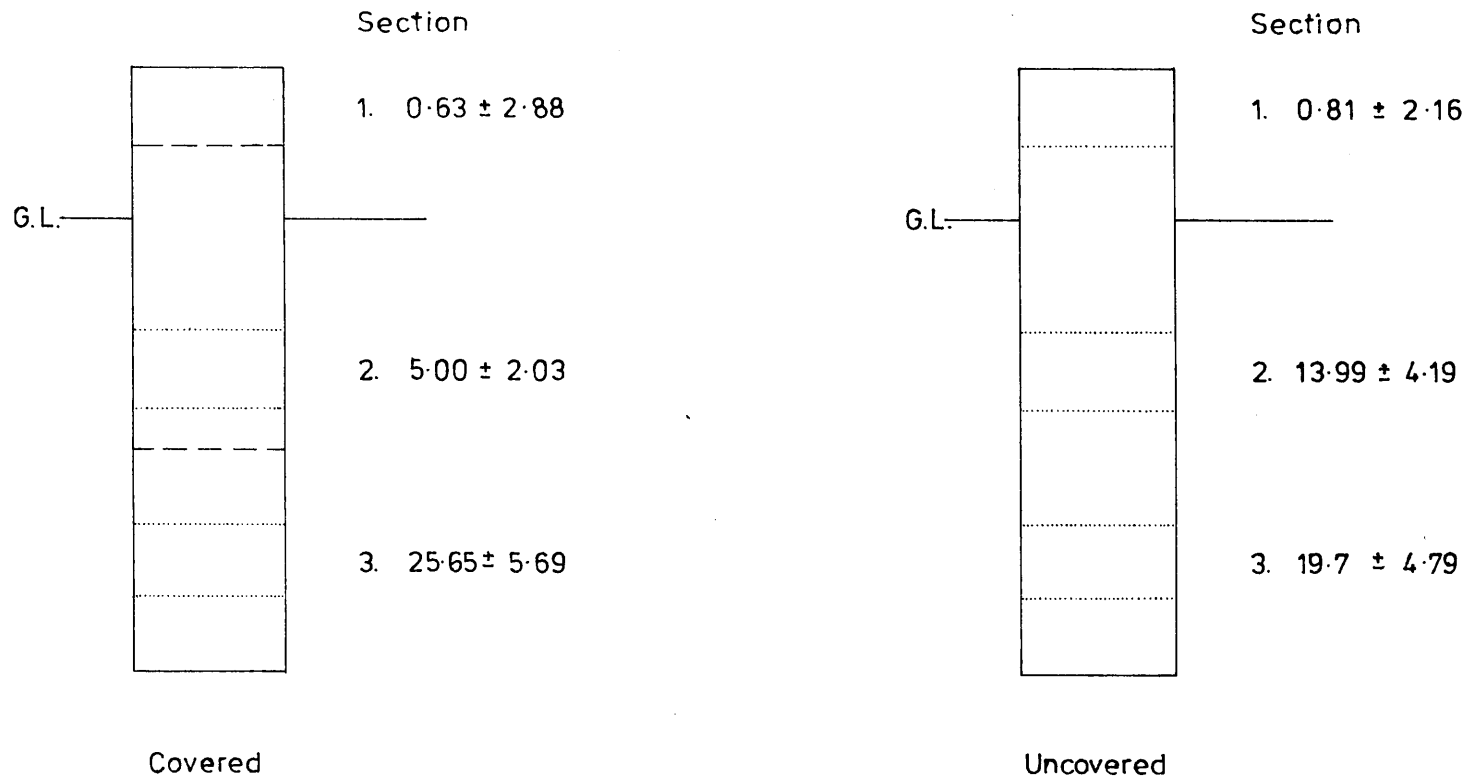


Fig. 6.2. Nitrogen contents (% m/m) in lime sapwood stakes determined at three indicated positions: 1. 10mm above ground line. 2. 15mm below ground line. 3. 40mm below ground line. Stakes were either uncovered or covered at the ground line with inert thermofit plastic (blocks were treated with 0·5% C.C.A. solution and figures represent means of seven replicates and their standard deviations).

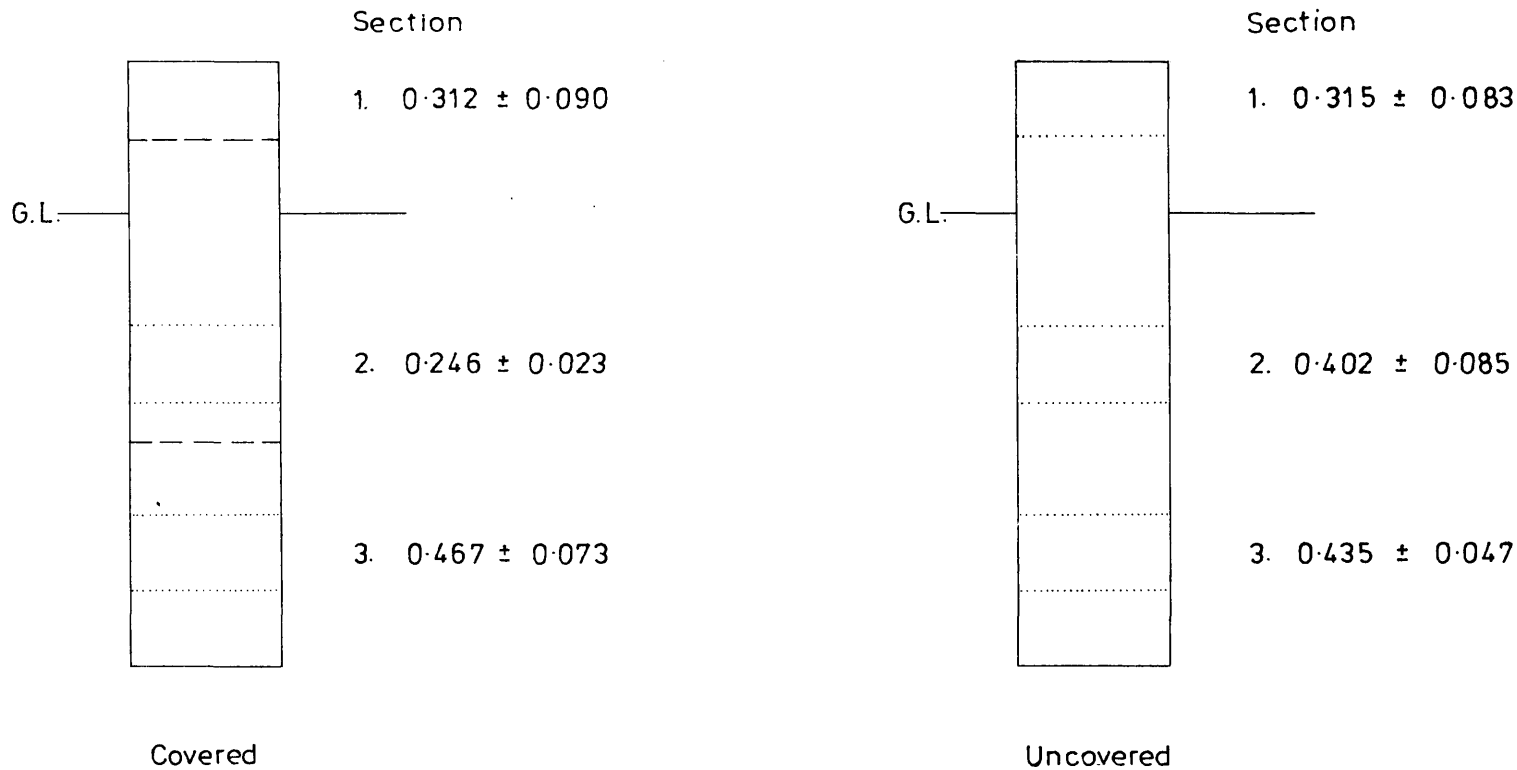


Fig. 6.3. Wood dehydrogenase activity (nm TTF/g dry wood/24hrs.) in lime sapwood stakes determined at two indicated positions: 1. 15mm below ground level. 2. 40mm below ground level. Stakes were either uncovered or covered at the ground line with inert thermofit plastic (blocks were treated with 0·5% C.C.A. solution and figures represent means of five replicates and their standard deviations).

