Table of contents continued

Table of contents continued

Table of contents continued

TABLE OF CONTENTS

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

_____ _____

Name of candidate Signature

Signed on this_____ day of _____ 19____.

sources as their sole source of these nutrients. All isolates were shown to utilize the majority or all of these sources, indicating that ectomycorrhizal fungi could play a significant role in providing N and P to *P. patula*, especially from organic and complexed sources from which the host plant would not normally have access.

Two ectomycorrhizal root types were collected from a sites in which litter had accumulated. These were identified using a combination of morphological and anatomical features, as well as chemical reactions. The ectomycorrhizas were compared with published identifications and factors such as sporocarp occurrence and fungal connections were considered. The mycorrhizas were identified as *Scleroderma citrinum* and *Boletus pinicola*. The *S. citrinum* were white, smooth and dichotomously branched with smooth, pale yellow, undifferentiated rhizomorphs. The mantle was plectenchymatous with an outer and inner layer. The inner layer showed a ring-like arrangement of hyphal bundles. The 'Hartig' net had a type A, palmetti shape. The *B. pinicola* mycorrhizas were brown with lighter coloured root tips, simple to dichotomously branched. The mycorrhizas were smooth with no distinct mantle, sparse hyphae occurred on the root surface. The shape of the 'Hartig' net was type A, palmetti with lobed haustoria.

On the basis of this study management strategies are proposed in order to improve litter decomposition in high altitude sites and include (i) controlled burning, to reduce loss of nutrients through volatilization (ii) increasing forest floor temperatures by altering the planting density and (iii) application of dolomitic lime to replace cations in order to improve the acidic conditions making the litter more favourable for microbial activity.

accumulation of litter in high altitude sites. Changes in litter quality during decomposition were also biphasic. Analysis of litter N indicated a rapid loss of N during the first 13 weeks (Phase N1) attributable to mineralization, followed by an increase in N which is attributable to assimilation or immobilization (Phase N2). Analysis of litter P indicated a rapid loss of P during the first 13 weeks (Phase P1), and a continual but more gradual loss of P thereafter (Phase P2). Phosphorus is continually being mineralized.

Reduced decomposition rates in high altitude sites might be linked to differences in microbial activity or in the composition of the microbial population. To test the possibility that ectomycorrhizas are able to retard decomposition because they are in competition with the decomposer microbial population, a trenching experiment which eliminated ectomycorrhizal roots from plots was performed. This showed that microbial activity and biomass were not depressed in the presence of active ectomycorrhizal roots. Plate counts were conducted to enumerate functional bacterial and fungal groups in both a low and a high altitude site and no evidence was found to suggest that these were significantly different between sites. It is suggested that reduced decomposition rates are more likely to result from changes in microbial activity, which are linked to environmental factors, such as cooler temperatures and acidic conditions in the litter layers of high altitude sites.

The cycling of nutrients was examined in a 42 year-old, high altitude *P. patula* site in which litter had accumulated. Samples of the vegetation, litter and soil components were collected and chemically analyzed for total N, total P and the major cations K^+, Ca^{2+} and Mg^{2+}. Complete nutrient budgets for N and P, and cation pool sizes were determined. It was evident from these studies that large reserves of N (1442kg ha^{-1}) and P (103kg ha^{-1}) are stored in the litter layers, with levels of cations being low. The majority of the fine feeder roots were distributed in the litter layers. This indicated a tightly closed plant-litter-plant nutrient cycle.

Approximately 50% of the fine feeder roots occurring in the litter layers were ectomycorrhizal. It was proposed that these fungi may provide access to both inorganic and organic sources of N and inorganic, complex inorganic and organic sources of P. *In vitro* studies examined the ability of ectomycorrhizal fungi isolated from *P. patula* plantations to utilize various N (ammonium sulphate, nitrate, BSA, glutathione and various amino acids) and P (potassium dihydrogen phosphate, aluminium phosphate, phytic acid, RNA and glycerophosphate)

ABSTRACT

Litter accumulates on the forest floor in *Pinus patula* plantations in Mpumalanga, South Africa and as a result nutrients become immobilized and site productivity is reduced. Studies have correlated litter accumulation with abiotic factors, such as high altitude sites, high rainfall soils derived from the timeball series (shale) have thick litter layers. This study focuses on the biotic factors involved in litter accumulation.

The forest floor mass is determined by the balance between the rates of litter production and decomposition. Litter accumulation may occur as a result of an imbalance between these processes. To determine the cause of the imbalance litter production and accumulation in an age series (5-30 years) of *P. patula* compartments growing at both low and high altitude sites was quantified and decomposition rates were measured. Litter production was shown to increase up to a stand age of 10 years, whereafter it continued to increase but at a slower rate. The average annual litter production figure ranged from 3.64 to 5.89t ha^{-1} yr^{-1} measured over a two-year period. No significant relationship existed between litter production and altitude but litter accumulation was shown to increase with increasing stand age and increasing altitude.

One of the factors affecting litter decomposition is litter quality. Litter collected from litter traps was analyzed for total N, total P, lignin, cellulose, polyphenolics and tannins. No significant relationships were evident between any of the litter quality variables and increasing altitude. To determine decomposition rates, litterbags were buried under the litter layer in a compartment of each age group, at both low and high altitudes. Litterbags were collected at intervals of 6 weeks, 3 months, 6 months, 9 months and 12 months and weighed to determine mass loss and chemically analyzed for total N and P, to determine changes in litter quality. Decomposition was shown to occur in two phases. During phase M1 (0-6 weeks) there was a rapid loss in litter mass after which mass continued to be lost but at a slower rate (Phase M2, 6-52 weeks). The average overall mass lost was 34% yr^{-1}. Examination of decomposition rate constants (k) indicated slower litter turnover in the high altitude sites (range -0.34 to -0.54). This combined with the trend of decreasing decomposition rates (Phase M2) with increasing altitude indicated that reduced decomposition rates were responsible for the

LITTER ACCUMULATION IN *Pinus patula* PLANTATIONS AND THE ROLE OF ECTOMYCORRHIZAL FUNGI IN A FOREST ECOSYSTEM

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LIST OF FIGURES

List of tables continued

List of tables continued

LIST OF TABLES

Table of contents continued

Table of contents continued

Table of contents continued

which includes both the amount of water absorbed and the rate at which water is absorbed (Moore, 1986). Water absorption is influenced by the morphological characteristics of the litter such as cuticle and leaf thickness (Taylor and Parkinson, 1988). Environmental factors also influence this initial phase, with leaching increasing with increasing temperature and rainfall (Buldgen, 1982).

The compounds released during leaching are readily metabolised by micro-organisms, who derive energy from the C for the subsequent breakdown of the litter. Leaching alters the chemical composition of the litter by lowering the sugar concentration (Ibrahima, Joffre & Gilion, 1995). The initial quantity of water-soluble substances in the litter is positively related to the decomposition rate; however, prolonged leaching could make litter less readily decomposable as the energy derived from the C compounds becomes deficient and the litter can no longer sustain microbial growth (Berg & Ekbohm, 1991; Ibrahima, Joffre & Gilon, 1995). Other chemical changes may occur depending on the species of litter e.g. cellulose and lignin concentrations and the lignin:N ratio may increase (Parson, Taylor & Parkinson, 1990).

Litter is broken down further by the action of decomposer organisms; this biological phase of decomposition proceeds concurrently with the leaching phase and beyond this initial phase. Litter serves two functions for the microflora, it provides energy and supplies C for the formation of new cell materials and catabolic enzymes. Microbial cells are approximately 50% C, the source of which is the substrate being utilized. The process of converting the substrate into protoplasmic C is called assimilation. At the same time as C is being assimilated, N and phosphorus (P), as well as other nutrients are required to sustain growth and reproduction. This uptake of nutrients is an important means of immobilization, *i.e.* the mechanism by which micro-organisms reduce the quantity of plant available nutrients in the soil (Alexander, 1977; Vogt, Grier & Vogt, 1986). Decreased availability of nutrients, or decreased litter quality, results in decreased decomposition activity by micro-organisms which ultimately influences the decomposition rate, as well as the chemical changes occurring during the decomposition process (Vogt, Grier & Vogt, 1986).

The relationship between FRP and aboveground litter production is not clear. Quantification of FRP is problematic, with several methods being followed. Nadelhoffer & Raich (1992) examining published data concluded that there was no correlation between FRP estimates and aboveground production. However, they stressed that the variability in the methods used to estimate FRP, may have obscured any relationship. Analysis of forest carbon (C) and nitrogen (N) budgets did, however, indicate that belowground production increased with aboveground production, suggesting that these two components of net primary production are linked and are possibly limited by the same factors (Nadelhoffer & Raich 1992).

1.3.2 Nutrient outputs

Decomposition

The decomposition of organic matter is an important means by which organically bound nutrients are released, returned to the soil, and made available for plant uptake. The measurement of litter decomposition is based on determinations of the amount of litter remaining after a period of time compared with the initial amount of litter. Expressions widely used for the decomposition rate are, the exponential decay constant (k) and the annual decay percentage (Olson, 1963).

The chemical constituents of litter can be separated into 3 groups namely, soluble substances; polymer carbohydrates and holocellulose; and lastly, acid insoluble aromatic compounds such as lignin and phenolic products (Mc Claugherty & Berg, 1987). Coniferous litter is characterized by low concentrations of nutrients and proteins and high levels of polyphenolic substances, factors which make pine litter resistant to decomposition (Berg & Staaf, 1980).

The initial phase of decomposition can be attributed mainly to the leaching of both inorganic elements, such as Ca^{2+}, K^{+} and Mg^{2+} and organic elements, such as organic acids, proteins and soluble sugars (Berg & Staaf, 1980; Ibrahima, Joffre & Gillon, 1995; Parsons, Taylor & Parkinson, 1990). Not all water-soluble compounds are leached at the same rate and the quantity released by the litter varies with species (Ibrahima, Joffre & Gillon, 1995). The physiochemical process of leaching is influenced by the litter's water absorption capacity,

are retained for approximately 24 months. A seasonal peak in needlefall occurs shortly after the maximum annual needle production (Morris, 1986). Factors such as climatic conditions, which delay needle production, will, in turn, affect needle longevity. It has been suggested that plant water stress is the possible proximal cue for needlefall (Wright & Cornejo, 1990).

Studies have shown that there is a negative linear relationship between total litter production and latitude, indicating an increase in litter production towards the equator (Bray & Gorham, 1964; Vogt, Grier & Vogt, 1986). Litter production has also been shown to be positively correlated with light availability (solar radiation) during the growing season, with equatorial regions having a longer growing season (Dickinson & Pugh, 1974). Litter production has also been positively correlated with temperature and precipitation during the growing season (Bray & Gorham, 1964; Kirschbaum, 1995). Meentemeyer, Box & Thompson (1982) examined the relationship between litter production and climate, concluding that it correlated well with the annual actual evapo-transpiration which combines both the energy and the moisture available in the ecosystem.

Litter production increases with stand age until canopy closure, after which the amount of litterfall remains the same. Although the quantity of litterfall remains constant in older plantations, changes may occur in the ratio of needles to larger litter such as cones and branches (Gholz *et al.*, 1985; Morris, 1986; 1992).

Belowground litter input

Little is known about root death and turnover, but the nutrient input from this belowground process may be as significant as, or more significant than the aboveground litterfall returns (Hendricks, Nadelhoffer & Aber, 1993). Belowground litter production is dependent on fine root production (FRP). Studies have suggested that FRP may contribute up to 75% of the annual net primary production in some ecosystems (Nadelhoffer & Raich, 1992). The aboveground litter input for *Pinus sylvestris* (Scots pine) was calculated as 71gC m^{-2} yr^{-1}, compared with the belowground inputs of 90gC m^{-2} yr^{-1} (Persson, 1980). The litter input from the roots is closely related to periods of drought, during which the mortality of roots increases. However, the seasonality of root litter production may be less pronounced than for aboveground litter production (Persson, 1980).

deposition in the afforested regions of Mpumalanga have been calculated as 8.6-32.9 SO_4^{2-}, 1.9-11.7 NO_3^-, 1.1-2.7 NH_4^+, 2.1-6.0 Ca^{2+}, 0.1-1.0 Mg^{2+} and 1.3-3.7 K^+ (Olbrich, 1995).

Forest canopies have a filtering effect on particles in the atmosphere. The canopy traps more aerosols and dust than a flat surface, due to the increased surface area. Accurate measurements of dry deposition are difficult to assess, because of problems with measurement techniques; some estimates (kg ha^{-1} yr^{-1}) of the major cations and anions contributed by dry deposition in the afforested regions of Mpumalanga have been calculated as 0.89-24.4 SO_4^{2-}, 0.62-24.9 NO_3^-, 1.22-17.0 Ca^{2+}, 0.1-0.8 Mg^{2+} and 0.5-1.3 K^+. This suggests that wet and dry deposition provides comparable quantities of nutrients for most elemental species (Olbrich, 1995).

Throughfall or canopy leaching is also regarded as a nutrient input process. Recent studies by Olbrich, Du Toit & Van Rensburg (1993) have examined the throughfall under *P. patula* in two sites in Mpumalanga. This study showed that, in general, concentrations of SO_4^{2-}, NO_3^-, Cl^-, Ca^{2+}, Mg^{2+} and K^+ ions in throughfall were enhanced relative to open (rainfall) samples, indicating that the canopy acted as a source rather than a sink of ions. This increase in nutrients was particularly evident with K^+, indicating the ability of K^+ to be leached from *P. patula* needles. These data support studies by Zamierowski (1975) which showed that considerable more K^+ than Ca^{2+} or Mg^{2+} can be extracted from *P. patula* needles with distilled water, indicating the leachable nature of the cation.

Atmospheric deposition has the potential to effect the long-term fertility of soil. Deposition acidifies the soil through the leaching of base cations from the soil, which results in decreased availability of cations and increased heavy metals, in particular aluminium, in the soil solution (Olbrich, 1995)

Aboveground litter production

One of the primary aboveground sources of nutrients entering the forest ecosystem is via the litterfall. Many factors have been shown to affect litter production. The longevity of needles is an important factor and is dependent on species. Son & Gower (1992) reported that needle longevity was 36 months for *P. strobus* (white pine), 46 months for *P. resinosa* (red pine) and 66 months for *Picea abies* (Norway spruce). *P. patula* needles

principles and concepts apply to the exotic, fast growing pine plantations in Mpumalanga. This has been demonstrated by Morris (1986; 1995) in the Swaziland *P. patula* plantations.

Nutrient cycling involves three main cycles; a geochemical, biological and a biogeochemical cycle (Attiwill & Adams, 1993). The geochemical cycle involves inputs of nutrients from atmospheric deposition, weathering and the outputs of nutrients through leaching. The biological cycle involves the movement of nutrients from soil to plant to soil, via the forest floor. The process includes the storage and retranslocation of nutrients within the tree. The biogeochemical process involves the movement of nutrients between the geosphere, the biosphere and the atmosphere (Attiwill & Adams, 1993; Charley & Richards, 1983; Helmisaari, 1995). The focus of this project is on the biological processes that control the rates of transfer within these cycles.

The functioning of the ecosystem depends largely upon the activities of soil micro-organisms found in the upper layers of soil. The storage of organically bound and exchangeable nutrients in the forest floor and underlying soil allows all systems to function in spite of the fact that nutrient inputs may not be in phase with the requirements of decomposer organisms or the release of nutrients by decomposers may not be synchronous with plant uptake (Gosz, Likens & Bormann, 1976). The following section discusses various aspects of nutrient inputs and outputs involved in the nutrient cycle.

1.3.1 Nutrient inputs

Wet and dry deposition

Nutrients which are dissolved in rain and mist contribute quantities of nutrients to the soil, and are regarded as inputs into the ecosystem. These nutrients are derived mainly from air pollutants and the quantities of nutrients can vary between rainfall events, seasons and years (Parker, 1990; Probert, 1976). The quality of rainwater is an important means of monitoring air pollution to which forests are notably sensitive. This sensitivity is attributed to the forest's capacity to absorb pollutants. The large crown volume of forest trees and the longevity of the trees and needles increases the absorptive surface and the exposure time of the trees to pollutants (Olbrich, 1995). Estimates (kg ha^{-1} yr^{-1}) of the major cations and anions contributed by wet

The accumulation of litter on the forest floor has been shown to increase with increasing distance from the equator. This can be illustrated by comparing *P. banskiana* (Jack pine) growing in Minnesota, USA which has a forest floor mass of 12t ha^{-1} with the 130t ha^{-1} under *P. banskiana* in New Brunswick, Canada (Vogt, Grier & Vogt, 1986). The increase in litter accumulation with increasing latitude indicates that the formation of 'mor' layers may be influenced by abiotic factors such as temperature and rainfall. The influence of these abiotic factors have been confirmed by Schutz (1990). In Schutz's (1990) study litter accumulation was positively related to both rainfall and altitude, among other factors. Examining the biotic influences on litter accumulation Schutz (1990) showed that accumulation could be correlated with several foliar nutrient concentrations, which include positive relationships with B, Mg, Al and Zn and a negative relationship with Ca.

Litter depth in managed, even aged plantations declines after clearfelling due to disturbances, then increases after the next rotation is planted until canopy closure. This increase reaches a maximum about 1/3 to 2/3 of the way through the rotation (De Ronde, 1993).

Litter accumulation may occur as a result of an imbalance in the input of organic material into the ecosystem (i.e. aboveground litterfall) and the breakdown or output of this material (i.e. decomposition). The relationship between litter accumulation and these biological processes in *P. patula* plantations has not previously been examined in Mpumalanga. If litter accumulation is a result of the imbalance between inputs and outputs, then it must influence the nutrient cycles in these *P. patula* plantations. This would in turn affect the long term productivity of these sites as well as their sustainability during future rotations. The following section examines the current literature pertaining to nutrient cycles in forest ecosystems.