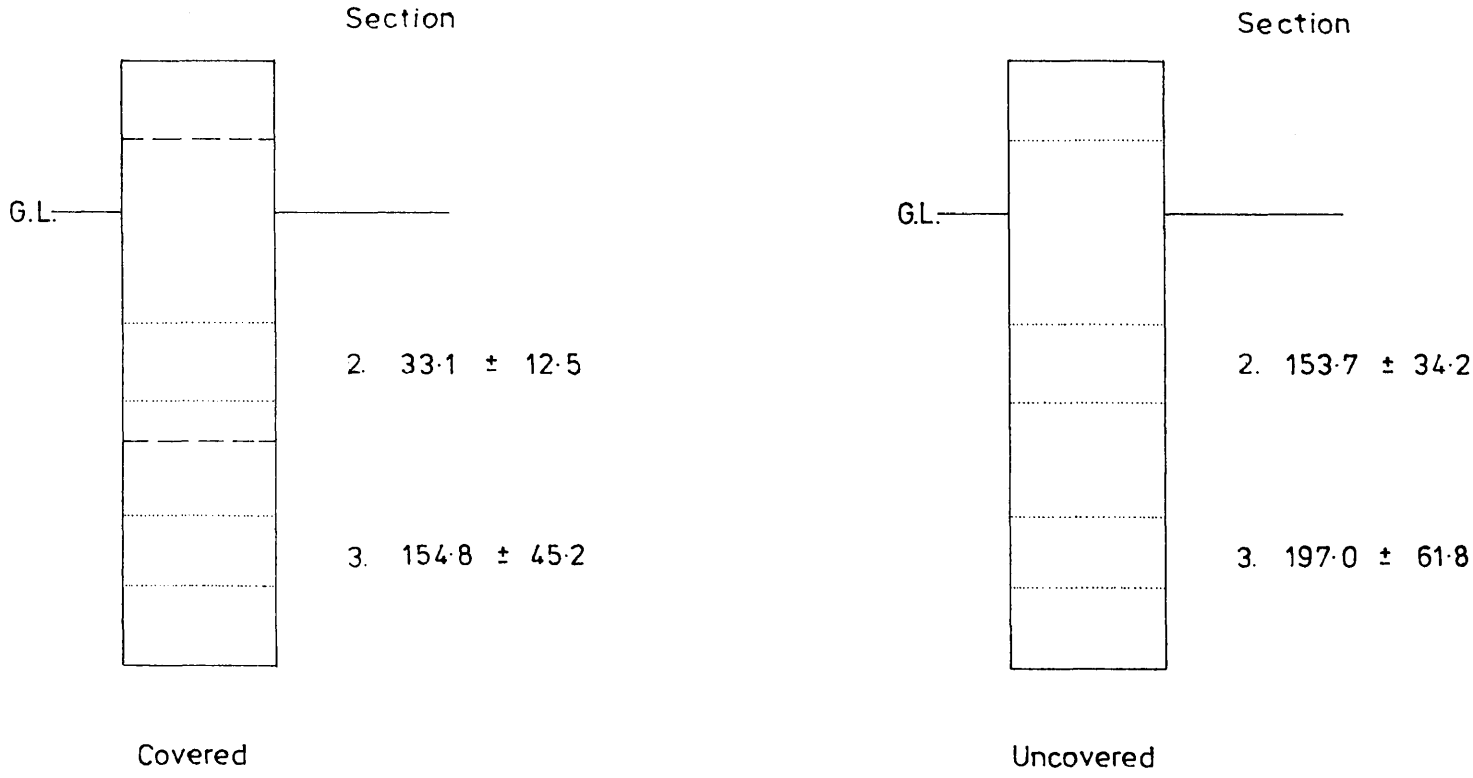


Fig. 6.4. Soil dehydrogenase activity (nm TTF / g dry soil / 24 hrs.) in soil within 3mm of lime sapwood stakes determined at two indicated positions: 1. 15mm below ground line. 2. 40mm below ground line. Stakes were either uncovered or covered at the ground line with inert thermofit plastic (blocks were treated with 0·5% C.C.A. solution and figures represent means of five replicates and their standard deviations).

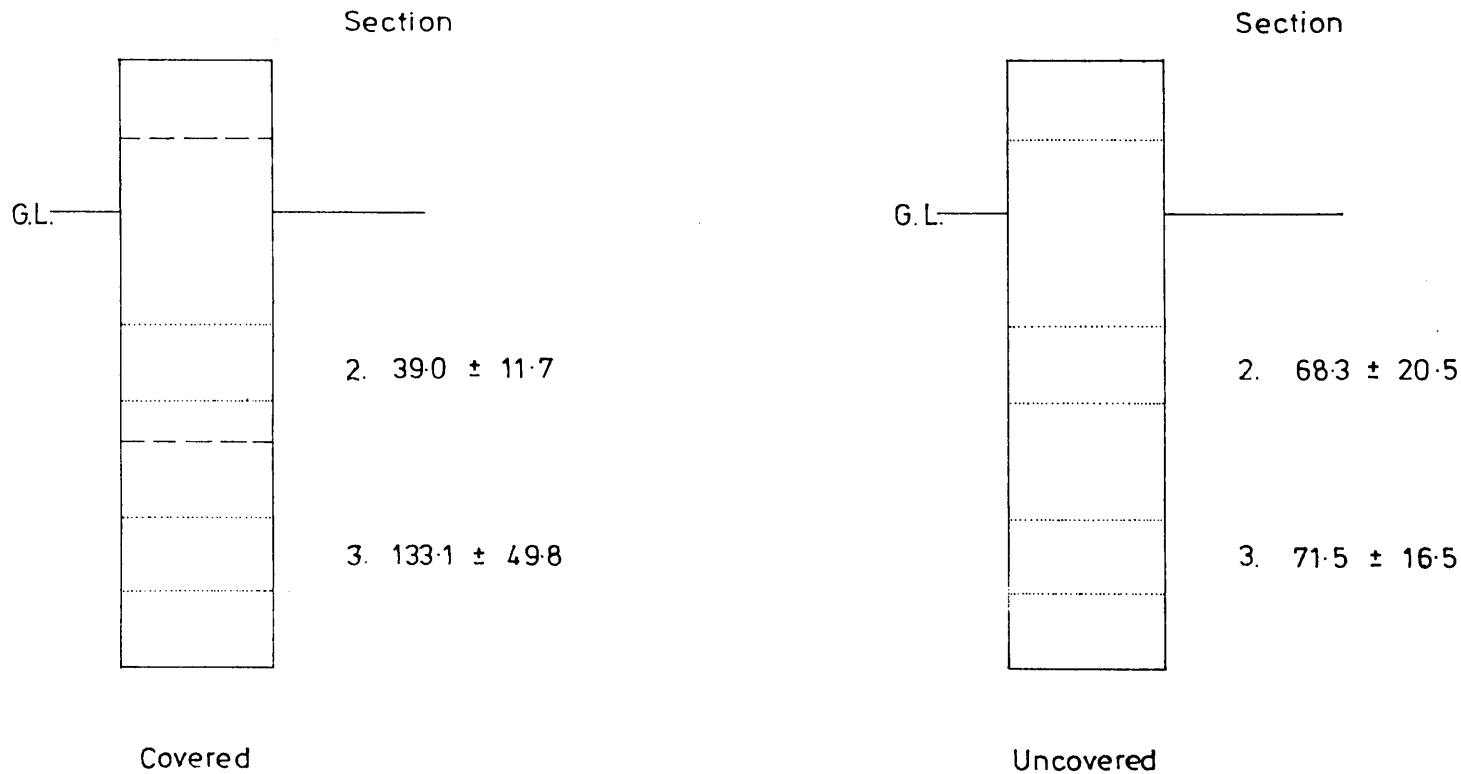
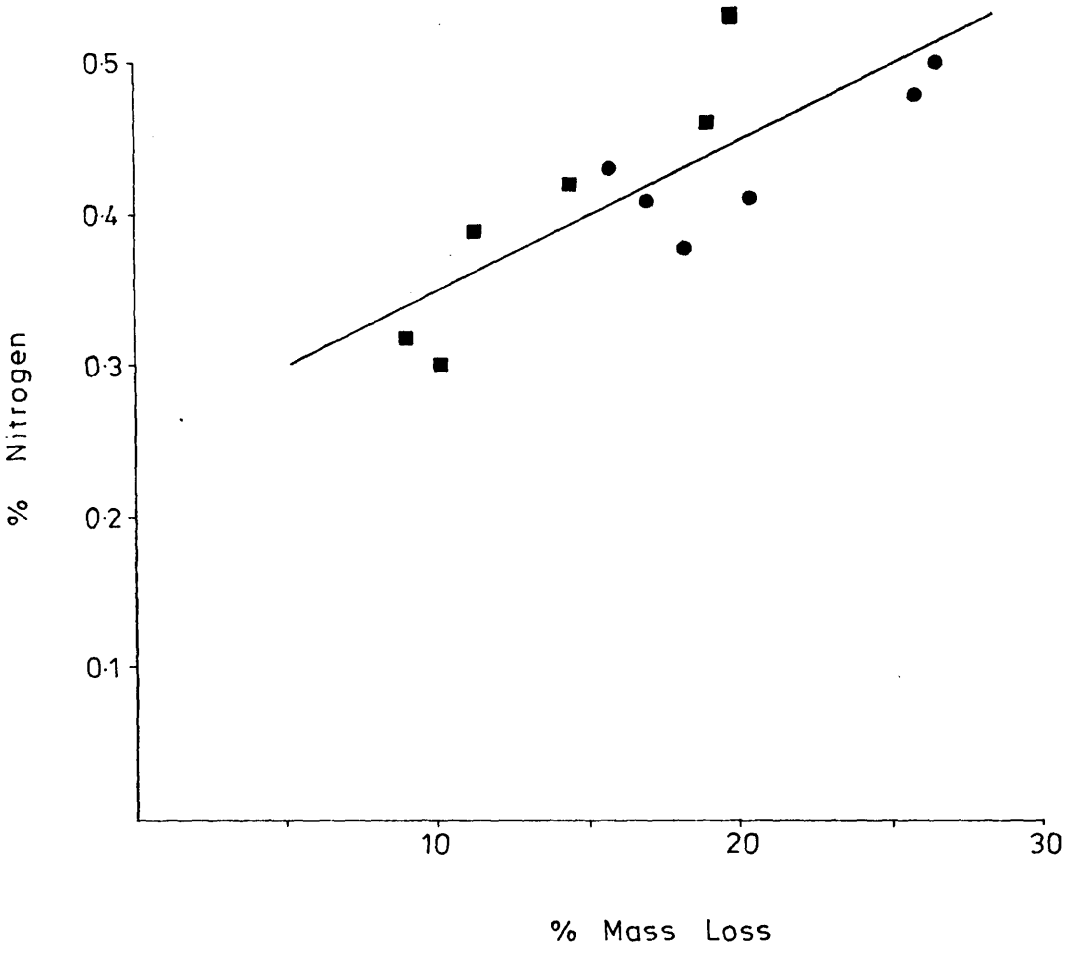


Fig. 6.5. Relationship between % mass loss and % nitrogen in 0·5% W/V CCA treated lime sapwood stakes at depths below groundline of 1. 15mm (■) and 2. 40mm (●).



r = 0·81 (p = 0·01)
Y = 0·251 + 0·0098 (X)

Fig. 6.6. Relationship between % mass loss and % nitrogen in 0·5% CCA treated lime sapwood stakes at depths below groundline of 1. 15mm (■) and 2. 40mm (●). Stakes were fitted with thermofit plastic barriers to a height of 10mm above and a depth of 30mm below groundline.

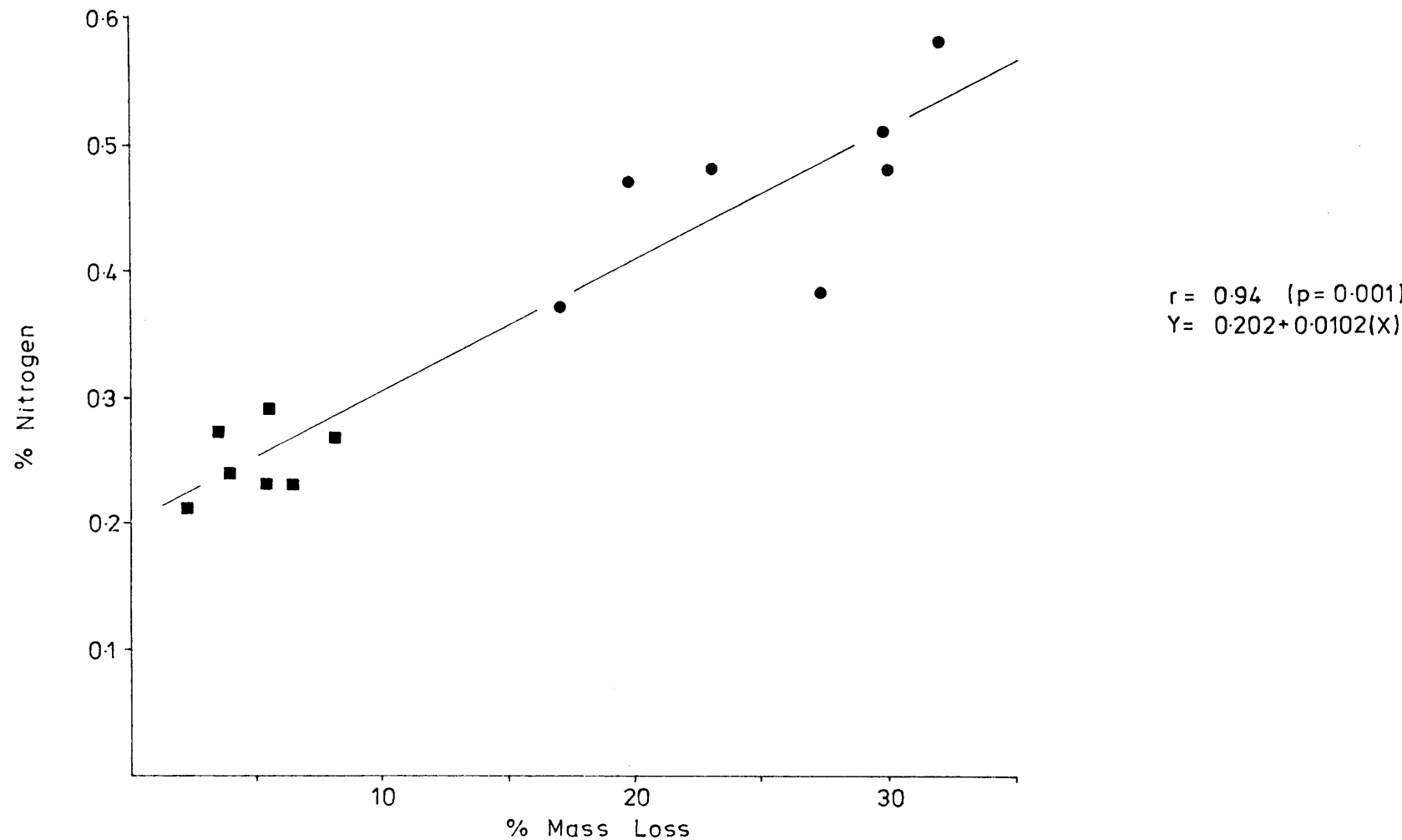


Fig. 6.7. Relationship between dehydrogenase activity in wood and 1. % mass loss, and 2. % nitrogen in matched 0·5% CCA treated lime sapwood stakes.