1.3 NUTRIENT CYCLING

Studies on the storage and movement of nutrients in forest ecosystems (i.e. nutrient cycling) have been conducted for more than 100 years (Attiwill & Adams, 1993). Although much research has been conducted in the temperate regions and mainly on natural forests rather than managed plantation forests, the basic

Litter serves many functions; it acts as an insulating layer which protects the soil from extreme changes in moisture and temperature; it protects the soil from erosion and frost and it improves water infiltration (Hering, 1982; Schutz, 1990). Biologically, litter provides energy and nutrients for heterotrophic organisms. The litter is metabolised by a host of organisms, resulting in the release of the nutrients which are then returned to the soil for reuse by the plant (Singh & Gupta, 1977). Litter, therefore, influences or regulates most of the functional processes occurring throughout the forest ecosystem (Gosz, Likens & Bormann, 1976). The conservation of essential nutrients and their efficient utilization in growth is required to sustain forest productivity, especially as many forest stands are located on nutrient poor sites (Attiwill & Adams, 1993; Vogt, Grier & Vogt, 1986).

If the decomposition or breakdown of litter slows down for any reason, litter or raw humus will accumulate resulting in a 'mor' layer (Hering, 1982; Schutz, 1990). Mor layers are regarded as a threat to site productivity, as nutrients are immobilized in the litter, organic acids are released and moisture penetration is altered. Practical problems also arise such as restriction of access, increased fire danger because of the increased fuel load and lack of wind firmness in newly planted trees of subsequent rotations (Schutz, 1990).

In an extensive research project carried out in the late eighties in South Africa litter accumulation was shown to be a potential problem in *P. patula* plantations in Mpumalanga, with a maximum thickness of between 60-65cm being recorded at Ceylon State Forest (Schutz, 1990). On average the litter thickness recorded under *P. patula* is 10cm (range of 3-35cm). Average litter thicknesses of 6cm (range of 3-13cm) have been recorded under *P. taeda* and 5cm (range of 3-14cm) under *P. elliottii*. The latter two species are also grown in the region (Schutz, 1990). Under *P. patula*, litter accumulation is manifested in there being little breakdown of litter, with only the formation of L and F layers (Schutz, 1990). In contrast to this, in the Western Cape, litter accumulation occurring under *P. pinaster* has an average litter depth of 13cm, the accumulation of the H layer being more prevalent (De Ronde, 1984; 1993).

The main silvicultural problems in growing *P. patula* in South Africa are the morphology of trees; fungal diseases such as *Rhizinia undulata* (root rot) which causes severe mortality of planted seedlings after slash burning (Germishuizen, 1984) and *Sphaeropsis sapinea*, which causes shoot blight and top die back becoming particularly prevalent after hail damage and drought (Swart & Wingfield, 1991; Smith *et al.*, 1996); and nutritional problems which may be associated with litter accumulation in the pine plantations (Wormald, 1975).

The establishment of many pine species in Southern Africa was initially hampered by the lack of suitable ectomycorrhizal fungi in the soil. It was only after the importation of humus and rootlets from native pine growing areas, which were then added to nursery soils, that success was finally achieved (Wormald, 1975). Presently, in South Africa, there is an increased demand for land to meet the needs of rural communities and as a result new land is no longer available for afforestation. The continued success of the industry lies in improving the sustainability of currently utilized forestry land.

1.2 LITTER ACCUMULATION

The term litter refers to dead plant material, both above- and below ground, such as dead needles, branches, twigs, cones and roots. Litter is an important characteristic which distinguishes forest soils from agricultural or cultivated soils (Satchell, 1974). In this thesis the term 'litter' refers to aboveground litter, unless otherwise stated. When aboveground litter falls it lies on the soil surface until it is covered by new litter. The mineral soil becomes permanently hidden by a series of these organic layers. These organic or 'mull' layers are described as;

- the litter (L) layer, which is composed of intact dead leaves;
- the fermentation (F) layer, which is composed of broken up organic matter that is still recognisable as litter and
- the humus (H) layer, which is composed of unrecognisable dark, powdery, organic matter (Hering, 1982).

1 INTRODUCTION

1.1 FORESTRY IN SOUTH AFRICA

The forestry and forest products industries are two of the largest and fastest growing sectors of the South African economy. Approximately 1.4 million ha are afforested with over 50% planted to pine (South African Forestry Facts, 1994). The genus *Pinus*, is of economic importance, with pine timber having a variety of uses from sawlogs, for the building industry, to pulp for paper production (Poynton, 1980).

P. sylvestris was the first pine species to be introduced into the country. This species was brought in by the Dutch East India Company; however, their initial attempts at establishment were unsuccessful. *P. pinaster* and *P. pinea* were later introduced and established by the French Huguenots towards the end of the 17th century. The first commercial plantation, Genadendal, was established between 1825-1830. Thereafter followed a rapid introduction of many pine species (Poynton, 1980).

Pinus patula Schlechtd. et Cham. was first introduced to South Africa from Mexico in 1907 and has since been widely used as a plantation species (Poynton, 1980). This species is also grown in Swaziland, Angola, Mozambique, Zimbabwe, Madagascar, East Africa, Malawi, Kenya, Tanzania, Uganda, Rwanda and Burundi (Wormald, 1975). The species thrives best on cool, moist sites in temperate humid regions and the mist belt of the summer rainfall regions. The species is not suited to areas with altitudes below 750m. The best growth rates are obtained in areas with an annual precipitation of ± 1800mm. The mean height increment during the first ten years is 1-1.5m per annum, and canopy closure is achieved at 4-5yrs. *P. patula* is resistant to frost, but on shallow soils the species suffers from drought during periods of abnormally low rainfall (Schonau & Fitzpatrick, 1981; Wormald, 1975). *P. patula* succeeds on a variety of soils, including those derived from dolerite, granite, dolomite, quartzite and sandstone (Schonau & Fitzpatrick, 1981).

Plate 8.8 WITS 06 - Autofluorescence of ectomycorrhizal root tip; *a* - 390-420nm, fluoresces green at tip. (Scale bar = 70μm).

Plate 8.9 WITS 06 - Cross section of ectomycorrhizal root; *a* & *b*; *hn* - common type A, palmetti 'Hartig' net; *lh* - lobed haustoria; *t* - tannin cells; *c* - cortical cells (Scale bar = 10μm)

Plate 8.10 WITS 06 - Longitudinal section of ectomycorrhizal root; *a* & *b*; *hn* - common type A, palmetti 'Hartig' net; *lh* - lobed haustoria; *t* - tannin cells; *c* - cortical cells; *rt* - root tip (Scale bar = 10μm).

Plate 8.11 WITS 01 - *Scleroderma citrinum* Pers. sporocarps; *a* & *b*; *sm* - dark coloured, internal, spore mass; *w* - wart-like protuberances.

Plate 8.12 WITS 06 - *Boletus piniciola* Vitt. sporocarps; *a* & *b*; *cp* - cap; *st* - stipe; *p* - pore layer; br - characteristic blue bruising of the pore layer.

LIST OF PLATES

Plate 7.1 Ectomycorrhizal fungal isolates growing on MMN medium; *clockwise from top right* - isolates WITS 01, WITS 02, WITS 04 and WITS 06.

Plate 8.1 WITS 01 - White, smooth, dichotomously branched ectomycorrhizal roots showing pale yel rhizomorphs; *a* & *b*; *dbr* - dichotomous branching; *m* - mantle; *r* - rhizomorphs (Scale bar = 150μm).

Plate 8.2 WITS 01 - Autofluorescence of ectomycorrhizal roots; *a* - 390-420nm, entire mycorrhiza fluoresces green; *b* - 450-490nm, entire mycorrhiza fluoresces yellow; *dbr* - dichotomous branching; *m* - mantle; *r* - rhizomorph (Scale bar = 120μm).

Plate 8.3 WITS 01 - Plectenchymatous mantle; *a* - loose outer mantle, no distinct pattern; b - inner mantle, hyphae more compact; *hb* - ring-like arranged hyphal bundles; *cl* - clamp connections (Scale bar = 10μm).

Plate 8.4 WITS 01 - Undifferentiated rhizomorphs, parallel hyphae compactly woven; *a*, *b* & *c*; *d* - emanating hyphae; *br* - branching; *icu* - inverted clamp anastomoses; *s* - septum; *cl* - clamp connections (Scale bar 10μm).

Plate 8.5 WITS 01 - Cross section of ectomycorrhizal root; *a* & *b*; *m* - mantle; *hn* - common type A, palmetti 'Hartig' net; *t* - tannin cells; *c* - cortical cells; *v* - vascular tissue; *cl* - clamp connection (Scale bar = 10μm).

Plate 8.6 WITS 01 - Longitudinal section of ectomycorrhizal root; *a*, *b*, *c* & *d*; *m* - mantle; *om* - loose outer mantle; *im* - compact inner mantle; *hn* - common type A, palmetti 'Hartig' net; *rt* - root tip (Scale bar 10μm).

Plate 8.7 WITS 06 - Brown, simple to dichotomously branched ectomycorrhizal roots; *a* & *b*; *c* - beaded ECM roots; *dbr* - dichotomous branching; *rt* - root tip (Scale bar = 150μm).

List of figures contined

it involves the utilization of organic acids in the tissue and the dark fixation of CO_2. Both the glutamate dehydrogenase and the glutamine synthetase pathways have been identified in mycorrhizas (France & Reid, 1983). The absorption of phosphates results in its conversion to nucleotides and sugar phosphates, followed by the accumulation of inorganic orthophosphates, organic phosphates and polyphosphates (Cox *et al.*, 1980).

Studies in temperate regions have shown that ECM dominate in environments in which nutrient supplies are seasonal, therefore, the storage of nutrients in the mycorrhizal structures decreases the loss of nutrients through leaching during periods of greater nutrient availability. A substantial amount of N has been shown to be cycled through the mycorrhizal system. Vogt *et al.*, (1983) found that mycorrhizal fruiting bodies returned twice as much N to the soil when compared with the aboveground litterfall in *Abies amabilis* forests. In a *Pseudotsuga* forest ecosystem mycorrhizas were reported to be responsible for 30% of the total N throughput (Fogel & Hunt, 1983).

The nutrients absorbed into the symbiotic system can be stored and recycled within the system. Furthermore, the presence of mycorrhizal hyphal linkages, along which both nutrients and C can pass from one host plant to another, and can result in a close cycling of nutrients within the ecosystem (Harley, 1989; Newman, 1988). The presence of mycorrhizal linkages may be less important in ectotrophic commercial plantations where host plants are grown in uniform stands and mycobionts show some degree of specificity. In this case it would be unlikely that nutrients would be transferred from the plantation trees to understorey plants such as *Solanum mauritianum* (bug weed), and *Acacia mearnsii* (Wattle) members of the Solanaceae and Fabaceae families, respectively, which are known to have endomycorrhizas (Harley & Harley, 1987).

Ectomycorrhizal role in decomposition

Several studies have shown that some ECM fungi are able to solubilize complex compounds such as organically bound N and P. Initially, it was thought that ectomycorrhizal fungi lacked the enzymes necessary to exploit complex organic sources of N and that they were dependent on simple organic forms of N such as amino acids, urea and glutamic acid (Marks & Kozlowski 1973). *In vitro* experiments have demonstrated that some species of ectomycorrhizal fungi are capable of using proteins as a sole N source. It has been confirmed that ECM

1.5.3 Ectomycorrhizas and the cycling of nutrients

Cycling of carbon

Mycorrhizal fungi obtain their C directly from the host plant and thereby have a constant supply of usable carbohydrate, allowing them to compete with non-mycorrhizal micro-organisms for inorganic nutrients (Harley, 1989). Many studies have examined the effect of mycorrhizas on carbon allocation and have shown that mycorrhizal plants have increased rates of photosynthesis and subsequently increased biomass as a result of the increased flow of nutrients to photosynthetically active leaves (Reid, Kidd & Ekwebelam, 1983). Under certain conditions mycorrhizas can drain host C because of the increased rate of respiration and the potential loss of soluble carbohydrates from the roots. Conversely, the loss of these soluble carbohydrates from the roots may provide a substrate for other soil microbes involved in nutrient transformations (Vogt, Publicover & Vogt, 1991).

The activities of the mycorrhizas and other soil microbes result in the release of CO_2 into the atmosphere. The amount of CO_2 respired can exceed (by 50-200%) the amount of C made available to the soil through the decomposition of both above- and below-ground litter (Marks & Kozlowski, 1973). The photosynthetic C diverted through rhizosphere and mycorrhizal organisms probably accounts for much of the respired CO_2 (Brundrett, 1991; Harley, 1989). More than 10% of the C photosynthesized by the host may be diverted through the mycorrhizal fungi and in one study by Fogel & Hunt (1983) 50% of the total annual throughput of C in a *Pseudotsuga* forest was respired by the mycorrhizal fungi. Studies in *Abies amabilis* (Pacific silver fir) forests estimate the C allocated to mycorrhizal fungi to be 15% (Vogt *et al.*, 1983). It is evident from these studies than ECM fungi play an important role in the C cycle.

Cycling of nutrients

It is well accepted that mycorrhizas enhance the uptake of nutrients from the soil, by absorbing nutrients at a distance and then translocating them to the root (Brundrett, 1991; Harley, 1989) Ectomycorrhizas absorb inorganic phosphates and ammonium compounds from soils of high phenolic content where nitrates are deficient. The mechanism of N uptake results in the rapid accumulation of glutamine in the fungal hyphae, and

1989). A number of ectomycorrhizas are smooth with few hyphal connections capable of exploiting nutrients from the soil. Evidence suggests that these fungi produce diffusible enzymes which provide the host plant with access to sources of N (Read, Leake & Langdale, 1989).

The formation of a fungal sheath around the roots contributes significantly to the amount of fungal tissue involved in mycorrhizal symbiosis as well as increasing the diameter of the root. Several studies have shown that the fungal sheath may account for as much as 39% of the ECM root mass (Harley, 1989). This figure excludes the contribution of fungal tissue made by the hyphal network and fruiting bodies. The sheath also has an accumulatory function and acts as an organ for the storage of nutrients and carbohydrates (Harley, 1989).

Most trees and shrubs initially develop tap roots in the seedling stage, which will later form the main axes, the apex of which will grow to extend the root system. From the main axes branches are formed, namely, long roots with unlimited growth and short roots with restricted growth and life-span. In the genus *Pinus* the short roots are sharply differentiated from the axes which bears them (Schenck, 1982). When infected by an ECM fungus, these short roots continue to grow slowly, branch dichotomously and form mycorrhizal systems. The dichotomous form of branching, typical of *Pinus* ECM, is rare or absent in other plant genera (Agerer, 1987-1995; Harley & Smith, 1983). This increased root branching increases the root surface area available for absorption of nutrients.

Mycorrhizal infection of the root system is permanent, although the longevity of mycorrhizas themselves may vary. It has been estimated that the longevity of *Pinus* mycorrhizal rootlets may be from several months to a year, although longer estimates have been made. Longevity depends on the climatic conditions as well as the position of roots in the soil horizon; for example, root growth may cease in surface layers during the coldest periods. This would result in a considerable mortality of roots, which ultimately would affect root turnover (Marks & Kozlowski, 1973; Harley & Smith, 1983). Another characteristic of ECM is their ability to grow apically, producing new active regions, while their proximal region ages and dies. During senescence the hyphae may remain active after the cells begin to degenerate (Harley, 1989).

In ectotrophic forests many types of mycobionts may occur, symbiosis is generally obligatory and the mycobiont is often specific to one or only a few host species. The hosts themselves may form associations with a wide range of mycobionts, often simultaneously. It is thought that these diverse associations alleviate the direct competition between individuals growing side by side (Malloch, Pirozynski & Raven, 1980; Vogt, Publicover & Vogt, 1991). Zak (1973) estimated that more than 100 fungal species have the potential to form ECM with *Pseudotsuga menziesii* (Douglas fir) some of which appear to be host specific while others are not. Trappe (1977) suggested that this was an under-estimation and that 2000 fungal species are potential mycobionts of this host.

Host plants may also be associated with different mycobionts at different times during their development, or depending on ecological conditions and climatic fluctuations. The mycobionts on seedlings are usually replaced as the plant matures, resulting in a succession of ectomycorrhizal types (Malloch, Pirozynski & Raven, 1980; Mason *et al.*, 1983).

1.5.2 The role of ectomycorrhizas in ecosystems

The effectiveness of mycorrhizas in ecosystems is influenced by a number of interacting factors regulated by the host plant, the mycorrhizal fungus, soil conditions and environmental factors (Brundrett 1991; Miller, Koo & Molina, 1991; Vogt, Publicover & Vogt, 1991). It has been well established that mycorrhizal fungi benefit their hosts by enhancing mineral uptake from the soil and thus contribute to cycling of nutrients in the ecosystem. The enhanced mineral uptake ability of mycorrhizal roots is related mainly to the increased surface area effective in ion absorption (Brundrett, 1991; Harley & Smith, 1983).

Most ECM have a considerable network of hyphae or hyphal strands which have long been regarded as extensions of the absorbing surface which serve to exploit the soil and translocate absorbed nutrients to the root. However, nutrient deficiency zones, especially of poorly mobile nutrients e.g. P, will develop around the hyphal network. Another function of these hyphal extensions is to provide new absorbing surfaces to cross the deficiency zones: the growth of hyphae is less expensive in material terms than the growth of roots (Harley,

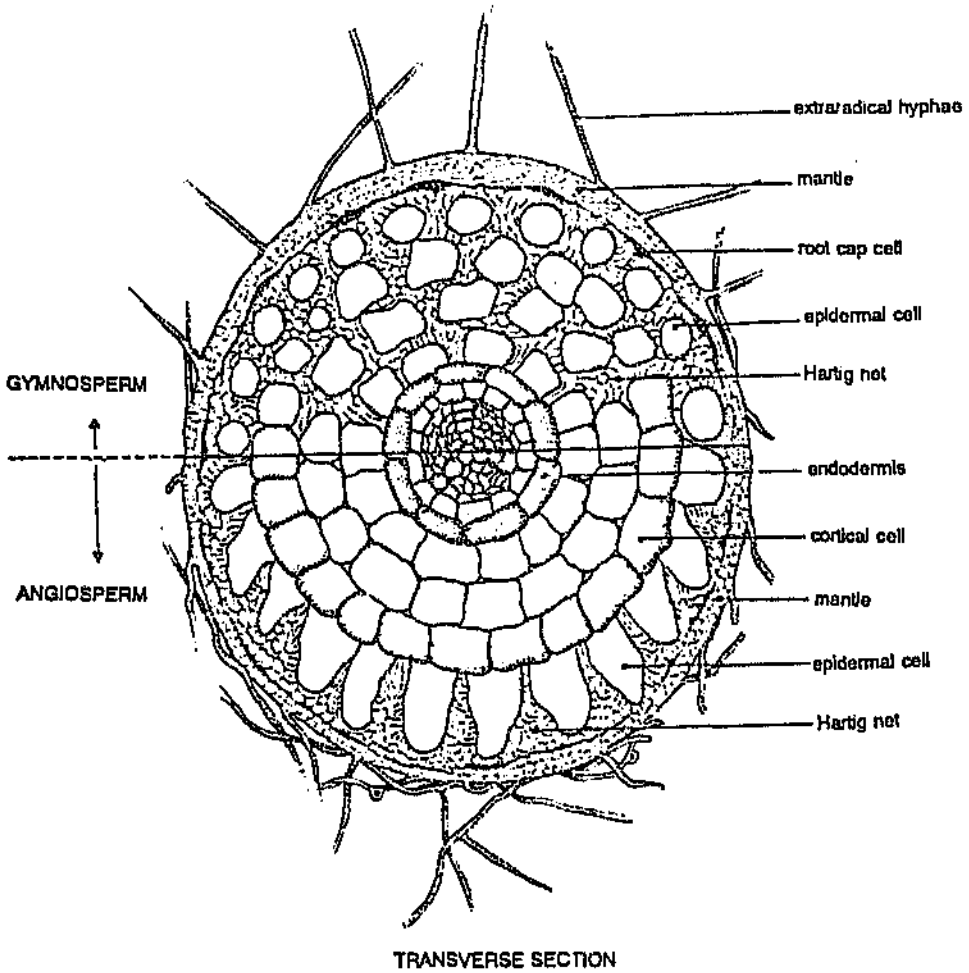
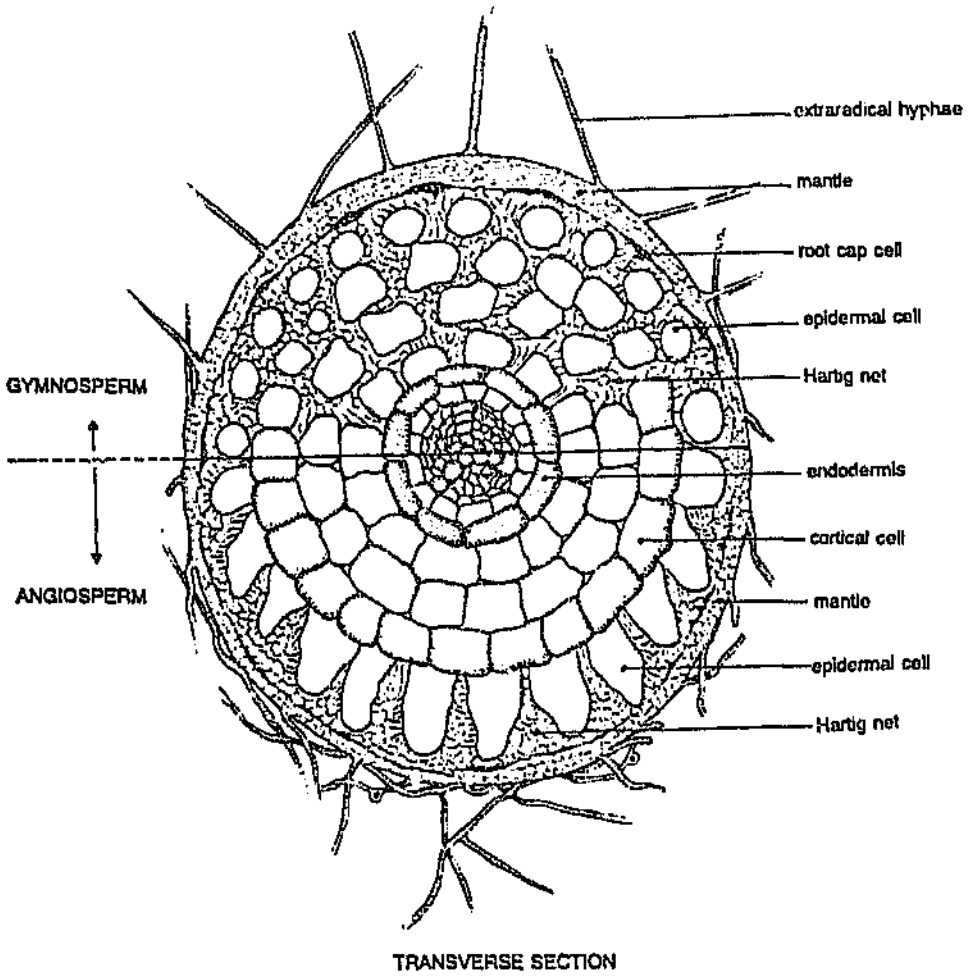


Figure 1.1 A diagram illustrating the anatomy of an ectomycorrhizal root (Brundrett, Melville & Petersen, 1994).

Ectomycorrhizal hosts are generally forest species occurring in the northern and southern temperate and sub-arctic regions and includes the genus *Pinus*. The genus *Eucalyptus*, a temperate and subtropical species from Australasia, is also ectomycorrhizal (Harley & Smith, 1983). These two genera are the main commercial forest species in South Africa. Tropical and subtropical trees may also be ectomycorrhizal *e.g. Casuarina* and *Allocasuarina* species, members of the Casuarinaceae and some members of the Caesalpinoideae (Harley & Smith, 1983; Thoen, Soygoufara & Dommergues, 1990).



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1.5 ECTOMYCORRHIZAS

1.5.1 Introduction

Symbiotic relationships between fungi and plant roots are ubiquitous in nature. Mycorrhizal associations are categorized according to their morphological characteristics. From these characteristics three main groups can be distinguished, the ectomycorrhiza (ECM), the endomycorrhiza and the ectendomycorrhiza (Harley & Harley, 1987).

Ectomycorrhizal associations are prevalent in *Pinus* as well as other forest ecosystems being identified by the presence of an external fungal sheath or mantle which encloses the roots of host plants in fungal tissue. Hyphae penetrate between the epidermal and cortical root cells forming a 'Hartig' net (Harley & Smith, 1983). A diagram illustrating the anatomy of an ectomycorrhiza is presented in figure 1.1 (Brundrett, Melville & Peterson, 1994). There is a considerable amount of variation between ECM in the development of the sheath, the development of the Hartig net, the extent of intercellular penetration and in the fungal connections between the sheath and the soil. It is these characteristics which have been used in the identification of ectomycorrhizas (Agerer, 1986; Zak, 1973).

The mycobionts associated with ECM include families of the Basidiomycota, Ascomycota, sterile Fungi Imperfecti and occasionally the Zygomycota (Harley & Harley, 1987). Field observations, such as the consistent association of fruiting bodies with a particular tree species and/or the presence of fungal hyphal attachments between fruiting bodies and the mycorrhizal root, have aided in the identification of the mycobiont. Pure culture isolations made from fruiting bodies, where possible, have been used to inoculate potential host seedlings under axenic conditions, the formation of ECM roots confirming the symbiotic relationship (Harley & Smith, 1983).

1.4.2 Fire

Slash burning after harvesting is no longer standard management practise in South African forests; however, forest wildfires still occur and it has been estimated that + 6430ha of plantations are burnt annually (van Wilgen & Scholes, cited in Olbrich, 1995). Burning is known to have a marked effect on nutrient cycling in ecosystems (Goh & Phillips, 1991). The temperatures attained during burning are determined by the organic matter content, its bulk density and moisture content. In *P. patula* compartments the forest floor is heterogenous in nature due to disturbances caused by thinning and pruning operations, changes in the microtopography and bushpig activity (Schutz, 1990); this results in hot spots occurring during forest fires. Substantial quantities of N and P are lost during burning with a loss of up to 66% and 35%, respectively, being recorded in New Zealand forests that have been clearfelled prior to burning (Goh & Phillips, 1991). Most of the N is lost as a result of volatilization (N_2), although a portion would be contained in the fly ash. Most of the P and cations are lost as particulate matter in the convection column, although volatilization of P has been reported to occur during fires of high intensity (Goh & Phillips, 1991).

1.4.3 Leaching

The leaching of nutrients from the soil profile represents a loss of nutrients from the ecosystem. This is of particular interest in areas were acid rain is prevalent, as in the escarpment areas of Mpumalanga. Studies by Carlson (1992) concluded that water percolating through the soil may become more acidic as the pH of rain decreases. Acidic precipitation could increase the amounts of Mg^{2+} and Ca^{2+} leached from the soil, reducing their availability to the plant and resulting in low basic cation concentrations in the soil.

1.3.4 Retranslocation

Before senescence of needles, nutrients are withdrawn and internally redistributed to support new growth (Proe & Millard, 1995). It is not clear whether this retranslocation occurs in response to site quality or environmental factors or whether the response is genotypic (Vogt *et al.*, 1995). In a ^{32}P study conducted by Proe & Millard (1995) no evidence was found to suggest that the amount of P remobilized was affected by current P supply; however, the timing and rate of N and P remobilization may differ. The phloem transport rate together with the rate of phloem loading and unloading which is partially a function of an osmotic gradient from source to sink, controls retranslocation. Almost no retranslocation takes place in the xylem (Chapin III & Kedrowski, 1983; Nambiar & Fife, 1991). This process of retranslocation is beneficial for the nutrient economy of the tree (Berg *et al.*, 1980).

1.4 NUTRIENT LOSSES

1.4.1 Harvesting

Under natural conditions forest trees would maintain the cycling of nutrients through the process of decomposition and subsequent return of nutrients to the forest soil followed by uptake. However, this is not the case when logs are harvested for commercial use. This represents a net loss of nutrients from the forest ecosystem, as nutrients contained in the logs are exported from the site. The potential annual export of nutrients (kg ha^{-1}) from *P. patula* sites range from between 10.2-15.1 N, 0.5-2.8 P, 4.1-9.4 K^+, 6.5-9.2 Ca^{2+} and 2.2-3.9 Mg^{2+} (Morris, 1986; 1992). Apart from the direct effect of harvesting on nutrient loss, the process also modifies many soil processes which subsequently affect nutrient availability (Goh & Phillips, 1991).

faeces which modifies the chemistry of the substrate, rendering it more available to microbial degradation; comminution, which breaks down litter into smaller pieces; and translocation which results in the redistribution of substrates, nutrients and micro-organisms (Setälä *et al.*, 1991; Shaw *et al.*, 1991).

1.3.3 Nutrient uptake

Terrestrial higher plants obtain the nutrients required for growth from the soil. These nutrients are mainly dissolved in the soil solution and solutes of low molecular weight are transported to the roots by diffusion or mass flow. This process is a non-metabolic, passive process dependant on availability of nutrients in the soil solution and the formation of root hairs (Jensén & Petterson, 1980; Marschner, 1995). Cell membranes act as a barrier, preventing the transport of nutrients against a concentration gradient. The movement of nutrients across the membrane involves specific binding sites or 'carriers' which facilitate the process. This is an active process and requires energy derived from ATP, the main source of which is respiration. Factors affecting respiration would therefore affect nutrient uptake. Some of these factors include:

- O_2 concentrations: a decrease in O_2 results in decreased uptake, particularly of K^+ and P;
- temperature: chemical reactions are temperature dependant, increasing temperatures would therefore enhance nutrient uptake;
- carbohydrates: this provides the substrate for respiration. There is a close interrelationship between light availability, carbohydrate supply to the roots, root respiration and nutrient uptake;
- pH: as H^+ concentrations increase pH decreases and cation uptake is generally inhibited at low pHs (Nye & Tinker, 1977; Marschner, 1995).

The size and distribution of the root system, as well as different physiological ages of individual roots are important in determining the magnitude of nutrient uptake (Jensén & Petterson, 1980).

Ectomycorrhizal (ECM) roots play an important role in the cycling of nutrients by increasing the surface area effective in ion absorption. In ECM this benefit is attributed to hyphal and mycelial strand formation, sheath formation, increased root branching, increased root diameter, and increased root longevity (Coleman, Reid & Cole, 1983).

Temperature has also been shown to be positively correlated with decomposition with increased temperatures stimulating microbial activity (Kirschbaum, 1995; Nicolardot, Fauvet & Cheneby, 1994). This relationship would only occur to an optimal limit as the microbial enzymes, such as cellulase, amylase, protease and phosphatase, to mention a few, which are responsible for decomposition would become inactive after a threshold temperature has been reached (Kshattriya, Sharma & Mishra, 1992; Sinsabaugh, Antibus & Linkins, 1991). These enzymes are also sensitive to pH, and microbial activity is enhanced under neutral to alkaline conditions (Alexander, 1977; Kshattriya, Sharma & Mishra, 1992). However, it must be remembered that microbial activity does not result only from the addition of simple effects, but is also influenced by interactions between the individual environmental factors (Nicolardot, Fauvet & Cheneby, 1994).

Microbial colonization of decomposing organic matter involves a number of species representing both the bacterial and fungal functional groups. During the initial phase of decomposition microbial communities able to degrade cellulose and presumably lignin are prevalent. As decomposition progresses changes in the microbial composition occur (Scheu & Parkinson, 1995). Changes in the microbial community occur as a result of chemical changes in the litter, notably the changes in the C to nutrient ratios. It is generally accepted that N is the element which becomes limiting during the early stages of decomposition, while C becomes limiting during the later stages (Enriquez, Duarte & Sand-Jensen, 1993). Coniferous forest litter is acidic and most studies have assumed that fungi are the main decomposer organisms. However, studies applying more direct methods of enumeration have suggested that bacteria may be more important in coniferous forests than previously thought (Persson *et al.*, 1980).

Soil fauna play an important role in the decomposition process and the cycling of nutrients. Soil fauna commonly distributed in coniferous forests include Nematoda (nematodes), Enchytraeida (potworms), Collembola (springtails), Acari (mites), Oligochaetes (earthworms) and Diplopoda (millipedes) (Shaw *et al.*, 1991). Soil fauna do not possess the range of enzymes required to breakdown organic matter and, therefore, do not influence decomposition directly. However, soil fauna can influence the mobilization and immobilization of nutrients through indirect methods. These methods include grazing on other micro-organisms, in particular fungi; predation, which can alter the balance between fungivores and bacterivores; conversion of litter into

Litter that decomposes slowly has been shown to have high C:N ratios, with N becoming limiting for microbial growth (Witkamp, 1966; Melillo, Aber & Muratore, 1982). The immobilization of N is generally considered to occur when the C:N ratio of litter is ≥ 30 (Parnas, 1975). Litter with high lignin and polyphenolic concentrations have also been shown to retard the decomposition rate (Fogel & Cromack, 1977; Mc Claugherty & Berg, 1987; Palm & Sanchez, 1991; Vanlauwe, *et al.*, 1995). The phenolic compounds generated by the oxidative degradation of lignin are known to have an inhibitory effect on the enzymes involved in degradation by micro-organisms (Kshatriya, Sharma & Mishra, 1992; Tian, Kang & Brussaard, 1992). Polymer carbohydrates decompose more rapidly, but because lignin and polymer carbohydrates are both physically and chemically linked, neither group of compounds decomposes independently of the other (Mc Claugherty & Berg, 1987).

Many studies have used ratios involving N, lignin and polyphenolics to predict the decomposition rate of various types of litter. Examples of these ratios are those of lignin:lignocellulose, lignin:N and polyphenol:N (Aber, Melillo & Mc Claugherty, 1990; Melillo, Aber & Muratore, 1982; Palm & Sanchez, 1991; Stump & Binkley, 1993; Upadhyay, Singh & Meentemeyer, 1989). However, other studies have not been able to show correlations between litter quality ratios and the rate of decomposition (Mc Claugherty, *et al.*, 1985; Moore, 1986). This can probably be attributed to differences in litter chemistry, particularly lignin and N, which varies widely among species (Aber & Melillo, 1980) and which may vary within species for stands of differing site quality and nutrient status (Lamb, 1976).

Rainfall not only influences the decomposition process during the initial leaching phase, but also influences the biological phase by maintaining the moisture content of litter at a level which sustains microbial activity. The number of rainfall events, rather than the total amount of rainfall, has been shown to be better correlated with decomposition. This indicates that regular moderate rainfall, which maintains the litter's moisture content, is more favourable than a small number of heavy rainfall events, which may lead to temporary litter dryness (Post *et al.*, 1985; Vanlauwe *et al.*, 1995). As the total amount of rainfall increases, anaerobic conditions may develop in the litter/microbial environment, retarding their activity. This relationship would presumably be affected by soil properties such as drainage.