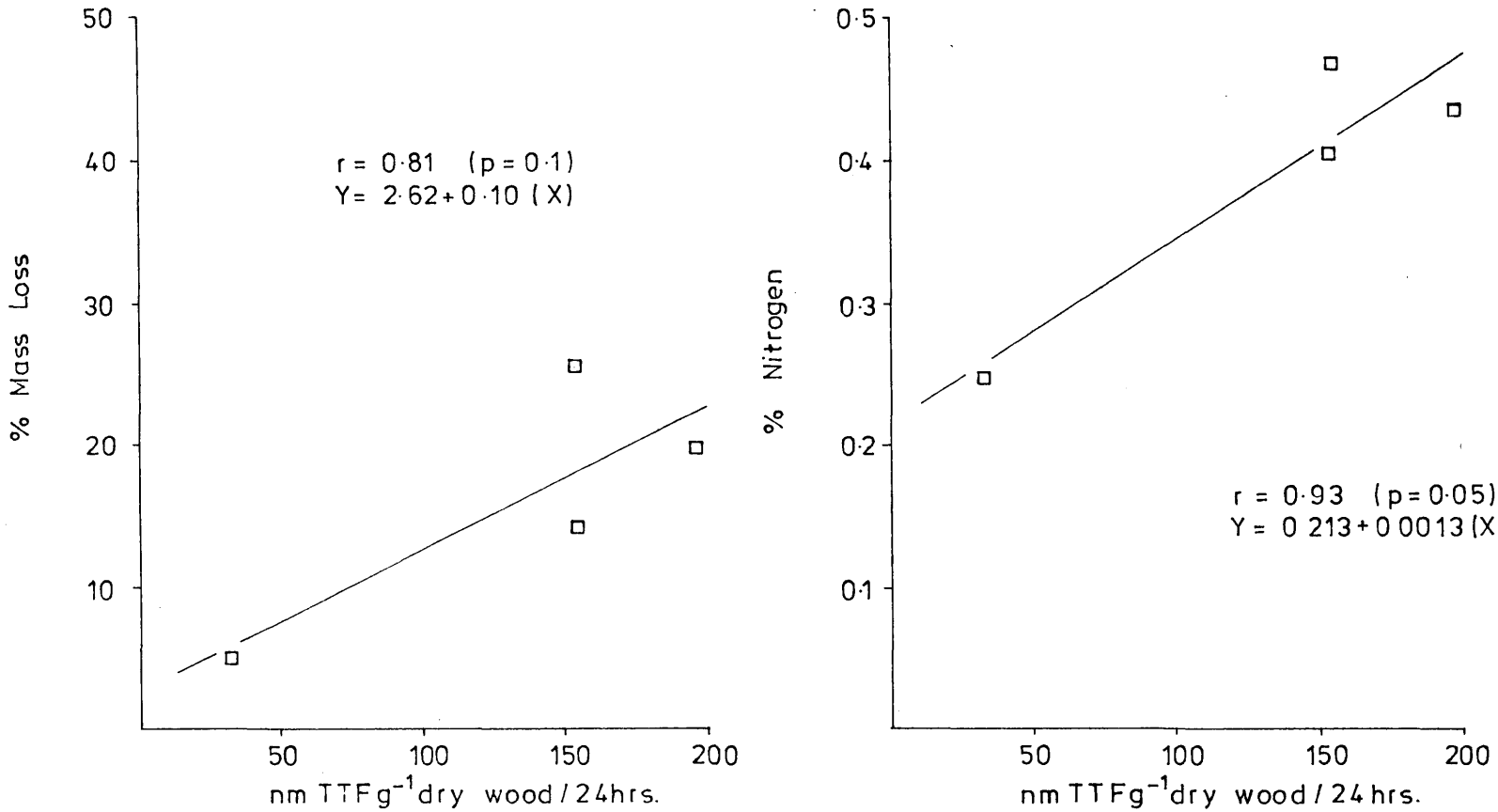


Fig. 6.8. Relationship between dehydrogenase activity in soil within 3mm of wood - soil interface and 1. % mass loss, and 2. % nitrogen in matched 0·5% CCA treated lime sapwood stakes.