Hypothesis 1: Litter production increases with increasing altitude (Fig. 2.1; chapter 2).

This chapter will examine litter production and accumulation in several *P. patula* compartments of various stand ages, at both low and high altitude sites. The relationships of these variables with stand age and altitude will be discussed.

4.2 METHODS

4.2.1 Study sites

A field study was established in March and April 1992, to monitor litter production in *P. patula* stands. Initially, thirty five *P. patula* compartments of stand ages between 5 and 30 years were selected from both low and high altitude plantations in Mpumalanga (Table 3.2; chapter 3). Compartments situated below an altitude of 1200m were designated as low altitude compartments, while those above this altitude were designated as high altitude compartments. At the beginning of the field study the quantity of organic matter on the forest floor, from all the compartments (Table 3.2; chapter 3), was assessed. Due to vandalism and the felling of drought damaged trees, a total of 15 compartments remained at the end of the first year of the study (Phase 1, ending March 1993). Several compartments were re-established in June 1993 to give a total of 24 compartments, two compartments in each age group at both low and high altitude sites (Table 3.2, compartments marked *) were selected. By the end of Phase 2 (June 1994) 22 compartments remained, two compartments being lost as a result of fire (Table 3.2; chapter 3).

4.2.2 Litterfall collection and quantification

Three litter traps (3m x 3m) were erected in each compartment. Traps were initially constructed from 40% shadecloth and were suspended 1m above the forest floor between groups of four *P. patula* trees. Litterfall, comprising mainly needles, branches and occasionally cones, was collected from the traps and quantified every

4 LITTER PRODUCTION AND ACCUMULATION

4.1 INTRODUCTION

Litter production is a collective term encompassing both the above- and belowground litter produced in an ecosystem. In this study the term litter production refers only to that contributed by the aboveground litterfall. Litter is an important source of nutrients and energy and is therefore a fundamental part of the nutrient cycling process (Meentemeyer, 1978). Litter production provides a major input of nutrients into the ecosystem. This input is balanced by the decomposition process or output of nutrients. Any disturbance of this balance could result in the litter accumulation observed under *Pinus patula* growing in high altitude sites, in the Mpumalanga plantations. Litter accumulation has been identified as a potential threat to long term site productivity, as nutrients may become immobilized in the litter layer resulting in nutrient limitations (Schutz, 1990).

Because of the role litter production may play in the accumulation of litter, this input process was quantified to determine whether increased litter production was responsible for the observed accumulation. Many studies have quantified litter production in forest ecosystems and global estimates have also been made to quantify annual organic matter production (Bray & Gorham, 1964; Singh & Gupta, 1977; Vogt, Grier & Vogt, 1986). However, in *P. patula* plantations in Mpumalanga, litter production has not been previously measured. In this study the annual litter production in *P. patula* compartments of various stand ages was quantified and seasonal trends were monitored.

Studies by Schutz (1990) showed that litter accumulation was correlated with several abiotic factors, one of which was altitude. Litter accumulation appeared to show an increase with increasing altitude. Keeping this relationship with altitude in mind as well as the importance of litter production in the ecosystem as one of the principal nutrient input processes, the following hypothesis was tested:

Plantation	Compartment	Age (yrs)	Altitude (m)	Rotation	Comment
Longtom	84	5	1374	1	*
	29A	9	1771	2	*
	6B	25	1647	1	
Brooklands	C1	5	1081	1	
	E15	5	1333	2	* #
	A42	10	1634	2	* #
	C25B	10	1087	2	
	A28B	15	1302	2	*
	B13	20	1278	2	
	B14A	20	1355	2	* #
	B9	21	1315	2	*
	D49	26	1351	1	* # ◆
	F2	42	1350	1	nutrient cycling/ trenching experiment
Berlin	E13*1	25	1520	2	*
	B4	15	1476	2	* #
	D15	30	1413	2	* #
	D16	30	1378	2	*

Compartments used for; * litter production study (Phase 2), # litter decomposition and ◆ microbial population study.

Table 3.2 Compartments used as study sites in several Mpumalanga plantations.

Plantation	Compartment	Age (yrs)	Altitude (m)	Rotation	Comment
Witklip	A4	5	1023	2	
	B42	5	1060	2	* #
	B34	9	1032	2	*
	B39	9	1041	2	* #
	B40A	9	1075	2	
	A2	15	1017	2	*
	D56A	25	1401	1	
	B55	30	1088	1	
Rosehaugh	B5	15	1085	2	* #
	C3	20	1029	2	* #
	F5	21	1161	2	*
	F6A	21	1180	2	
	E2A	25	992	2	* } lost in fire
	E3B	25	1011	2	* # ◆ } lost in fire
	C10	30	1099	2	* #
Mariti	C38	5	1000	2	*
	D48	30	1069	1	*
Swartfontein	D16B	15	950	2	

Compartments used for; * litter production study (Phase 2), # litter decomposition and ◆ microbial population study.

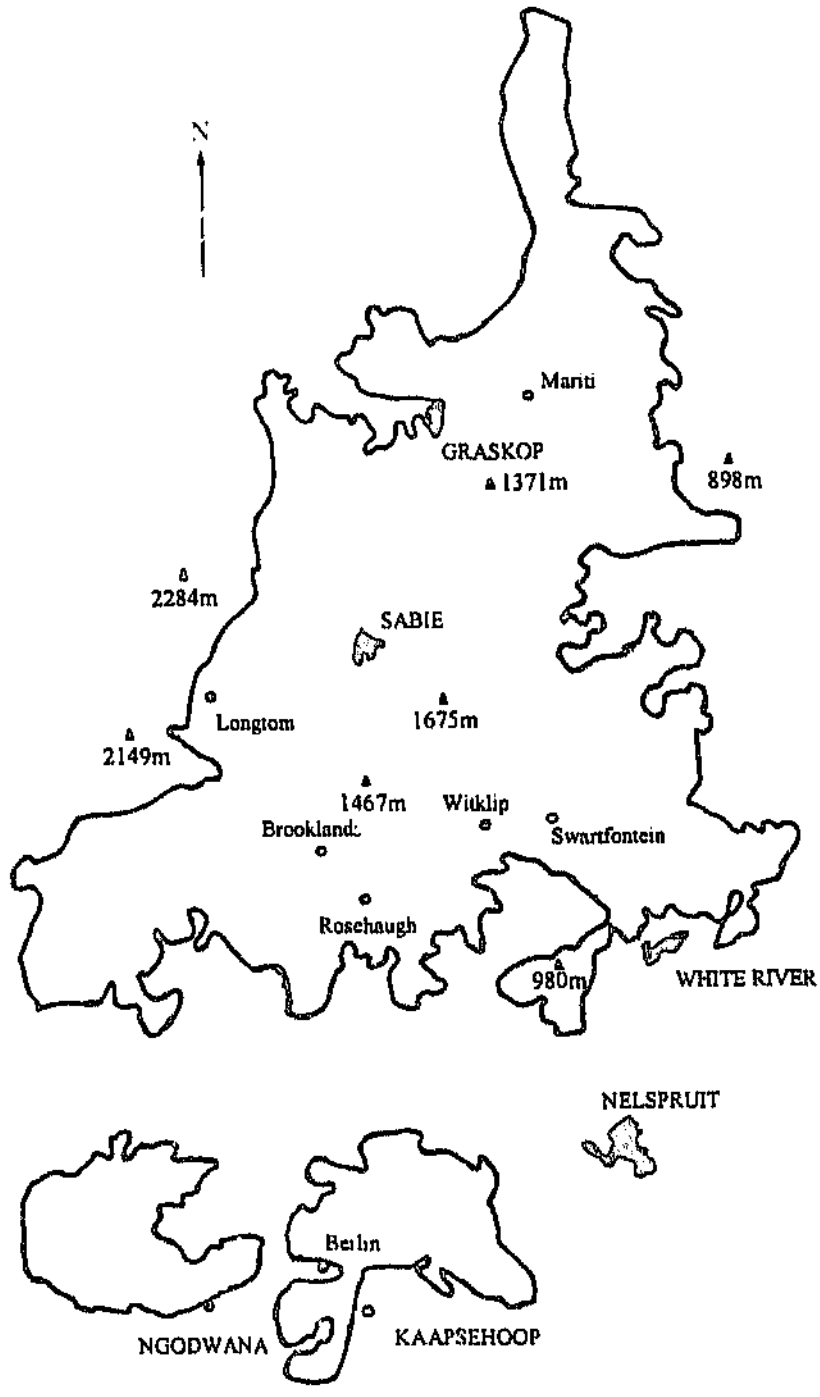


Figure 3.2 Location of study areas within the Mpumalanga plantations.

The timber industry began around the mining town of Sabie, owing to the demand for mine pit props. It became apparent that the soils, climate and rainfall made the escarpment an ideal area for plantation forestry. The government forestry department followed the lead of the mining companies and large plantations of pines and gum trees were begun after the Anglo-Boer war (Poynton, 1980).

Approximately 17 000ha contribute to the forestry zone in Mpumalanga, 21% of the total afforested area in South Africa (South African Forestry Facts, 1994).

3.2 STUDY SITES

Study sites included several *P. patula* plantations in the province namely. Witklip, Rosehaugh, Mariti, Swartfontein, Longtom, Brooklands and Berlin (Fig. 3.2). These plantations, previously managed by the Department of Forestry, have now been privatized to form the South African Forestry Company Ltd. (SAFCOL).

A total of 35, *P. patula* compartments were selected from the plantations. Criteria for selection included stand age, ...ation and altitude. Compartment information is presented in Table 3.2. Several problems were experienced during the field studies, these included removal of drought damaged trees, vandalism and fire. As a result not all the compartments were used throughout the study period; those relevant to a particular section are indicated on the table and further information is provided in the relevant sections. Field studies commenced in March 1992 and continued until July 1994.

Mpumalanga is a summer rainfall region, precipitation occurring mainly in the form of thunderstorms. The mean annual rainfall varies from 350mm in the NE to 1600mm on the escarpment. The region's proximity to the Tropic of Capricorn and the warm Mozambique current of the Indian ocean results in a sub-tropical, frost-free climate in the low lying areas of the Lowveld. Cold winters with heavy frosts and occassional snowfalls are experienced on the escarpment, which is often cloaked in mist (Schul · 1972).

Climate statistics, for the Sabie region, are presented for the D. R · 'et weather station, situated to the east of Sabie (Table 3.1) (Weather Bureau, 1986). Data from this station is provided as an indication of the average long-term climatic conditions prevailing in this forestry region.

Table 3.1 Climate statistics for the D.R. De Wet station, latitude 25° 06'S, longitude 30° 28'E, situated at an altitude of 915m (Weather Bureau, 1986).

Climate statistics	D R. De Wet Station
Temperature	
Min. (°C)	12.2
Max. (°C)	23.9
Range (°C)	11.7
Precipitation	
Rainfall (mm)	1200
No. of days with ≥ 0.1mm rainfall	114.1
No. of days with mist	72.3

3 STUDY AREA AND SITES

3.1 STUDY AREA

The province of Mpumalanga is situated in the eastern part of southern Africa (Fig. 3.1). The Drakensberg escarpment runs roughly north to south through the province and the plateau or Highveld stretches westward to the province of Gauteng. The land drops sharply from the mountains to the foothills, with the steep slopes gradually levelling off to form the low lying bushveld plains which constitutes the Lowveld.

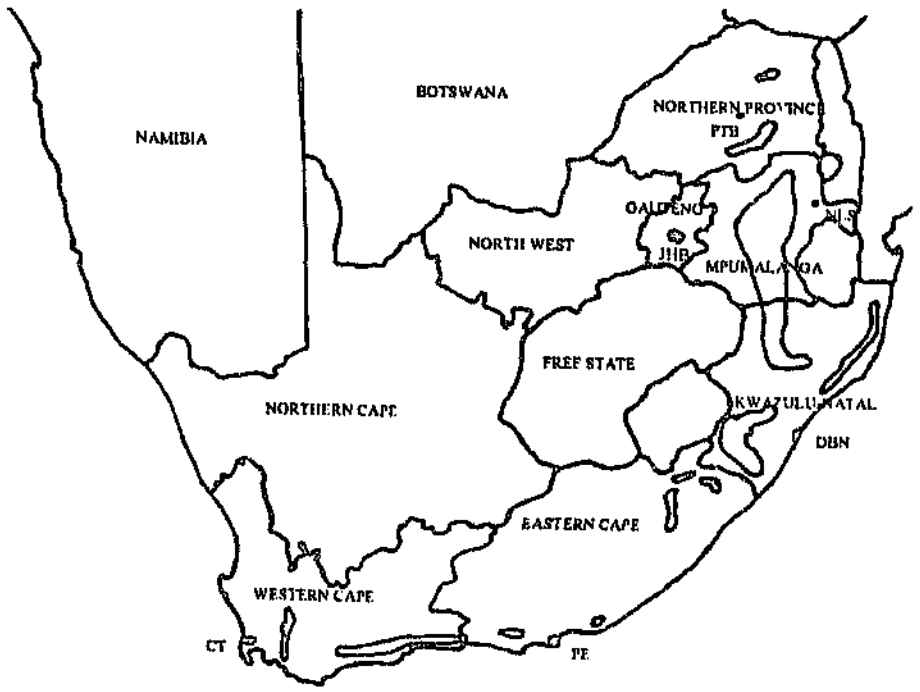


Figure 3.1 Map of South Africa indicating the major forestry regions. *CT* - Cape Town; *PE* - Port Elizabeth; *DBN* - Durban; *JHB* - Johannesburg; *NLS* - Nelspruit and *PTB* - Pietersburg.

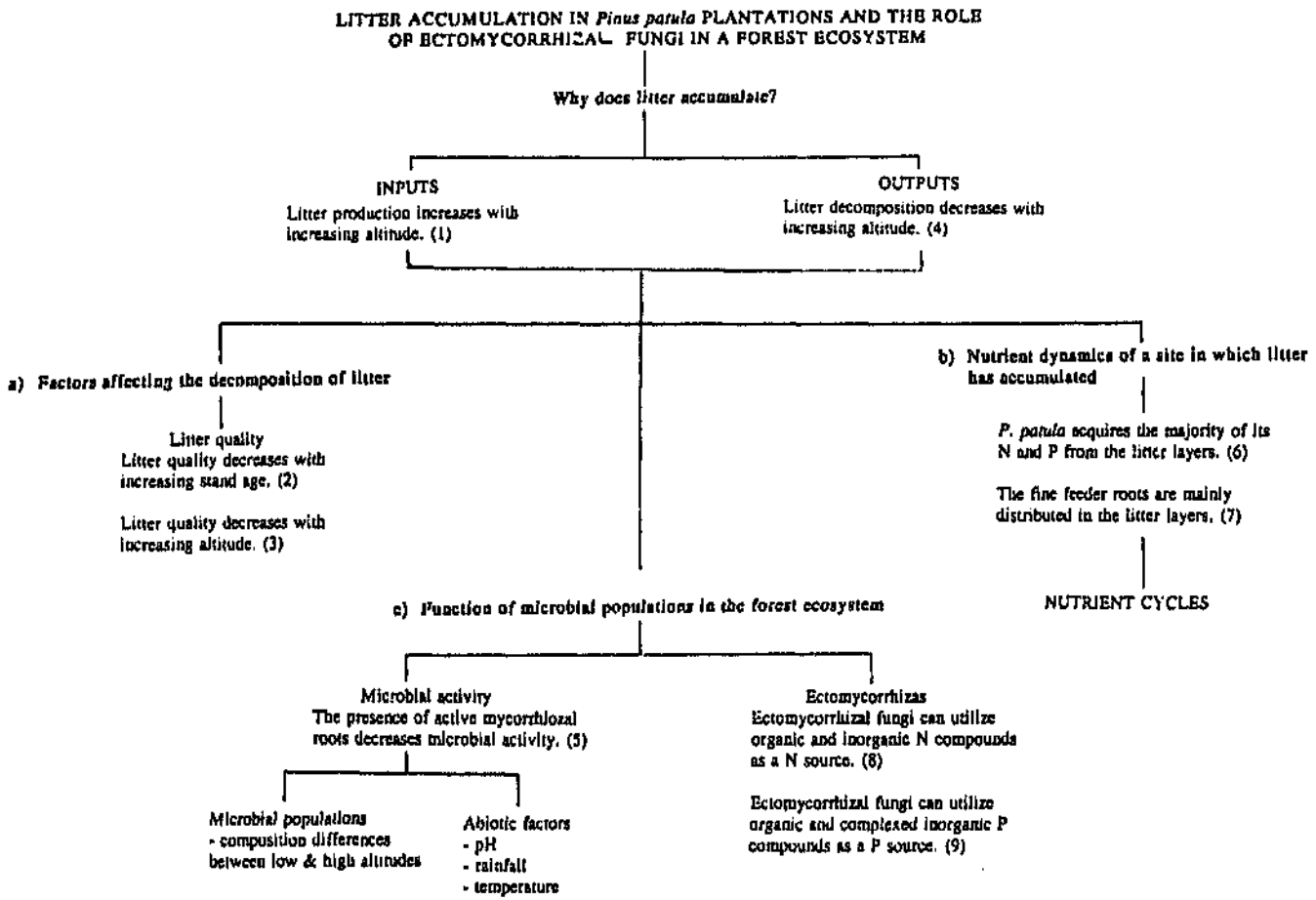


Figure 2.1 Flow diagram illustrating the links between the study objectives. Numbers in brackets represent hypotheses tested.

Hypothesis 5: The presence of active mycorrhizal roots in the litter decreases microbial activity.

Hypothesis 6: The fine feeder roots of *P. patula* are distributed mainly within the litter layer.

Hypothesis 7: *P. patula* acquires the majority of its N and P from the litter layer.

Hypothesis 8: Ectomycorrhizal fungal isolates of *P. patula* can utilize some organic N, as well as, inorganic N compounds as their sole source of N.

Hypothesis 9: Ectomycorrhizal fungal isolates of *P. patula* can utilize some organically bound or complexed P compounds as their sole source of P.

The relevance of these hypotheses and their relationship to the main objective will be expanded on in the introduction of the various chapters. An outline of the thesis layout is presented in the following section.

2.2 THESIS OUTLINE

In the introduction (chapter 1) a brief literature review is given, covering aspects related to litter accumulation, decomposition, nutrient cycling and ectomycorrhizas. The chapters that follow address these aspects separately. Chapter 3 provides information regarding the study sites used in the Mpumalanga plantations. Litter production (nutrient inputs) and accumulation in the study sites are examined in Chapter 4. Aspects of litter decomposition are addressed in Chapter 5. The cycling of nutrients, particularly N and P, within a site in which litter has accumulated, are discussed in Chapter 6. Chapter 7 examines the role of ECM fungal isolates within the forest ecosystem. The characterization of ECM roots, collected from a site in which litter has accumulated, is the subject of Chapter 8. All the chapters are presented in standard scientific format having an introduction, methods, results, discussion and conclusions section. As the specific subjects discussed are often inter-related, cross referencing within the individual chapters is provided. Chapter 9 synthesises the project conclusions as a whole and puts forward recommendations regarding the management of sites in which litter has accumulated. Areas which require further research are also highlighted.

2 OBJECTIVES AND THESIS OUTLINE

2.1 OBJECTIVES

Litter accumulation may occur as a result of an imbalance between the input of organic material (i.e. aboveground litter production) into the ecosystem and the rate of breakdown (or output) of this material (i.e. decomposition). The relationships of litter accumulation with these biological processes in *P. patula* plantations have not been previously examined in Mpumalanga. If litter accumulation is a result of an imbalance between inputs and outputs, then it must influence the cycling of nutrients within these *P. patula* plantations. This would in turn affect the long term productivity of these sites, as well as, their sustainability over future rotations. The main objective of this study was to determine the factors responsible for litter accumulation under *P. patula*. This required an understanding of;

a) how these factors affect the decomposition of litter;

b) the nutrient dynamics of a site in which litter had accumulated and

c) the functioning of the microbial population, in particular the ectomycorrhizal fungi, occurring in a site in which litter had accumulated.

To achieve the main objective various hypotheses were tested covering aspects of litter production, litter decomposition, nutrient cycling and the role of ectomycorrhizal fungi in the forest ecosystem. Hypotheses are numbered and their relationship to other aspects of the project are presented in a flow diagram (Fig. 2.1).

The hypotheses addressed were as follows;

Hypothesis 1: Litter production increases with increasing altitude.

Hypothesis 2: Litter quality decreases with increasing stand age.

Hypothesis 3: Litter quality decreases with increasing altitude.

Hypothesis 4: The litter decomposition rate decreases with increasing altitude.

produce external acid proteases that breakdown protein to amino acids and short chain peptides (Abuzinadah & Read, 1985; Abuzinadah, Finlay & Read, 1986).

Mycorrhizal fungi have also been shown to produce surface phosphatases capable of hydrolysing phytates. Mycorrhizas have been shown to have the ability to decompose organically bound P as a result of this phosphatase activity (Häussling & Marschner, 1989; Vogt, Publicover, Vogt, 1991). In forest soils, 50% of the phosphorus can be in an organic form, which is generally regarded as being unavailable for plant uptake. Soil minerals are weathered by microbial activity as well as physical processes. Mycorrhizal hyphae have been shown to aid weathering by the production of calcium oxalate resulting in the solubilization of mineral P (Lapeyrie, 1988).

Some ECM fungi express pectinolytic activity, as is evident by the breakdown of pectins in the middle lamella of the host during mycorrhizal formation. It has also been shown that some ECM fungi can decompose lignin, holocellulose and lignocellulose (Maijala, Fagerstedt & Raudaskoski, 1991).

If ectomycorrhizal fungi are capable of utilizing organically bound N and P compounds in the field, they would be capable of successfully competing with the decomposer micro-organisms for nutrients. This would result in decreased decomposer activity. Studies by Gadgil & Gadgil (1975) have suggested that forest soils permeated with ECM fungi show reduced rates in litter decomposition, as measured by mass loss, and accumulation of litter. Whether this close cycling of nutrients has an adverse effect on the nutrients dynamics of a forest ecosystem are unknown (Harley, 1989; Newmann, 1988; Vogt, Publicover & Vogt, 1991).

4.3.3 Prediction of litter depth

A multiple regression model using stepwise, forward variable selection, was used to determine which of the variables; stand age, altitude and litter production, could best predict litter depth. The variables altitude and litter production entered the model, accounting for 41% of the variability observed in litter depth (Table 4.2). However, this model showed no improvement on the relationship between litter depth and altitude (R^2 0.41), indicating that altitude accounted for 41% of the variability in the litter depth observed.

Table 4.2 Model predicting litter depth, using multiple regression analysis, with stepwise variable selection.

Independent variables	Regression coefficient	Std. error	t-value	*p*
Constant	-34.21	25.35	-1.35	0.19
Altitude	0.44	0.02	2.40	0.03
Litter production	9.47	3.45	2.75	0.01
	R^2 0.41	S.E. 18.67	MAE 14.65	

4.4 DISCUSSION

4.4.1 Litter production

A full two year period of data collection would have been preferable for the quantification of litter production. Studies in the literature recommend a minimum of three years in order to overcome annual variations and obtain a precise estimate (Bray & Gorham, 1964; Birk & Simpson, 1980). Annual variability was evident on

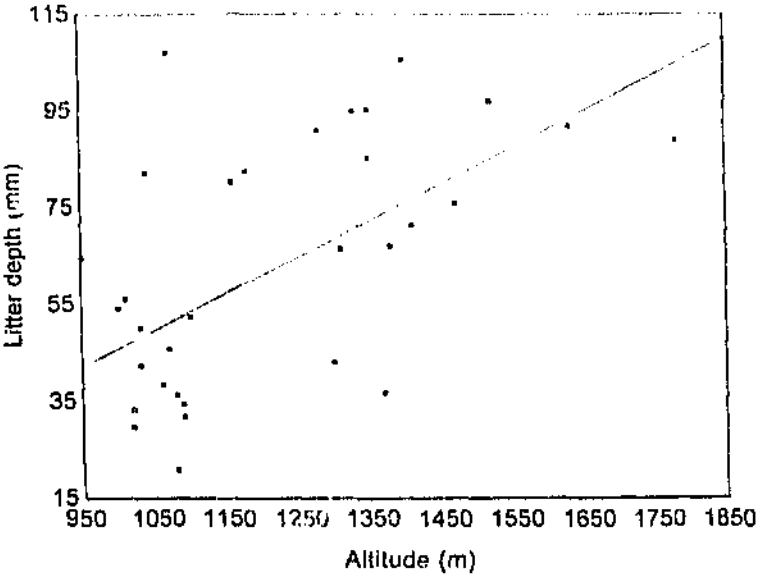


Figure 4.10 Linear regression of litter depth and altitude. $y=-30.14+0.08x$; $F_{1,30}=20.28$; $p\leq 0.0001$; $R^2=0.41$.

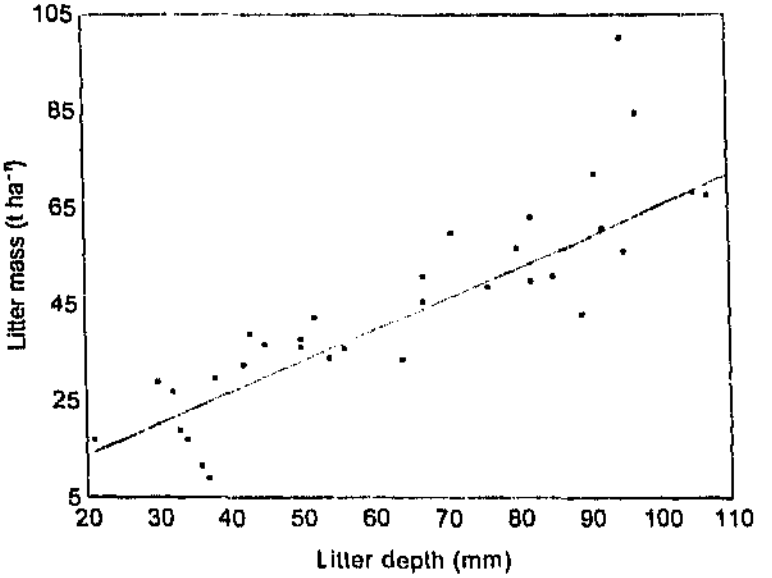


Figure 4.11 Linear regression of litter mass and litter depth. $y=2.83+0.65x$; $F_{1,32}=126.76$; $p\leq 0.00001$; $R^2=0.80$.

contributing overall to between 20-50% of the litter on the forest floor. The F layer contributed between 30-40%, being highest in 5 and 10 year old stands, thereafter declining. The contribution of WL was variable between 20-40%, being highest in 5 year old stands. The contribution made by NP litter was between 1-10%, the highest occurring at 5 and 15 years.

Simple regression models were used to determine the relationships between (i) litter depth and stand age, (ii) litter depth and altitude, (iii) litter mass and stand age (iv) litter mass and altitude, and (v) litter mass and litter depth. Analyses of relationships with stand age used pooled data, irrespective of altitude. Analyses of relationships with altitude use pooled data, irrespective of stand age.

Analysis of (i) and (ii) revealed significant but weak positive linear relationships, indicating that litter depth increased with stand age (Fig. 4.9) and with altitude (Fig. 4.10). Analysis of (iii) and (iv) also showed litter mass to increase with stand age ($y=23.54+1.13x$, $F_{1,29}=11.12$, $p\leq 0.002$, $R^2=0.28$), and with altitude ($y=27.58+0.06x$, $F_{1,29}=18.31$, $p\leq 0.0002$, $R^2=0.39$). Analysis of (v) showed a high positive relationship with the result that litter depth can be used as a predictor variable for litter mass (Fig. 4.11).

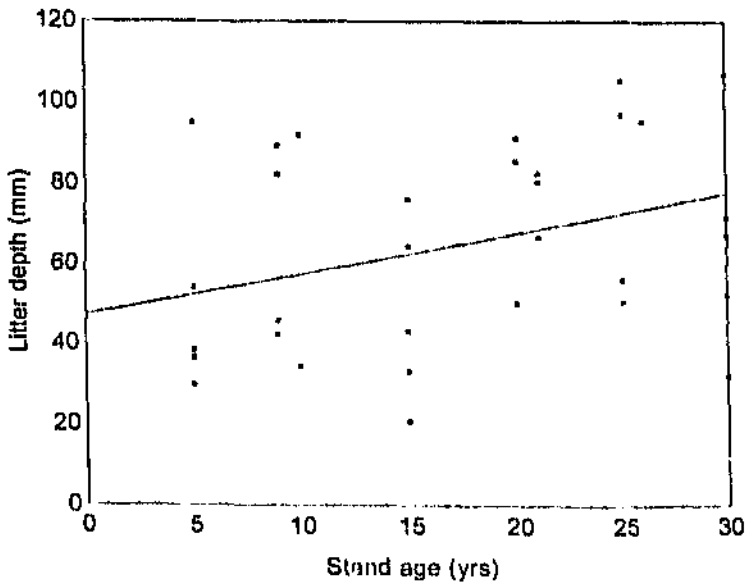


Figure 4.9 Linear regression of litter depth and stand age. $y=47.13+1.02x$; $F_{1,30}=4.28$; $p\leq 0.05$; $R^2=0.12$.

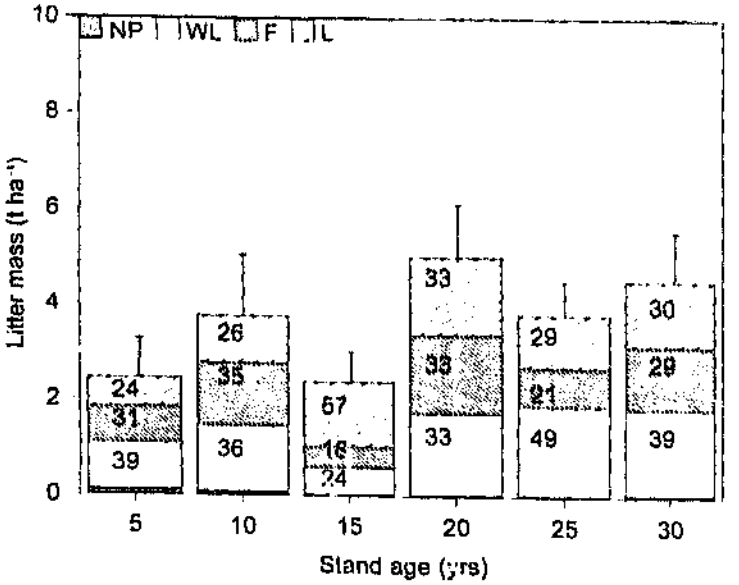


Figure 4.7 Forest floor composition in low altitude *P. patula* sites at various stand ages. Standard deviations (+) of total litter mass shown on bars. Numbers within bars indicate percentage contribution of each litter fraction.

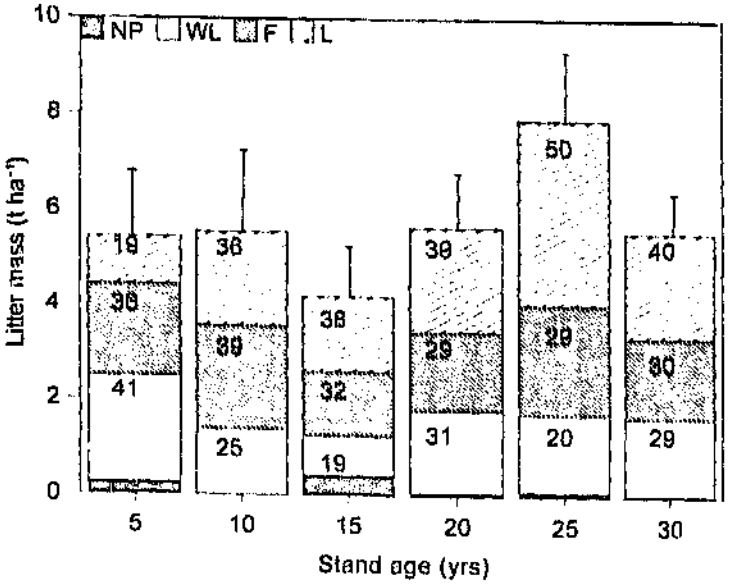


Figure 4.8 Forest floor composition in high altitude *P. patula* sites at various stand ages. Standard deviations (+) of total litter mass shown on bars. Numbers within bars indicate percentage contribution of each litter fraction.

Table 4.1 Litter depths under *P. patula* at various stand ages for low and high altitude sites. Mean values, standard deviations and number of replicates are presented.

Stand age (yrs)	Low altitude Litter depth (mm)	High altitude Litter depth (mm)
5	43±18	66±36
	n=60	n=30
10	51±24	91±38
	n=60	n=30
15	39±24	60±28
	n=45	n=30
20	71±29	81±30
	n=45	n=45
25	53±14	121±50
	n=45	n=45
30	42±17	69±19
	n=45	n=30

15 years where the L layer made up 56% of the litter. The F layer contributed 30-35%, with values of approximately 20% being recorded for 15 and 25 year old stands. The contribution made by WL was between 20-50%, with the highest percentage occurring at 25 years and lowest at 15 years. The contribution made by NP litter was low (1-5%), occurring mostly in 5 year old stands and declining as the stand ages. In high altitude sites (Fig.4.8) the L layer increased up to a stand age of 10 years and then remained constant

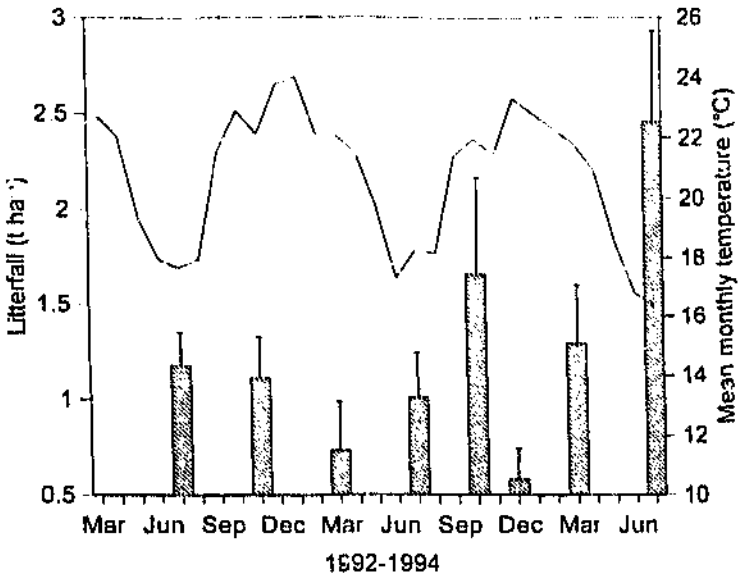


Figure 4.5 Seasonal trends in litter production (1992-1994) in low altitude sites. Bars represent the mean litter production of all stand ages collected at 3-4 month intervals. Standard deviations (+) shown on bars. Line graph represents mean monthly temperatures recorded at the D.R. De Wet station.