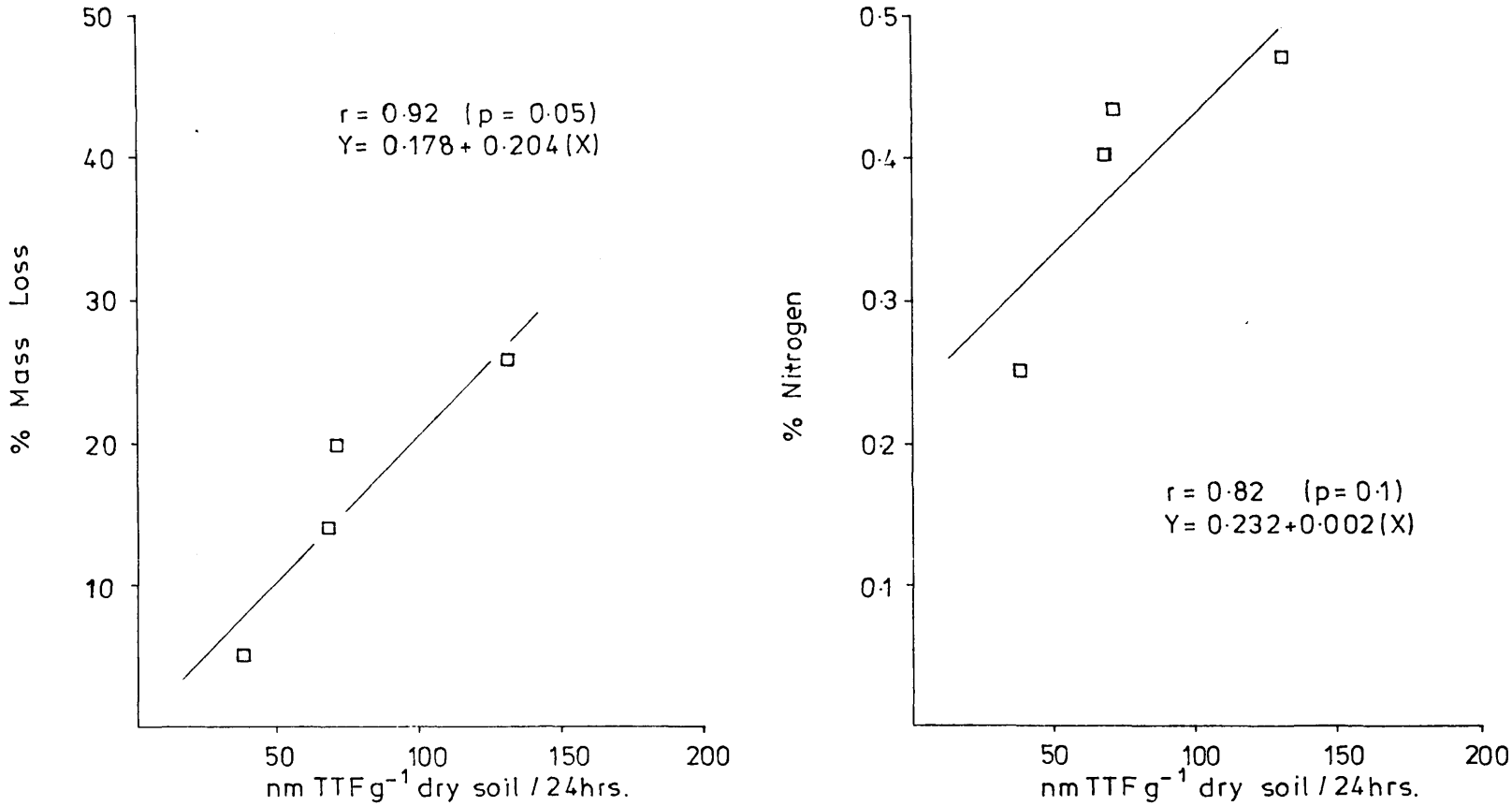
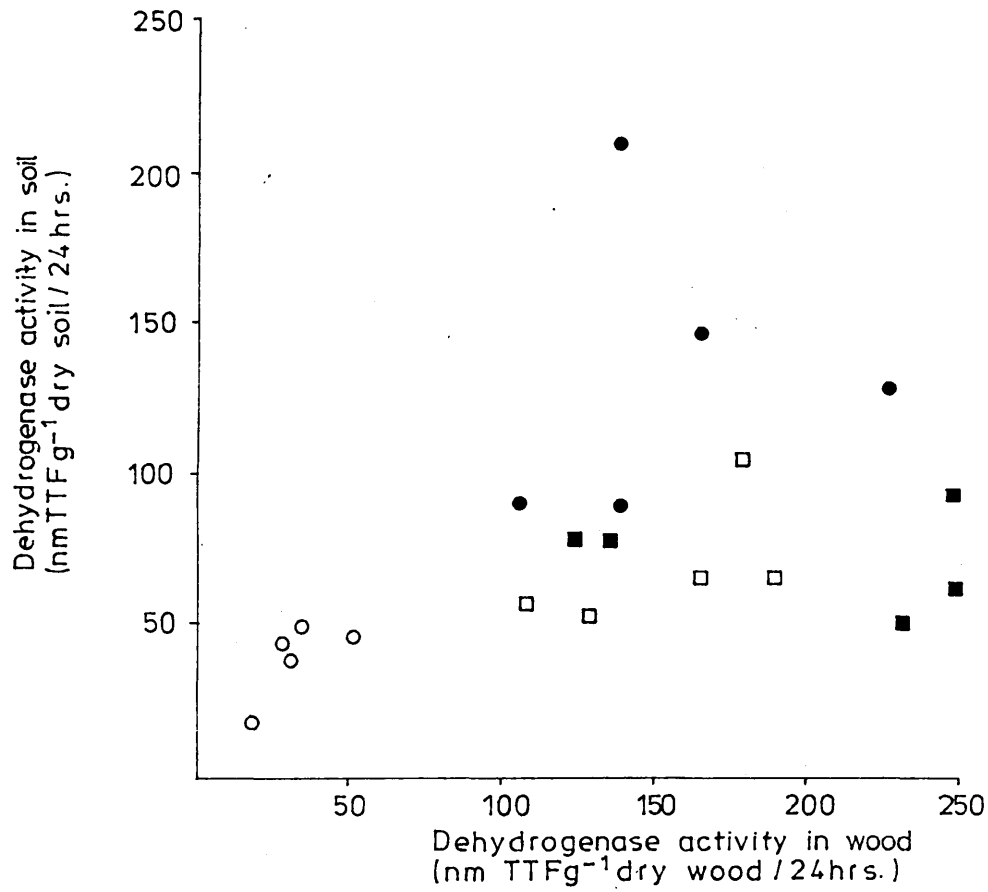


Fig. 6.9. Relationship between dehydrogenase activity in wood and in soil within 3mm of wood-soil interface at two positions below groundline: 1. 15-25mm (○), and 2. 40-50mm (●) for 0·5% CCA treated lime stakes fitted with thermofit plastic barriers to a depth of 30mm below groundline and for 0·5% CCA treated lime stakes without such barriers at matched positions: 1. 15-25mm (□), and 2. 40-50mm (■).



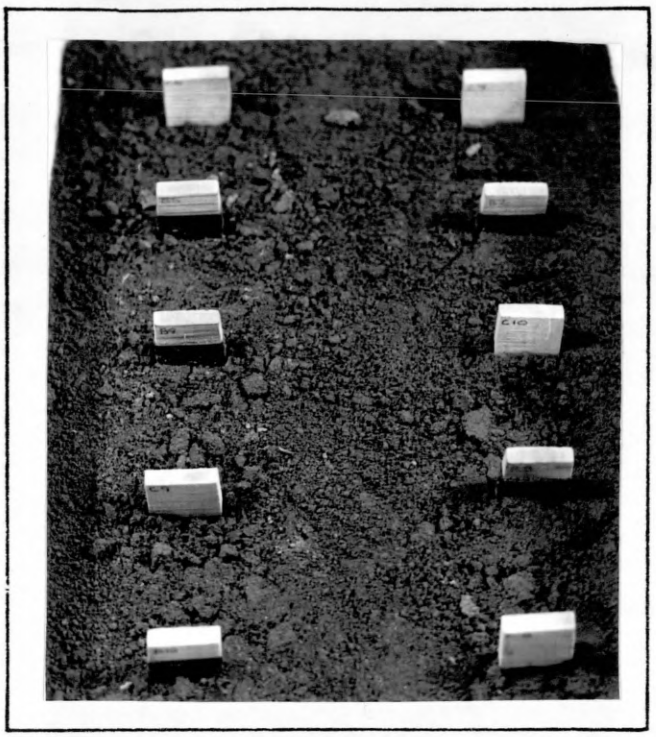


Fig. 6.10(a). Soil box showing arrangement of emplaced 0·5% copper chrome arsenic treated lime sapwood stakes. Stakes are either fitted with thermofit plastic barriers at the groundline, or do not have such barriers.

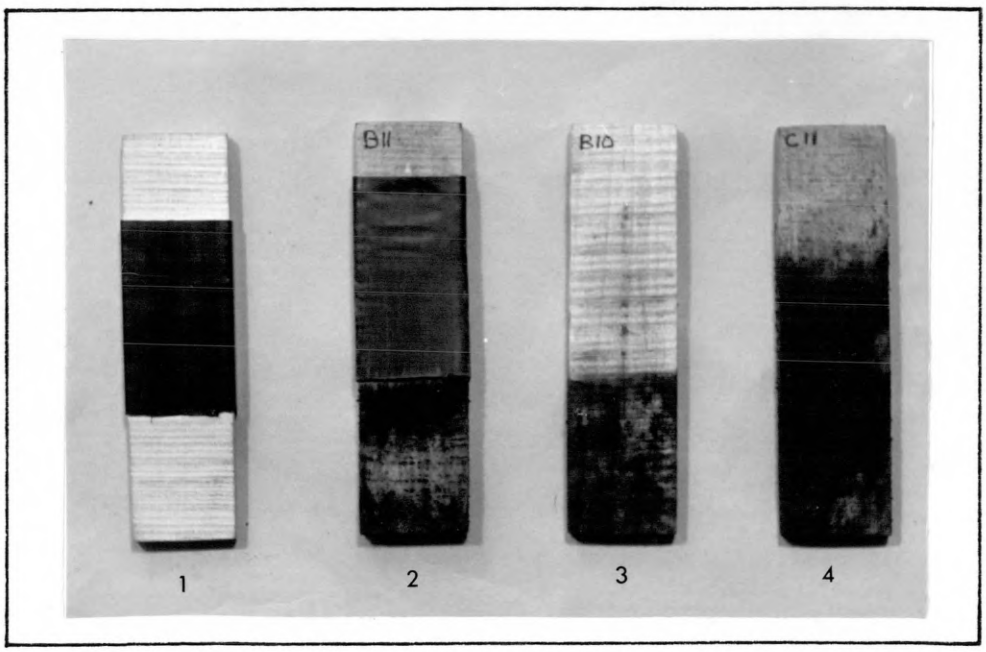


Fig. 6.10(b). Lime sapwood stakes (impregnated with 0·5% copper chrome arsenic) showing: 1. Stake (with thermofit plastic barrier), prior to emplacement in soil, 2. Stake (with thermofit plastic barrier), after having been emplaced in soil for 24 weeks at 25°C, 3. Stake (as for stake 2), with plastic barrier removed, and 4. Control stake (i.e. without plastic barrier), after 24 weeks in soil at 25°C.

6.4. Discussion

In chapter 5 microbial biomass accumulation in soil about decaying wood in soil was shown to occur both in untreated and 0.5% copper chrome arsenic treated lime sapwood blocks completely buried in soil.

As decay status of wood blocks increased there was a contemporaneous increase in nitrogen content of wood and it was hypothesised that such nitrogen increases were due to an increased microbial population invading wood from soil. The aim of the investigation undertaken and described in this chapter was to determine the effects physical barriers imposed between the wood and soil would have on wood decomposition processes. It was considered that such barriers would act not only to disrupt actual movements of microbial populations from soil to wood but also act to disturb chemostimulatory processes mediated by wood volatiles and nutrients.

The results presented in this chapter clearly demonstrated that lime sapwood stakes which were shielded at the groundline with closely fitted inert plastic barriers underwent only limited decay in that section of the stake enclosed by such barriers. Dehydrogenase activity in soil adjacent to plastic barriers was significantly less than enzyme activity in soil adjacent to the same sections of matched copper chrome arsenic treated stakes and such actively was shown in the previous chapter to be related to microbial density in soil. The use of dehydrogenase assay for determining microbial activity in wood was developed and

shown to be a useful indicator of microbial presence. In those areas of wood which underwent significant decay i.e. in those sections of stakes which were in direct soil contact, dehydrogenase activity was very significantly above dehydrogenase activity in wood sections sampled beneath plastic barriers.

The correlation coefficient for dehydrogenase activity in soil and dehydrogenase activity in wood is not significant. Further studies are necessary with greater replication to elucidate the more precise relationship between dehydrogenase activity and microbial population densities. The five replicate assays performed on both wood and soil showed too great a variability for a correlation with statistical significance to be drawn. The observations however are clearly arranged into two sets. Where enzyme activity in wood is low the corresponding value in the soil is equally small. In those sections of wood where enzyme activity is elevated then a similar increase is noted for soil.

The effect of the barrier is to both limit mass loss and increases in the nitrogen content of the wood. Where there were no barriers fitted decay proceeded at rates similar to those for completely buried 0.5% copper chrome arsenic treated blocks. The barrier inhibits the development of an increased microbial population in the soil as measured by dehydrogenase assay and prevents significant transfer of microorganisms from soil to wood.

There were significant increased levels of nitrogen

in all sections of stakes sampled. Decay, however, was not even over the length of stakes but was maximised at bases of stakes. (Fig. 6.10) 40 - 50mm below the soil surface. Conditions for decay must have been optimised at such areas and not at the soil surface or groundline as postulated. Further studies are therefore necessary to delimit the area of greatest decay risk. Monitoring the effects of physical barriers extending far enough into the soil so as to afford protection to those lengths of stakes susceptible to greatest decay hazards is therefore required to evaluate the true effectiveness of such inert barriers.

The increases in wood nitrogen were considered to be due largely to an increased microbial presence, however large increases in nitrogen occured on those sections of stakes above the soil surface and not in soil contact. Such sections were not decayed and the nitrogen accumulated in these areas may well be explained by wick action (Uju et.al, 1981). Where decay of wood had occurred however nitrogen contents were significantly higher than in those undecayed sections of wood above the groundline. The mechanism of 'wick' action may thus well deposit nitrogen at evaporative areas in the wood, however in such cases these may well occur above the groundline and this is consistent with nitrogen concentrations observed in those sections removed from tops of stakes. The very high levels of nitrogen in the lower sections of stakes may not be explained in this way. In response to the loss caused by evaporation of water above the groundline the lower sections might be considered to be in a constant state of

elution by soil solution and might be expected to have lower nitrogen values. They did, however, have as stated previously significantly elevated nitrogen contents with very significant correlation between nitrogen contents and mass loss. Furthermore, as nitrogen values of the most decayed sections of stakes were similar to those of buried 0.5% copper chrome arsenic treated lime blocks at similar mass losses recorded in chapter 5, these increases were considered to be a direct consequence of increased microbial populations associated with the decomposition process.

Nitrogen content of wood beneath the barriers was increased by approximately 90% to a mean value of 0.246% at a mass loss level of 5%. That decay had occurred in these sections meant that invading microorganisms had gained access from the unprotected wood surface 5mm below the sampled area. It was noted on removal of barriers that some discolouration had occurred 2 - 3mm beneath the lower edge of the plastic barriers. On shrinking the thermofit fitted very tightly at corners of stakes but in some cases the barrier was relatively less tight on radial faces due to stresses at the corners. To secure better adhesion stakes of round cross section would distribute stress during shrinkage more equally and thus might have reduced the incidence of decay observed below barriers.

Smith (1980) proposed that wood decomposition in soil could be divided into two phases. A decay phase delimited to that period of time when mass loss was measurable followed an induction phase. Smith considered the induction phase to consist of a lag period during which soil microorganisms

responded to wood presence in soil. Such responses consisted of recognition of proximity of nutrient resource followed by invasion, colonisation and utilisation of such resources.

The presence of physical barriers between wood and soil may prevent microorganism recognising the presence of the newly emplaced resource. The dehydrogenase assays indicated that in the soil adjacent to barriers microbial densities did not increase. Chapter 5 has shown that dehydrogenase assays correlate well with fungal hyphal length and could be used as a direct measure of fungal biomass in soil. Enzyme activity was very significantly increased only in soil adjacent to decaying wood. The possibility exists therefore that analysis of soil microbial biomass and activity could be developed as a method to determine the degree to which decay has occurred in wood. Such predictive testing of wood decay in soil would therefore not require actual wood sampling procedures.

Results presented in this chapter clearly demonstrate that:

1. Inert plastic barriers about wood in soil afford a degree of protection against decay as measured by mass loss.
2. Barriers inhibit the transfer of nitrogen from soil to wood.
3. The density of microbial populations measured by dehydrogenase assay are reduced in both wood and soil about wood in those sections of stakes covered by plastic barriers.

Chapter 7.

General discussion.

7. General Discussion

Carbon mineralisation in soil is the decomposition of carbon containing organic resources to inorganic forms of carbon compounds such as carbon dioxide. Wood is a carbonaceous resource containing an heterogenous amalgam of organic substrates including cellulose, lignin hemicelluloses and proteins in complex inter-relationship with each other and decay of wood in soil is inevitable unless environmental parameters are altered making decomposition processes non-sustainable. Generally, environmental conditions in most soils are stable, although quite pronounced cyclic variations may take place, and allow complex matter to be degraded to simpler inorganic forms.