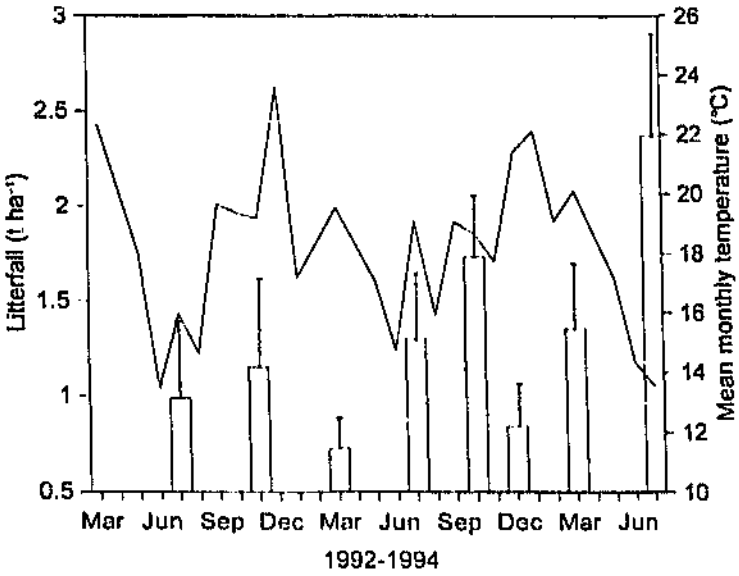


Figure 4.6 Seasonal trends in litter production (1992-1994) in high altitude sites. Bars represent the mean litter production of all stand ages collected at 3-4 month intervals. Standard deviations (+) shown on bars. Line graph represents mean monthly temperatures recorded at Makobulaan station.

year-old stands, high altitude 1[illegible] year-old stands *etc.*) ($F_{11,54}=4.03$; $p \leq 0.0003$). Multiple comparison revealed that these differences were attributed to significantly ($p \leq 0.05$) lower litter production in 5 year old stands, when compared with the other age groups. There was a significant increase in litter production in low altitude 20 and 30 year old stands, when compared with the corresponding high altitude stands, and a significant increase in litter production in the high altitude 25 year old stands, when compared with the corresponding low altitude stands.

Seasonal trends in litter production for both the low (Fig 4.5) and high (Fig. 4.6) altitude sites were obtained by combining all the data regardless of age groups. Line graphs of the average monthly temperatures (1992-1994) are provided as an indication of seasonal changes. Temperature data were obtained from the D. R. De Wet and Makobulaan stations, for the low and high altitude sites, respectively (Forestek, Climatic Group). In both low and high altitude sites (Figs. 4.5 & 4.6) litter production increased from March to September, with the highest litterfall being measured in September, during the early spring season. However, the mid-winter (June) collections of 1994 showed the highest levels for both low and high altitude sites.

4.3.2 Litter accumulation

The average litter depths recorded under *P. patula*, in stands of various ages, at low and high altitudes, are presented in Table 4.1. The variability around the means as indicated by the standard deviations might be attributed to the heterogeneous nature of the litter and changes in the micro-topography. The table does indicate a weak trend of increasing litter depth with stand age, as well as thicker litter layers in the high altitude sites. The maximum litter depths recorded in low and high altitude sites were in 20 and 25 year-old stands, respectively.

Histograms, for low (Fig. 4.7) and high altitude (Fig. 4.8) sites are presented to illustrate the composition of litter on the forest floor. It is evident that the accumulated litter mass in the low altitude sites was lower than that in the high altitude sites, which confirms the trends shown in Table 4.1. In low altitude sites (Fig. 4.7) the L layer contributed between 24-32 % of the forest floor at all stand ages, the exception being in stands of

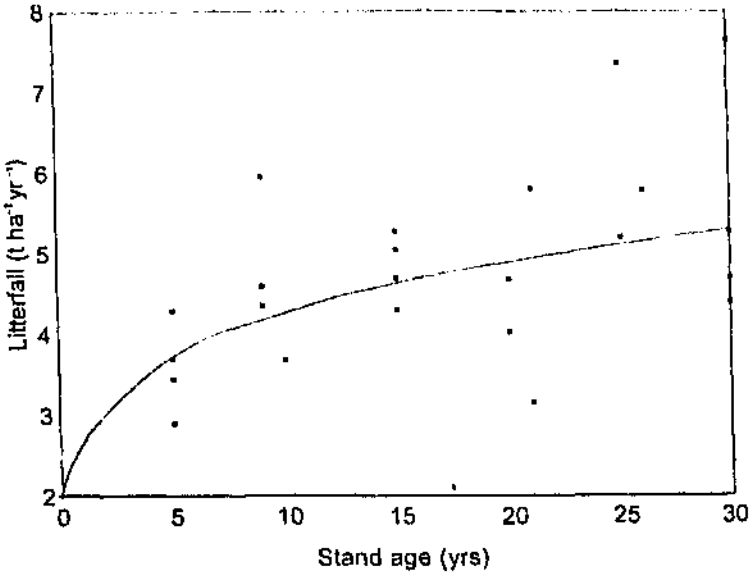


Figure 4.3 Multiplicative regression (or power fit curve) of litter production (Phase 1 & 2 combined) and stand age. $y=2.7\ (x^{0.2})$; $F_{1,21}=8.18$; $p\leq 0.01$; $R^2=0.28$.

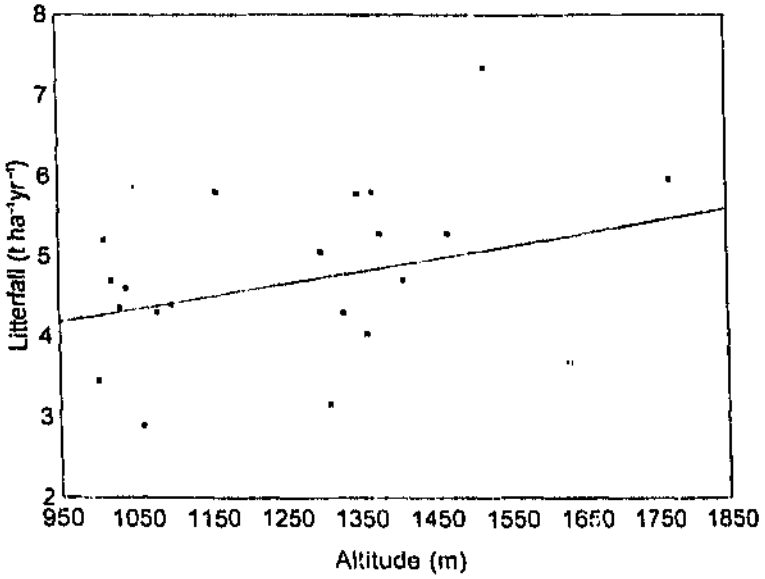


Figure 4.4 Linear regression of litter production (Phase 1 & 2 combined) and altitude. $y=2.68+0.016x$; $F_{1,21}=2.63$; $p\leq 1.12$; $R^2=0.12$. Relationship not significant.

fairly constant at 20 and 25 years with a decline at 30 years. At high altitudes, litter increased in production up to 15 years, followed by a decline at 20 years, and a dramatic increase at 25 years, declining again at 30 years. Litter production in 5, 10 and 20 year-old, low altitude sites was higher than the corresponding high altitude sites. At 25 and 30 years high altitude sites produced more litter with a very distinctive peak occurring in high altitude 25 year-old stands. It is important to note that the number of replications for each age/altitude group was seriously compromised throughout this collection period although it never fell below three. During phase 2 (Fig. 4.2) a similar increase in litter production up to age 10 for both low and high altitude sites was shown. Thereafter, litter production remained fairly constant with dips in production for low altitude sites at 25 years and for high altitude sites at 15, 20 and 30 years. Litter production was slightly higher in high altitude sites up to age 15, and again at age 25. Higher litter production was observed in the low altitude 20 and 30 year old stands.

Simple regression models were used to determine the relationships between (i) litter production and stand age and (ii) litter production and altitude. The average annual litter production data for each compartment, from both phase 1 and 2, were used both separately and in combination, for the analyses.

Analysis of (i) revealed a significant positive relationship between litter production and stand age during phase 1 ($y=1.68+0.06x$, $F_{1,17}=5.21$, $p\leq0.04$, $R^2=0.23$). However, no significant linear relationship was evident when analysing the phase 2 data. Examination of the combined data set (phase 1 and 2) revealed a significant positive, multiplicative relationship (Fig. 4.3) which indicates an overall increase in litter production with increasing stand age. This increase was rapid up to 10 years after which litter production continued to increase, but at a slower rate. Analysis of (ii) revealed that there was no relationship between litter production and altitude from either phase 1, phase 2 or combined (phase 1 and 2) data sets (Fig. 4.4). Therefore, litter production did not significantly increase with increasing altitude.

The relationships were clarified by one-way ANOVA (Phase 2 data only) which revealed that there were significant differences between litter production at different stand ages ($F_{3,60}=3.22$; $p\leq0.01$) and age/altitude groupings (*i.e.* data grouped as low altitude 5 year-old stands, high altitude 5 year-old stands, low altitude 10

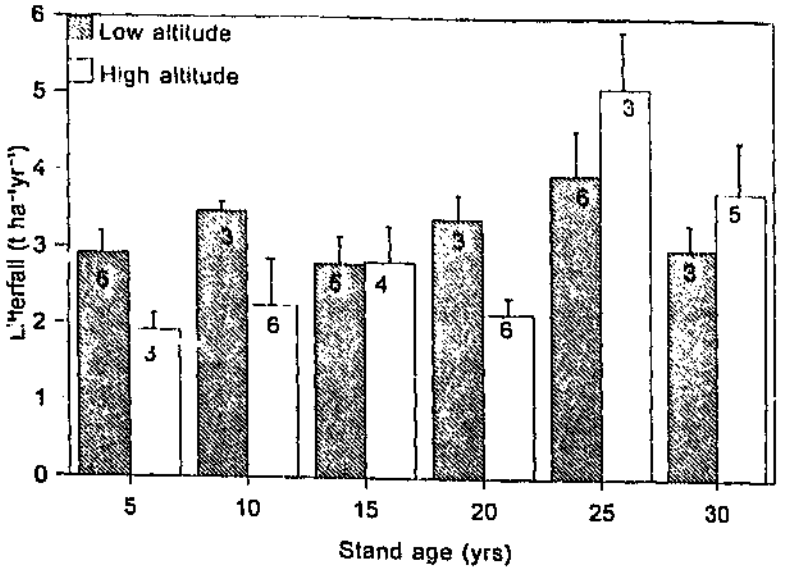


Figure 4.1 Litter production, March 1992-March 1993 (Phase 1), in both low and high altitude *P. patula* sites. Numbers on bars represent number of replicates, standard deviations (+) shown on bars.

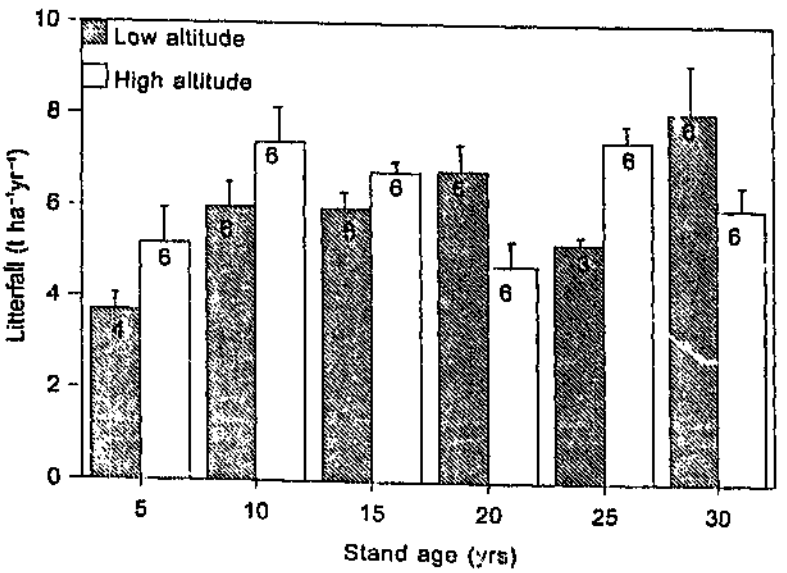


Figure 4.2 Litter production, June 1993-June 1994 (Phase 2), in both low and high altitude *P. patula* sites. Numbers on bars represent number of replicates, standard deviations (+) shown on bars.

described above. To obtain the total litter mass, the masses of the various components were summed. Ash free dry weights (atdw, g in $0.9m^2$) were then converted to litter mass (t ha^{-1}) by multiplying these by a correction factor (0.0111) (modified from Schutz, Bredenkamp & Herbert, 1983).

4.2.4 Data analysis

Statistical analyses were conducted using Statgraphics version 5.1. Analyses of litter production and litter accumulation (litter depth and mass) data included ..uple regression models to determine whether there were any trends between these variables and stand age or altitude. One-way analysis of variance (ANOVA) was used to determine whether there were any significant ($p \leq 0.05$) effects between litter production, age and altitude. Where significant differences were evident multiple comparisons, using least significant differences were conducted between all variables (Sokal & Rohlf, 1981). Because of the problems experienced during phase 1 of the litterfall collection, statistical analyses were only conducted on the averaged two year data where indicated. Generally, analyses were conducted on the data from the two phases separately or only from phase 2. The 1993/1994 data set (Phase 2) was considered to be representative of the annual litter production. To determine whether litter depth could be predicted from a model using the variables, litter production, stand age and altitude a multiple regression analysis, using stepwise, forward variable selection was conducted (Sokal & Rohlf, 1981).

4.3 RESULTS

4.3.1 Litter production

Litter production for the period March 1992-March 1993 (Phase 1) and June 1993-June 1994 (Phase 2) are presented as histograms. Bars represent litter production for each age group at both low and high altitudes (Figs. 4.1 & 4.2). In phase 1 litter production at low altitudes increased up to age 10, thereafter a slight decline in litter production at age 15 occurred (Fig. 4.1). Litter production then increased again, and remained

3 to 4 months. In July 1993 as a result of thefts of the traps, steps were taken to ensure the reliability of the data for phase 2 of the study. These measures involved removing all remaining littertraps and clearing the forest floor directly beneath the position of the traps. In compartments where traps had gone missing, the forest floor was cleared in the exact location of the previous trap and to the same size. Litter was collected every 3 months, directly off the soil surface.

Litterfall was quantified by measuring the field wet weight of the litter. A subsample of litter was returned to the laboratory for dry mass determination, samples were dried at 60°C for 72hrs. Samples from the first collection were heated in a muffle furnace for 8hr at 400°C. The organic matter content was determined by quantifying the loss of mass after ignition (Hesse, 1971). The average organic matter content (96%) was used to convert all dry mass determination into ash free dry weight (afdw). During phase 2 an added problem was experienced, especially during the rainy season, in that wet soil was collected with the litter. Litter was then sieved, through a 2mm sieve, after drying. Field wet weight measurements were corrected for moisture, soil and organic matter content. To determine the annual litter production, data from each collection period were summed giving a measurement in g m^{-2}, data were then converted into tons per hectare (t ha^{-1}).

4.2.3 Assessment of litter accumulation

In order to quantify litter accumulation on the forest floor, five trees were randomly selected around each littertrap, giving a total of 15 sites per compartment. A 0.3m x 0.3m frame (surface area 0.9m^2) was placed on the forest floor, approximately 1m from the base of the tree (Schutz, 1990). A sharpened long bladed knife was used to cut into the litter. The knife blade was guided by the frame. Litter was collected to the mineral layer, placed in polythene bags and returned to the laboratory. Depth measurements, down the middle of each of the litter sample walls, were taken and averaged to obtain litter depth (mm). Litter samples were separated into various components, these representing the litter (L) layer, fermentation (F) layer, woody litter (WL) and non-pine litter (NP). The components were dried, weighed and the organic matter content was determined as

5.2 METHODS

5.2.1 Study sites

To assess *P. patula* litter quality, needle litter was collected from all initial littertraps in July 1992 (Table 3.2; chapter 3). In order to measure the decomposition of *P. patula* litter, decomposition experiments were established in July 1993 for which one compartment in each age group at both low and high altitudes was selected (Table 3.2, marked *). To determine whether there were any differences in the soil microbial or soil faunal populations, litter/soil core samples were collected in August 1994 from two 25 year-old stands, one representing a low altitude site and one a high altitude site (Table 3.2, marked ◆; samples taken prior to loss of one compartment in a fire). These two sites were chosen as the high altitude, 25 year-old stand produced the maximum amount of litter and had the thickest litter layers (Table 4.1; section 4.3.2; chapter 4). An assumption was made that if differences in the microbial populations existed, they would be more readily detected by comparing these two sites. To determine whether ECM roots retarded microbial activity in the litter of a *P. patula* compartment a field study involving the trenching of sites was undertaken in a compartment in which litter had accumulated (Brooklands, F2; Table 3.2; chapter 3).

5.2.2 Litter quality

To assess litter quality, needle litter samples were oven dried at 60°C for 72hrs, crushed and chemically analyzed for total nitrogen (N), total phosphorus (P), lignin, cellulose, polyphenolics and tannins.

Digestion of litter for determination of total N and P

The analysis of total nutrients in the litter samples required the complete oxidization or breakdown of the organic matter. A modified Kjeldahl, wet oxidation procedure was used to oxidize the litter samples. This was achieved by using concentrated H_2SO_4, with an additional oxidizing agent, H_2O_2. To promote the process a selenium catalyst was added. The samples were digested on a heated block until the acid cleared (Anderson & Ingram, 1989).