The degradation of carbonaceous compounds in soil is undertaken by diverse groupings of saprophytic microorganisms of which fungi and bacteria are considered to be of primary importance. Such breakdown is effected by enzymes which are produced extracellularly and which must have physical contact with their substrates before decomposition can take place. The long filamentous growth habit of fungi and small discrete nature of bacterial cells ensures that they can pervade a new resource and make intimate contact with carbonaceous substrates which comprise wood cell walls.

Both fungi and bacteria in soil are considered to exist mainly as inactive or dormant propagules, conditions

known as fungistasis and bacteriostasis (Dobbs & Hinson, 1953; Lockwood, 1977; Lynch, 1982). Lynch (1982) found that sterilisation of soil by autoclaving or irradiation alleviated fungistasis and release from fungistasis was considered by him to be a response to increased nutrient levels in soil following cell death and lysis after sterilisation. Soil bacteriostasis can also be counteracted by nutrient addition to soil (Ko & Chow, 1977; Lynch, 1982). Lynch concluded that microbiostasis in soil could be explained by soil being nutrient depleted and Gray & Williams (1971) suggest that soils do not have enough input in the form of plant litter to even satisfy maintainance energy requirements of the microflora, thus necessitating that heterotrophic organisms grow actively only at sites of high nutrient concentrations such as plant rhizospheres.

Smith (1980) proposed that when wood is emplaced in soil small traces of nutrients diffuse from the wood into soil, counteracting fungistasis and stimulating germination of dormant fungal propagules although he also considered that little work had been done in this area. Hardie (1979) showed that water soluble leachates of sapwood of several wood species stimulated germination of ascospores of Chaetomium globosum although lime (Tilia vulgaris Hayne) inhibited germination. King et al (1983) showed that wood with high soluble nitrogen content stimulated rates at which nitrogen was transferred from soil to wood compared to wood with low soluble nitrogen content, and these workers considered that soluble wood nutrients may have pronounced

effects on soil microbial populations.

Chapter 2 of this thesis demonstrated clearly that dried wood in the vicinity of fungal inocula stimulated hyphal growth. It was also shown that such growth was oriented towards wood even when wood was treated with a non-volatile preservative such as copper chrome arsenic. Such oriented growth even occurred towards material subjected to thorough aqueous leaching. It is well known that wood volatiles can inhibit or stimulate growth of many wood decay fungi; however, the results presented in chapter 2 show for the first time that this response may be directed towards the source of those volatiles, i.e. wood.

Smith (1980) proposed that nitrients diffusing from wood into soil initiate germination of dormant fungal propagules and that emerging hyphae grow onto wood. The results presented in this thesis suggest that such emerging hyphae could orientate their growth in response to volatiles emanating from wood into soil. Such orientation of hyphae taking place along concentration gradients of volatiles which would be most concentrated at wood-soil interfaces and decreasing with increasing distance into the soil. Furthermore, Emerson (1948) showed increased germination of ascospores of Neurospora crassa when furfural was incorporated in basal media, and Harman et al (1980) showed that many C_5 - C_{10} aldehydes and ketones stimulated conidiospore germination in both Alternaria alternata and Fusarium solanii when in soil imposed stasis. Furfural and C_5 - C_{10} aldehydes and ketones are common volatile compounds

found in heated, and to a lesser degree unheated, Scots pine wood (Flodin & Andersson, 1977). Similar aldehydes and ketones (C_5- C_{10}) have been shown to be autoxidation products of unsaturated fatty acids (Swift et al, 1949; Swoboda & Lea, 1965) such as oleic and linoleic acids, both commonly found in wood (Rice, 1970).

It is therefore considered that it may not be necessary, as proposed by Smith (1980), for nutrients to diffuse from wood to soil as precursors to stasis release. As leached blocks have been shown by the work described in this thesis to elicit tropic responses, it is difficult to accept this postulate of Smith's as the sole cause of decay initiation. The topic responses demonstrated by fungi were clearly stimulated by volatile emissions from the wood as other possible transfer routes, e.g. through the agar, were eliminated in the experimental design. It is postulated in this thesis, therefore, that wood volatiles might not only initiate stasis release and germination of fungal spores in soil as described by other authors but also stimulate hyphae germinating from such spores to orientate their direction of growth towards the wood.

The importance of soluble nutrients in wood emplaced in soil may not be limited just to the role of easily assimilated organic nutrients but, as shown in chapter 3, also act to stimulate bacteria to accumulate in areas where soluble nutrient concentrations are greatest. All four bacteria tested are commonly found in terrestrial habitats and the strain of Bacillus subtilis used was

isolated from wood (Ward & Fogarty, 1972). Each of these bacteria demonstrated positive chemotactic responses to aqueous extracts of lime, pine, beech and eucalypt sapwood as well as to defined single nutrients such as amino acids and monosaccharides. Pseudomonas aeruginosa also responded to ammonium ions and all bacteria were more attracted to amino acids than sugars at the concentrations used in this investigation; nitrogen sources may be more attractive chemotactically than sugars and further investigations are required in this area.

Clubbe (1980) identified bacteria as the primary colonisers of both untreated and copper chrome arsenic treated wood in soil. King et al (1980) showed that the nitrogen content of wood could be significantly increased by a bacterial presence. It is also clear from the literature that bacteria isolated from wood and litter are predominantly flagellate and motile with Bacilli and Pseudomonads being especially numerous (Greaves, 1971; Gray et.al., 1974) and that colonisation of freshly emplaced litter in soil can be very rapid with tenfold increases in bacterial numbers in the first 3 days (Gray et. al., 1974). Such increases in numbers may obviously be due to proliferation by vegetative reproduction; however, it is well known that qualitative differences exist between the structure of bacterial populations distributed throughout soil and those in the zone of plant rhizospheres. Random coincidence of growing root with soil bacteria may lead to increased numbers of those groups of organisms better able to compete in the root zone

environment, therefore explaining such qualitative differences. However, Bowra & Dilworth (1981) and Gotz et. al.(1982) have demonstrated that Rhizobium spp. are stimulated by plant root exudate to show positive chemotactic behaviour, and such responses may be of fundamental importance in the development of plant rhizosphere populations. Similarly nutrients leaching from wood to soil may elicit positive chemotactic responses in motile soil bacteria thereby initiating invasion of wood substrates emplaced in soil, and this invasion may have significant implications for preservative performance. That this invasive process may be very rapid has been demonstrated by Gray et. al., (1974), and simple nutrients in wood may act not only to cause release of both fungal and bacterial stasis as proposed by Lynch (1982), but also to direct bacteria to wood resources.

The presence of copper chrome arsenic preservative in wood in soil may have a significant influence on bacterial responses to leachates from wood. Bacterial motility is severely inhibited in the presence of heavy metal ions due to such ions complexing with the bacterial flagella, even at concentrations as low as 10^{-6}M (Adler & Templeton, 1967). Furthermore, the effect of free copper or chromium ions in wood would render motile bacteria immotile, preventing further colonisation, and if copper should leach from wood to soil the invasion of wood by bacteria may be prevented as much by inhibition of motility as to direct toxicity. Thus migration of copper to soil and its accumulation therein with associated changes in microbial

biomass require much further investigation before the full process of decomposition of wood in soil when preserved can be fully elucidated.

The results presented in chapters 5 and 6 show that even when treated with copper chrome arsenic wood in soil decays, although the rate at which such decay occurs is slowed by preservative presence. It is therefore possible that microbial mechanisms exist which detoxify preserved wood in soil environments prior to decomposition processes taking place. Many biochemical compounds have chelating properties, e.g. organic acids and amino acids, and such compounds are continuously produced in soil due to the activities of soil microorganisms. Death of bacteria due to the toxic nature of copper may cause lysis of the bacterial cell releasing a wide variety of biochemical molecules into the environment, many of which would chelate or complex and solubilise or stabilise the heavy metals present. Solubilisation would render metal ions susceptible to leaching and removal from the wood environment. Secondary colonisers of wood such as fungi would not, therefore, enter a material as toxic as when initially emplaced in soil, or alternatively those toxicants present may be chelated or complexed by microorganisms or microbial products so that they are no longer effective as toxicants.

Beveridge & Murray (1976) have shown that isolated cell walls of Bacillus subtilis can retain, complexed onto the surface, substantial amounts of heavy metal ions such as copper. Such ions are held tenaciously on such surfaces and

render the copper ions immobile and therefore non-toxic to further colonising microorganisms. The role of bacteria as the primary colonisers of preserved wood in soil might therefore be of greater significance than previously supposed.

Chapters 2 and 3 have shown for the first time that both fungi and bacteria show positive directional responses to wood. These responses are moderated by wood volatiles or aqueous extracts of wood and it is proposed that wood chemicals play an active role in stimulating and initiating the invasive process by microorganisms of wood in soil. The movement of microbial biomass from soil to wood would increase the nitrogen content of the wood thereby increasing the quality of the resource in terms of recycling of their nutrients for further invading microorganisms. Primary colonisers such as bacteria can solubilise or immobilise elements of copper chrome arsenic preservative, making such preservative formulations less toxic. It is therefore proposed that the induction phase of Smith (1980) consists of that period of time necessary for microbiostasis release which occurs when wood is emplaced in soil and the time taken for both bacteria and fungi to invade the wood under the stimulus of wood chemicals and to initiate utilisation. The induction phase occupies a longer time period in copper chrome arsenic treated wood due to the necessity for detoxification mechanisms to occur; however, such detoxification is an inevitable occurence and although copper chrome arsenic preservatives may delay decay processes they cannot prevent them.

The transfer of microorganisms from soil to wood

during decay has been monitored using changes in nitrogen concentrations in wood with time (King & Waite, 1979). Such a measurement relies on the assumption that the nitrogen changes in wood during decay are correlated only with microbial biomass presence. Such assumptions were justified by King et. al.(1981) who argued that nitrogen increases only occurred in wood undergoing decay and not in blocks remaining undecayed, and that such increases could not be due to a 'wick' mechanism (Uju, 1981) as totally buried blocks were utilised. The importance of nitrogen to soft rot decay of wood in soil was also stressed by King et. al. (1981); therefore, a preliminary investigation, described in chapter 4, was carried out to monitor nitrogen differences in soil adjacent to decaying wood in soil as a measure of differences in microbial population densities. These investigations indicated elevated nitrogen concentrations in soil within 1mm of severely decayed beech posts, and this increased level was postulated to be due to increased microbial biomass. There was considerable variation in soil nitrogen content between the International Research Group on Wood Preservation test sites, varying from a minimum of 0·032% at site 29 to as high as 0·555% at site 2; however, no correlation exists between these values with decay estimates published by Levy & Dickinson (IRG/WP/3164). There was difficulty in comparing soil nitrogen contents with wood decomposition at the sites, however, because no standard existed for sampling of soil. To obtain meaningful data in future, therefore, such a standard would need to be specified.

A more detailed examination of wood decay in soil was undertaken to determine changes in microbial populations in soil during decomposition and described in chapters 5 and 6. The changes in microbial population densities in soil were measured by three different methods. Nitrogen content of soil was monitored to determine whether it correlated with the more conventional method of direct observation of soil biomass using agar films and a biochemical assay method monitoring dehydrogenase activity was also used, both these latter methods being used for the first time in wood decay studies. The extent of wood decay was monitored using mass loss and nitrogen concentrations in the wood.

Results presented in chapter 5 showed for the first time that fungal hyphal lengths in soil were very significantly correlated with dehydrogenase activity in soil, and also with mass loss and nitrogen concentration in wood. There was a build up of both bacterial numbers and fungal biomass in soil about wood as decay progressed with time, and such bacterial numbers and fungal biomass were related to mass loss from the wood. That events occurring in wood and in soil are related have thus been positively established for the first time, and the hypothesis that wood, even when preserved or leached, strongly influences the microbial patterns in soil confirmed. When wood is emplaced in soil it is therefore concluded that both bacteria and fungi may respond by directional movement or growth respectively towards the wood, and it may therefore be assumed from this that both bacterial numbers and fungal hyphal lengths would increase in soil contiguous with wood-soil

interfaces.

As decay proceeds and wood polymers are degraded, simpler soluble nutrients would be released, possibly initiating further stimulation of the soil microflora, resulting in increased soil microbial biomass and increased invasion and colonisation of the wood. If the mechanism of decay occurs as described, then an inert barrier around wood in soil would have a twofold influence. Such barriers would act to prevent both wood volatiles and nutrients from diffusing into the soil, thereby preventing microbiostasis release, and secondly, such barriers would prevent the invasive process. Barriers were investigated and the results presented in chapter 6 indicate that microbial biomass transfer from wood to soil was prevented, and that microbial population densities do not increase in soil contiguous with wood-soil interfaces covered by bariers. For the first time dehydrogenase assays were carried out in wood; these preliminary studies suggest that dehydrogenase assays may be useful in quantifying microbial presence in wood.

As stated earlier, microbial population densities in soil and wood was only seen to increase in those areas where decay had been initiated. The technique used in this investigation sampled soil within 3mm of wood-soil interfaces. If sampling had been limited to 1mm it is possible that numbers of bacteria and hyphal lengths would have been seen to increase before decay initiation, and further work is necessary in this area. It may be possible, therefore, to investigate numbers and activity of the soil microbial population about wood as a predictive determination of the

extent to which decay hass occurred in the wood. Further work is necessary therefore to study methods by which quantification of microbial biomass in wood can be accurately undertaken, especially by direct observation. In soil this is achieved by agar films and possibly thin sections of wood could be used to measure hyphal lengths and bacterial numbers by the grid method described for agar films. Such measurements are essential in order to correlate dehydrogenase assays with microbial biomass presence.

The thesis postulates a mechanistic model for wood substrate utilisation in soil. Microbial growth patterns in soil adjacent to wood are radically altered by behavioural responses produced by both fungi and bacteria to chemostimulants in the microenvironment by which they first detect and then locate wood substrates in soil by chemotropic and chemotactic responses, and that emplacement of wood in soil initiates a sequence of events which lead to complete mineralisation of the wood substrate with time.

Studies of wood decay in soil have been largely concentrated upon colonisation and succession patterns of the microbial community in wood. Events occurring in soil have largely been neglected. The results presented in this thesis reveal that a strong relationship exists between wood substrate utilisation and changes in microbial population densities and activity in soil. Consideration should, therefore, be applied to the events which occur in soil in any study of wood decomposition in soil.

The terrestrial environment is considered to have

a remarkable capacity to degrade large quantities of substrates with diverse chemical and physical properties. Such diversity exists between hardwoods and softwoods and when treated or untreated with heavy metal preservative formulations. However, microbial communities are equally diverse and various combinations of different microorganisms are capable of degrading all wood substrates. An obvious extension of this thesis is that members of such communities are selected by the chemostimulatory nature of the substrates colonised, and that microbial communities and the climax populations are functions not only of the chemostimulatory nature of wood, but also by the chemostimulatory characteristics of previous colonisers and microbial residues, all of which contribute to the ultimate decay of wood in soil.

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