Hypothesis 2 : Litter quality decreases with increasing stand age (Fig. 2.1; chapter 2).

Hypothesis 3 : Litter quality decreases with increasing altitude (Fig. 2.1; chapter 2).

Hypothesis 4 : The litter decomposition rate decreases with increasing altitude (Fig. 2.1; chapter 2).

Ectomycorrhizas (ECM) and their associated microbial population are an important component of the *Pinus* forest ecosystem. The role that ECM play in the uptake of nutrients has been well documented (Harley, 1989). Studies which excluded roots of *P. radiata* from areas of the forest floor have shown that the presence of living roots and their associated micro-organisms suppressed litter decomposition (Gadgil & Gadgil, 1971; 1975). As the ECM root/fungus partnership is the dominant biotic influence in the main rooting zone, Gadgil & Gadgil (1971; 1975) attributed this reduced activity of the decomposer organisms to the competition between these organisms and ECM roots with their associated external mycelia, for both nutrients and moisture. In view of their importance in the ecosystem, and the abundance of ECM roots observed in the accumulated litter layers (Chapter 6), the microbial activity in a site in which litter has accumulated was assessed and the following hypothesis was tested.

Hypothesis 5 : The presence of active ectomycorrhizal roots in the litter decreases microbial activity (Fig. 2.1; chapter 2).

Other micro-organisms are associated with ECM roots and organic matter in the forest ecosystem. The composition and quantity of the soil micro-organisms and soil fauna are also factors which influence the decomposition rate. This study briefly compared, by means of enumeration, the soil microbial and soil faunal populations in both a low and a high altitude *P. patula* site to determine whether there were any significant differences in the decomposer populations. The results from these studies and their influence on the decomposition process and ultimately on litter accumulation will be discussed in the following sections.

5 LITTER DECOMPOSITION

5.1 INTRODUCTION

The input of nutrients into an ecosystem occurs in a number of ways, one of which is through aboveground litter production. The nutrients in the litter are released by the process of decomposition, which represents the output of nutrients. These nutrients are returned to the soil and are made available for plant uptake, completing the nutrient cycle (Attiwill & Adams, 1993; Dickinson & Pugh, 1974).

The accumulation of litter on the forest floor, under *P. patula* in high altitude sites, may occur as a result of an imbalance between the input and output processes operating within the ecosystem. Chapter 4 examined the litter input process and litter production; and concluded that litter accumulation, in high altitude sites, was not a result of increased litter production. This chapter will examine the decomposition of *P. patula* litter, a process which if retarded, will result in the accumulation of organic matter on the forest floor (Swift, Heal & Anderson, 1979). Litter accumulation results in the immobilization of nutrients within the litter layer. This is a situation which could potentially threaten the longterm productivity of plantation sites (Schutz, 1990).

This section of the study examined the quality of *P. patula* litter collected from various stand ages at both low and high altitude sites. The decomposition process was also monitored to determine the decomposition rates of the litter at various sites and the changes in litter quality.

Litter decomposition is a complex process and is regulated by a number of abiotic and biotic factors. Abiotic factors include the litter's physical structure, temperature, moisture, relative humidity and pH, while biotic factors include litter quality, microbial activity and the composition of the soil micro-organisms and soil fauna (Dickinson & Pugh, 1974; Swift, Heal & Anderson, 1979). This study not only monitored the decomposition of *P. patula* litter but also assessed the relationship between decomposition and various regulatory factors. These studies were conducted to address the following hypotheses.

4.5 CONCLUSIONS

P. patula litter production was quantified for various stand ages with the maximum, overall average of 5.89t ha^{-1} of litter being produced annually for 25 year-old plantations and with production increasing during the cooler, drier winter to early spring season. Litter production was shown to increase up to a stand age of 10 years, with a continual but more gradual increase in litter production thereafter. No significant relationship existed between litter production and altitude therefore **hypothesis 1** which states that litter production increases with increasing altitude can be rejected. The relationship between increased litter accumulation with increasing altitude, previously reported by Schutz (1990), was confirmed. Increased litter production, in high altitude stands, was not responsible for the litter accumulation observed in these sites. The input of organic matter into these *P. patula* plantations was not responsible for the imbalance proposed between the input and output processes. Chapter 5 will examine the output or decomposition processes within the *P. patula* ecosystem to investigate what causes litter to accumulate.

Litter depth (Fig. 4.10) and mass were also shown to increase with altitude which confirms Schutz's (1990) findings which indicated that altitude was an important variable influencing litter accumulation under *P. patula*. Multiple regression analyses (Table 4.3) confirmed the influence of altitude on litter depth. Altitude should not, however, be considered in isolation. Climatic changes occur with increasing altitude with higher altitude sites experiencing cooler minimum temperatures, mist and higher rainfall (Schutz, 1990).

Litter depth can be used to predict litter mass (Fig. 4.11). Litter depth measurements are more convenient to obtain for study purposes and can then be converted to litter mass using the regression equation presented in figure 4.11. A similar model obtained by Schutz, Bredenkamp & Herbert (1983) was based on a quadratic relationship resulting in a curvilinear plot between Log litter depth and Log litter mass data. This quadratic relationship was explained as resulting from the presence of compacted, more dense litter in thicker litter layers. Log transformations of the data obtained in this study did not improve the model presented (Fig. 4.11). This may be a result of differences in stand age, which was not a variable in the study conducted by Schutz, Bredenkamp & Herbert (1983).

Comparisons of the litter composition in the low altitude (Fig. 4.7) and high altitude (Fig. 4.8) sites indicates that the litter component contributes substantially to the forest floor composition, particularly in high altitudes. The variability in the WL component can be attributed to silvicultural practices, the presence of slash in second rotation sites and increased natural pruning in older compartments. The decline in NP litter, particularly in the low altitude sites, results from increased shading as the canopy closes. The NP litter recorded in the high altitude sites was composed not only of litter from understorey plant growth, but also from partially decomposed grass which was found occurring under the litter layer in some compartments. This represented the original vegetation occurring at the site prior to afforestation, the presence of which was also noted by Schutz (1990). From the data collected it was not possible to determine whether seasonal changes occurred in the composition of the forest floor. However, data from litterfall collections suggests that any changes to the forest floor composition would mainly result from silvicultural practices and local climatic conditions such as occasional heavy storms and strong winds.

4.4.2 Litter accumulation

The collection of litter depth measurements is time consuming, as the forest floor has a high spatial variability resulting in large numbers of samples being required (Schutz, 1990). The large standard deviations observed in depth measurements presented in Table 4.1 are attributed to the accumulation of litter in high altitude sites; to disturbances of the litter caused by removal of logs from thinning operations and to the action of bushpigs which turn over the litter in search of food (Schutz, 1990). Other factors which influence litter accumulation include changes in the micro-topography, the rotation of the stand and whether the stand was subjected to slash burning after clearfelling or wildfires, which would result in a reduced litter layer (De Ronde, 1993). Information regarding burning is difficult to obtain for a particular compartment. However, the presence of charcoal within the litter did indicate that for at least a few of the compartments used in the study fire had caused a disturbance in the forest floor development. The majority of the compartments selected were in their second rotation, however, to obtain older stands, as well as some of the 5 year old stands, first rotation compartments had to be used (Table 3.2; chapter 3).

The litter depth measurements (Table 4.1) recorded in this study (39-121mm) were within the lower range (30-350mm) reported by Schutz (1990). These low range values can be accounted for by the younger stand ages selected for this study: in Schutz's (1990) study, the average stand age was 38 years. Litter mass figures reported for other *Pinus* forests are presented, for comparison, in Table 4.4. Comparing these reported figures with litter mass recorded in this study (Figs. 4.7 & 4.8) emphasises the high levels of litter accumulation under *P. patula* in Mpumalanga: only New Mexico shows higher levels under *P. ponderosa*.

Litter depth (Fig. 4.9) and mass were shown to increase with stand age. This trend was expected as litter production generally increased with stand age (Fig. 4.3). Singh (1982) reported that forest floor accumulation under *P. patula* in India reached a maximum at a stand age of 34 years. The development of the forest floor has been reported to increase up to canopy closure, reaching a maximum about a 1/3 to 2/3 of the way through a rotation (De Ronde, 1993). This developmental pattern, which is similar to reported litter production trends, would be influenced by thinning and pruning.

Significantly more litter was produced in low altitude 20 and 30 year old stands, when compared with high altitude stands. This may be accounted for by faster growth rates in low altitude sites, which experience warmer climatic conditions (Miller *et. al.*, 1979).

The significantly higher litter production in the high altitude, 25 year-old sites, when compared with their corresponding low altitude sites (Figs. 4.1 & 4.2), could be a result of the problems experienced during collection. During phase 1, litterfall was obtained only from a single low altitude 25 year-old stand whereas during phase 2 although litterfall was collected from two stands the last three months data was not obtained because of fire. However, if a 12 month period from March 1993 - March 1994 is taken for the 25 year old stands annual litter production figures of 5.21 ± 0.103 and 7.79 ± 1.32t ha^{-1} is obtained for the low and high altitude stands, respectively. Comparison of these figures still results in significantly more litter being produced in the high altitude stands. Therefore, the problems experienced during collection would not account for the difference observed. The reasons for this increase in litter production are unclear but may possibly be related to local wind conditions which may have contributed to increased woody litter production.

No relationship was evident between litter production and altitude, thus, increased litter production would not explain why litter accumulates in high altitude stands. Further examination of the balance between nutrient inputs and outputs are required. Therefore, as litter production did not increase with increasing altitude, **hypothesis 1** was rejected.

Litter production was found to be continual throughout the year, although production increased from March to September, with high levels in June and September which correspond to the winter/spring months (Figs. 4.5 & 4.6). This confirms observations made by Morris (1992), that litter production peaked after the summer rainfall season. Because litter production was only assessed quarterly, precise seasonal trends, related to either rainfall or temperature, could not be made. However, the trend in *P. patula* litter production indicated an annual peak during the cooler, drier season. The second peak (June-July 1994) observed in these graphs (Figs. 4.5 & 4.6) probably reflects the higher litterfall of Phase 2 which followed the proceeding season's low rainfall (Table 4.3).

The initial increase in litter production with stand age (up to 10 years) confirms previous studies (Bray & Gorham, 1964; Morris, 1986, Millar, 1974). This increase occurs until canopy closure, which for *P. patula* is reported to be 4-5 years (Poynton, 1980). However, in the compartments studied, trees are thinned and pruned at approximately 5-8 years and again at 15-18 years. This will have an effect on litter production and would account for the decline observed in 15 year old stands recorded during the 1992-1993 collection period (Fig. 4.1). These silvicultural practices would also account for the general trend of higher levels of litterfall observed in stands older than 10 years. Morris (1992) reported that litter production increased up to 10 years in the Usutu plantations in Swaziland, and thereafter remained constant. However, these *P. patula* trees are grown for pulp production and are not thinned.

Litter production is dependent on needle production and hence crown biomass, which has been shown to increase to a peak followed by a decline (Morris, 1992). This is important considering the relationship of leaf area to photosynthetic rates, both of which will decline as a result of water or nutrient deficiencies in the stand. As the stand ages, needle biomass declines, this combined with a reduced rate of litter production results in reduced litterfall (Morris, 1992). However, the influence of stand age on the total annual litter production may be masked, after canopy closure, by the increased contribution of branches to the litterfall. This trend has been observed in other pine stands (Das & Ramakrishnan, 1985; Gholz, *et al.*, 1985) but the trend in this study (Fig.4.3) indicates a continual increase in litter production, although this increase occurs at a slower rate after 10 years. This may be attributed to increased woody litter production because of increased natural pruning in older stands (Morris, 1992) and silvicultural practices, which promotes needle production and hence litterfall.

During this study the contribution of branch litter to the total litter production was not measured, however, from the observations made, increased branch litter was evident in older compartments and thinned or pruned compartments, comprising approximately 7% of the total litter production. On occasions when strong winds and heavy storms occurred, this percentage may rise to approximately 10% in the older compartments.

Species / Location	Stand age (yrs)	Latitude	Litter production (t ha^{-1} yr^{-1})	Litter accumulation (t ha^{-1})	Reference
P.elliotti / Western Cape	8	35°S	1.00		Wienand (1992); Wienand & Stock(1995)
	20		2.90		
	25		2.40		
P. radiata / Australia		36°S	3.69	11.83	Vogt, Grier & Vogt (1986)
P. taeda / Carolina		35°N	5.41	18.44	Vogt, Grier & Vogt (1986)
P. ponderosa / Arizona		35°N	2.12	32.83	Vogt, Grier & Vogt (1986)
P. ponderosa / New Mexico		36°N	2.32	113.00	Vogt, Grier & Vogt (1986)
P. radiata / New Zealand		38°S	2.22	2.86	Vogt, Grier & Vogt (1986)

Table 4.4 Litter production and accumulation figures for various *Pinus* species including *P. patula*.

Species / Location	Stand age (yrs)	Latitude	Litter production ($t\ ha^{-1}\ yr^{-1}$)	Litter accumulation ($t\ ha^{-1}$)	Reference
P. patula / Mpumalanga	5	25°S	3.64±1.64	35.50±33.30	This study
	10		4.83±2.32	42.20±17.63	
	15		4.84±1.37	31.42±13.45	
	20		4.39±2.15	52.23±11.61	
	25		5.89±2.25	65.52±29.50	
	30		5.52±2.31	49.58±15.86	
P. patula / India	10	27°N	3.75	8.82	Singh (1982)
	12		4.15	7.22	
	17		4.67	7.91	
	25		11.42	18.84	
	34		8.75	23.24	
P. patula/Tanzania	19		6.22		Lundgren (1978)
P. elliotti	1		0.83		Nemeth (1973) cited in Morris (1986)
	5		3.70		
P. elliotti / Florida	15	30°N	4.99		Gholz *et al.*, (1985)
	27		4.99		
	36		4.99		

Table 4.3 Monthly rainfall data recorded at the D.R. De Wet for 1992-1993 and 1993-1994 seasons, compared with the long term average (Forestek, Climatic Group; Weather Bureau, 1986).

Month	Rainfall		
	1992-1993 (mm)	1993-1994 (mm)	Average (mm)
Mar	68.8	429.2	104
Apr	24.2	46.6	98
May	5.4	23.8	31
Jun	1.8	6.0	17
Jul	3.4	2.4	19
Aug	26.8	19.4	14
Sep	35.4	18.0	42
Oct	136.0	112.6	94
Nov	184.6	191.6	170
Dec	242.6	286.2	213
Jan	97.5	0	204
Feb	18.4	53.8	194
Total	844.9	1189.6	1200

examination of the y axis scaling for the phase 1 and 2 histograms (Figs. 4.1 & 4.2). The overall average annual litter production during phase 1 was 2.85t ha^{-1} and during phase 2, 6.29t ha^{-1}. This increase in litterfall may be an artifact related to the change in collection technique although every precaution was taken to ensure that both the location and size of traps, *i.e.* the collection areas, remained constant. Therefore, it was unlikely that the collection technique was responsible. It was expected that a reduced quantity of litter would be collected during phase 2, as a result of the loss of finer litter which is difficult to collect off the soil surface and the loss of mass due to the onset of decomposition which occurs as the litter comes into contact with the soil surface.

Annual variation has been reported to be considerable in other forest ecosystems, with as much as a 5 fold difference between the maximum and minimum annual litter production being recorded (Millar, 1974). This difference is normally between 1.5 to 3.0 fold, a range within which the 2.2 fold difference between phase 1 and 2 falls. The increase observed during phase 2 may be attributed to variations in litter production as a result of severe environmental factors such as drought (Millar, 1974). The 1992/93 rainfall season was a drought season, with higher rainfall occurring during the 1993/1994 season (Table 4.3); only rainfall data for the D.R. De Wet station are presented as Makobulaan rainfall data was sparse. During drought conditions needles are retained on the tree for longer. Once the stress has been alleviated the needles retained from the previous season as well as the current season's senescent needles will drop, resulting in increased litter production (Hendricks, Nadelhoffer & Aber, 1993; Turner, Lambert & Holmes, 1992).

The average annual litter production of *P. patula* compares favourably with that reported for *P. patula* by Singh (1982) in India and Lundgren (1978) in Tanzania up to a stand age of 20 years. The average annual litter production in older stands was lower in Mpumalanga than that reported by Singh (1982) (Table 4.4). This may be attributed to the variability in litter production in these older stands between low and high altitude sites (Figs. 4.1 & 4.2). The latitude of the site also accounts for differences observed in litter production, with litter production increasing towards the equator (Bray & Gorham, 1964). Table 4.4 includes, for comparison, litter production figures for other *Pinus* species.

Table 5.3 Model predicting litter depth from litter quality variables and site characteristics, using multiple regression analysis, with stepwise variable selection.

Independent variables	Regression Coefficient	Std. error	t-value	*p*
Constant	8.02	40.92	0.20	0.85
N	35.27	12.58	2.80	0.009
Tannins	-21.08	6.68	-3.15	0.004
Altitude	0.09	0.02	5.35	0.000
	R^2 0.56	S.E. 21.84	MAE 16.92	

Substitution of litter mass for litter depth in the multiple regression model resulted in no litter quality variables being selected when these were entered alone. However, on addition of the site characteristic variables, the model selected the variables tannins and altitude to predict litter mass, accounting for 34 % of the variability (Table 5.4)

Table 5.4 Model predicting litter mass from litter quality variables and site characteristics, using multiple regression analysis, with stepwise variable selection.

Independent variables	Regression Coefficient	Std. error	t-value	*p*
Constant	35.89	34.71	1.03	0.31
Tannins	-12.60	5.82	-2.16	0.04
Altitude	0.07	0.02	4.15	0.0003
	R^2 0.34	S.E. 19.27	MAE 13.91	

$F_{1,31}$=7.58; $p \leq 0.01$; R^2 0.21); lignin:N ratios and stand age (y= [illegible]17-0.16x; $F_{1,31}$= 11.54; $p \leq 0.002$; R^2 0.28); lignin + cellulose + polyphenolics:N ratios and stand age (y=59.41-0.59x; $F_{1,31}$= 15.84; $p \leq 0.0005$; R^2 0.37); and N:tannin ratios and stand age (y= 107.12-12.02x; $F_{1,31}$=4.58; $p \leq 0.04$; R^2 0.13). Analysis of (iii) indicated that there were no significant relationships between any of the litter quality variables and altitude.

5.3.2 Relationships between litter quality variables and litter accumulation

Litter accumulation, as assessed by both litter depth and litter mass was examined in chapter 4. In order to determine which litter quality variables and site characteristics (stand age and altitude) could best predict either litter depth or mass, multiple regression models, using stepwise, forward variable selection, were used.

When only litter quality variables were entered into the analysis, the final model used to predict litter depth selected litter N and polyphenolics, which accounted for 24% of the variance observed (Table 5.2). When the site characteristics, age and altitude, were included as variables in the model, the final model used to predict litter depth selected the variables litter N, tannins and altitude. This model accounted for 56% of the variability observed (Table 5.3).

Table 5.2 Model predicting litter depth from litter quality variables, using multiple regression analysis, with stepwise variable selection.

Independent variables	Regression Coefficient	Std. error	t-value	*p*
Constant	66.45	37.80	1.76	0.09
N	51.77	16.79	3.08	0.004
Polyphenolics	-2.55	1.05	-2.42	0.02
	R^2 0.24	S.E. 28.79	MAE 20.96	

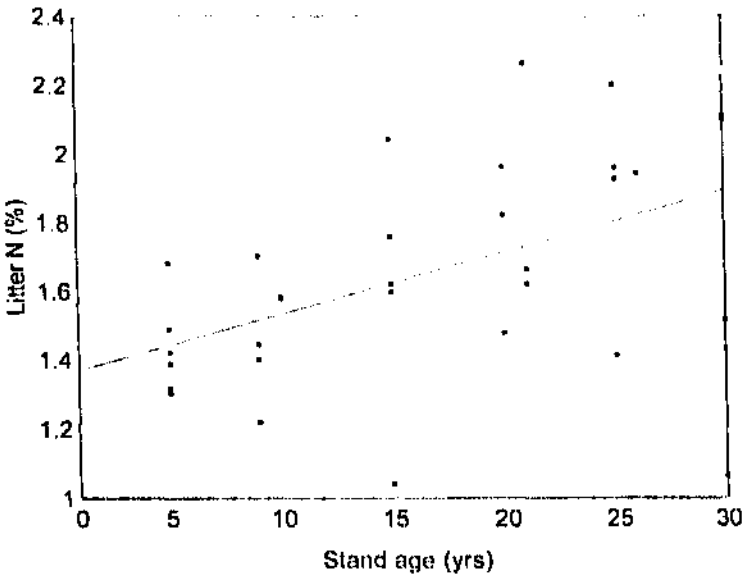


Figure 5.2 Linear regression of litter quality variable, N and stand age. $y=1.37+0.018x$; $F_{1,31}=12.05$; $p\leq 0.002$; $R^2=0.29$.

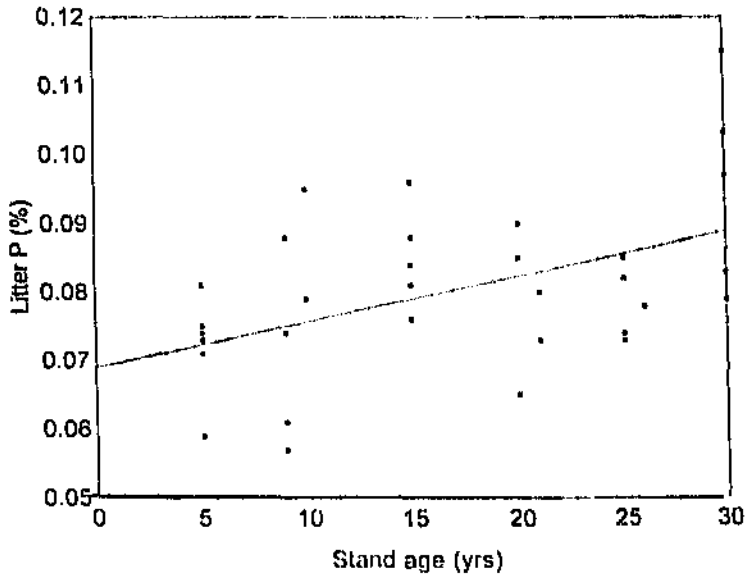


Figure 5.3 Linear regression of litter quality variable, P and stand age. $y=0.06+0.0007x$; $F_{1,31}=9.19$; $p\leq 0.005$; $R^2=0.23$.

Table 5.1 Litter quality of *P. patula* at various stand ages. Mean values, standard deviations and the number of replicates are presented.

Stand age (yrs)	N (%)	P (%)	Lignin (%)	Cellulose (%)	Polyphenolics (%)	Tannins (%)
5	1.43 ± 0.14 n=18	0.07 ± 0.01 n=18	22.0 ± 2.2 n=6	29.3 ± 3.2 n=6	34.3 ± 3.2 n=6	5.8 ± 0.5 n=6
10	1.49 ± 0.17 n=18	0.07 ± 0.01 n=18	20.7 ± 2.3 n=6	27.0 ± 1.5 n=6	32.1 ± 5.2 n=6	5.6 ± 0.9 n=6
15	1.61 ± 0.36 n=15	0.08 ± 0.01 n=15	19.6 ± 2.3 n=5	29.4 ± 5.2 n=5	31.7 ± 8.3 n=5	5.6 ± 0.7 n=5
20	1.80 ± 0.28 n=18	0.08 ± 0.02 n=18	21.3 ± 0.9 n=6	27.0 ± 2.7 n=6	33.7 ± 3.1 n=6	5.6 ± 0.5 n=6
25	1.89 ± 0.29 n=15	0.08 ± 0.01 n=15	21.4 ± 1.8 n=5	27.1 ± 1.6 n=5	21.1 ± 5.2 n=5	5.3 ± 0.3 n=5
30	1.64 ± 0.45 n=15	0.09 ± 0.01 n=15	20.1 ± 1.7 n=5	28.4 ± 2.1 n=5	33.2 ± 3.5 n=5	5.9 ± 0.9 n=5

N and P concentration. Where significant differences ($p \leq 0.05$) were evident multiple comparisons using least significant differences, were used to establish which variables contributed to the differences observed (Sokal & Rohlf, 1981; Zar, 1984). The overall decomposition rate of the litter i.e. the k value, was determined using non-linear regression analysis (Weider & Lang, 1982; Woomer, *pers. comm.*). The Systat statistical package was used for this purpose. To determine whether there were any significant differences in microbial populations between the two sites and whether there were differences in microbial activity in the trenching experiment, two sample t-tests were used (Sokal & Rohlf, 1981).

5.3 RESULTS

5.3.1. Litter quality

The average initial chemical composition of *P. patula* needle litter at the various stand ages is presented in Table 5.1, which shows litter N ranging from 1.5% to 2.0% of the dry mass of the litter and P ranging from 0.07% to 0.09% both elements tending to increase with increasing stand age. The percentage polyphenolics, cellulose, lignin and tannins, in decreasing magnitude, make up the bulk of the chemical components in the litter, but they show no specific trends with regards to stand age.

Simple linear regression models were used to determine the relationships between (i) litter quality variables and stand age, (ii) ratios of litter quality variables and stand age and (iii) litter quality variables and altitude.

Analysis of (i) showed significant positive relationships between litter N and P with stand age (Figs. 5.2 & 5.3, respectively). However, no significant relationships were evident between either lignin, cellulose, polyphenolics or tannins with stand age. Analysis of (ii) showed significant negative relationships between C:N ratios and stand age ($y = 30.08 - 0.27x$; $F_{1,11} = 13.09$; $p \leq 0.001$; $R^2 = 0.31$); C:P ratios and stand age ($y = 593.55 - 4.108x$;

K_2SO_4 (Mueller, Joergensen & Meyer, 1992). Non-fumigated (Time 0) and fumigated (Time 1) samples containing 200ml 0.5M K_2SO_4, were placed on a shaker for 1 hr to extract the C. Extracts were filtered using Whatman No. 1 filter paper (Vance, Brookes & Jenkinson, 1987).

The total organic C in the extracts was then determined using a method by Heanes (1984). A 10ml aliquot of extract was placed in digestion tubes, to which was carefully added 10ml $K_2Cr_2O_7$, and 20mls 98% H_2SO_4. Tubes were placed on a digestion block, preheated to 135°C, and digested for 30min after which they were removed and allowed to cool. Samples were made up to 100ml with deionized water. A blank and sucrose standards were also digested. After complete oxidation the difference between added and residual $K_2Cr_2O_7$ gives a measure of C (Okalebo, Gathua & Woomer, 1993). Absorbance was measured at 600nm on a visible light spectrophotometer. The concentration of C was determined from a sucrose standard curve (Heanes, 1984).

Assessment of mycorrhizal root colonization

To determine whether trenching had successfully eliminated active ECM roots, a subsamples of roots (50g) from each sample (T and CON plots) were examined using the gridline intersect method (Kormanik & Mc Graw, 1982). Roots placed on a grid were scanned under a dissecting microscope. To determine the percentage mycorrhizal roots both the vertical and horizontal gridlines were scanned and the absence and presence of ECM root tips was recorded at each point where the root intersected a line. A minimum of 100 gridline intersects were counted. Results were expressed as percentage active mycorrhizal roots.

5.2.6 Data analysis

Statistical analyses were conducted usin· ·phics version 5.1. Linear regression models were used to determine whether there were any relationships between the litter quality variables and stand age or altitude; and between litter decomposition rates, as determined by mass loss and changes in the N and P concentrations, and stand age and altitude. A multiple regression analysis, with stepwise variable selection, was used to predict litter depth from the above mentioned variables. Litter decomposition data were subjected to one-way ANOVA to determine the effects of stand age and altitude on mass loss during decomposition as well as, the changes in

Substrate Induced respiration (SIR)

A sample block of litter was removed from each plot (T & CON) and placed in plastic bags. Samples were stored at 4°C and returned to the laboratory, where they were divided into L and F layers. From each sample four subsamples of approximate 1.0g (d.w. equivalent) were placed into test tubes (5cm x 14cm). Samples were moistened with sterile water containing 0.01ml of Tween 20, and incubated overnight, at 4°C (Beare, *et al.*, 1990; Beare, *et al.*, 1991).

Samples were brought to room temperature and 2.5ml of glucose (16g l^{-1}) was added to each sample. Sample tubes and tubes containing only glucose (blank) were secured to a continuous air flow apparatus (Cheng and Coleman, 1989). Tubes were allowed to stabilize for 30min after which time they were connected to CO_2 traps containing 0.04M NaOH. Respiration was allowed to continue for two hours (Beare, *et al.*, 1990; Beare, *et al.*, 1991).

To prevent further absorption of CO_2, 3M $BaCl_2$ was added to each sample to precipitate out the carbonate as $BaCO_3$ (Page, Miller & Keeney, 1982). The concentration of CO_2 in the solution was determined by titrating with 0.04M HCl, using phenolphthalein as the indicator. Blanks were titrated and used to correct all values for CO_2 absorbed during handling.

Chloroform fumigation extraction technique

Two 50g (w wt.) samples of both the L and F layers were placed into 100ml beakers. Samples were moistened and beakers were covered with parafilm and were left in at 5°C for 7 days. A subsample was oven dried (at 60°C for 72hr) to determine the moisture content. One beaker of each sample was placed in a desiccator containing a beaker of alcohol-free chloroform. Wet blotting paper was placed around the inside of the desiccator to keep the chamber moist. The desiccator was closed and evacuated using a vacuum pump, until the chloroform started to boil. The tap on the outlet was closed and the desiccator was kept in the dark for 5 days (Anderson & Ingram, 1989; Vance, Brookes & Jenkinson, 1987). Chloroform fumigation destroys cell membranes and causes autolysis thereby increasing the amount of organic carbon rendered extractable by 0.5M

Soda lime was oven dried at 100°C overnight and 30g was placed into tins (5cm x 8cm). The tins were weighed, sealed and placed in a container containing silica gel, for transportation to the field. One tin was placed in the centre of each plot (T and CON). On placement, the lids of the tins were removed and a 5l plastic bucket was placed upside down over each tin. Prior to placement the top 10cm rim was cut off the buckets. This rim was placed correct way up onto the litter mat. To ensure a tight fit on the litter a shallow cut (±1cm) was made into the litter around the rim. The rim was then pressed down into the litter. The remainder of the bucket was placed upside down over the tin, fitting firmly into the rim. To ensure that air trapped during placement could be released a small hole had been drilled at the base of the bucket. After placement this was plugged with a rubber bung and sealed with silicon grease (Fig. 5.1). The soda lime tins were left in the field for 6 hours. On removal of the tins, they were sealed and returned to the laboratory where they were oven dried overnight at 100°C. After drying the soda lime was reweighed and the difference *i.e.* the increase in weight was recorded. To determine the amount of CO_2 absorbed the following equation was used;

$$mgCO_2\ hr^{-1} = (W1-W2) \times \frac{1.4}{hrs} \qquad \text{(Equation 5.1)}$$

where, W1 = initial weight of the soda lime (g)

W2 = dry weight (g) after the measurement period

(Edwards, 1932; Raich, Bowden & Steugler, 1990).

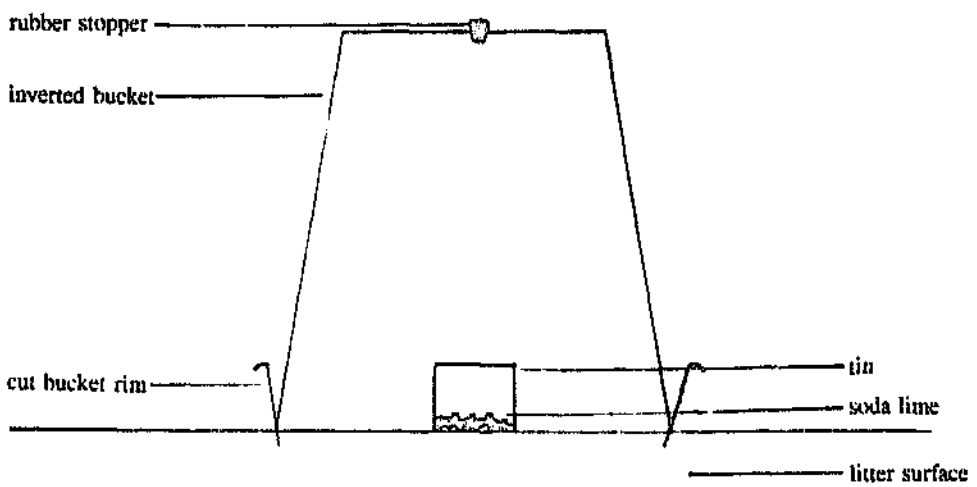


Figure 5.1 Diagrammatic representation of the soda lime technique.

5.2.5 Microbial activity and biomass

To assess whether the presence of mycorrhizal roots retards decomposer activity in a *P. patula* site in which litter had accumulated, (Brooklands, F2 - Table 3.2; chapter 3) a trenching experiment was conducted. A description of the experimental design and the site preparation follows. Twelve 1.5m^2 plots were randomly selected between *P. patula* trees within a 1ha study site. Trenches were dug around the perimeter of six of these plots. The trenches were approximately 45cm wide and 70cm deep, down to the underlying shale. These trenched (T) plots were left to stand for 7 months (August 1992 to March 1993). The purpose of trenching was to sever all roots entering the plot and to exclude active ECM roots from within the plots. The remaining six control (CON) plots were not disturbed in any way.

After the stabilization period, the microbial activity and biomass of the T and CON plots was assessed. Three techniques were used to assess microbial activity and microbial biomass accumulation, these included two methods to determine respiration and one to determine microbial carbon (C_{micro}). The methods used were;

- the soda lime technique to quantify the amount of CO_2 evolved from the forest floor *in situ* (Edwards, 1982);
- the substrate induced respiration (SIR) technique to assess CO_2 respired from the litter in the laboratory (Beare, *et al.*, 1990; Beare, *et al.*, 1991);
- the chloroform fumigation extraction technique to quantify C_{micro} (Heanes, 1984; Vance, Brookes & Jenkinson, 1987).

Details of these techniques are provided below.

Soda lime technique

Soda lime is used for absorbing moisture and CO_2, the CO_2 becoming chemically bound to the soda lime. The principle of the technique was to dry and weigh the soda lime before and after a measurement period. The difference in weight represents the amount of CO_2 absorbed minus the water released during the chemical reaction. The amount of chemically released water is predictable and a correction factor of 1.4 is incorporated into equation 5.1 (Edwards, 1982).

As the litterbag experiment could be conducted for a period of only one year, information regarding the decomposition of recalcitrant compounds such as lignin during subsequent years could not be obtained. To provide some information on the long term decomposition, ten litter core samples were collected from a site in which litter had accumulated (information regarding this site is provided in Chapter 6). It was assumed that newly fallen litter occurred at the top of the core with an age sequence of litter occurring further down the profile. The litter cores were divided into various layers (Chapter 6) and bulked samples were chemically analyzed for lignin, cellulose and polyphenolics (Section 5.2.2). Foliar samples collected for the nutrient cycling study (Chapter 6) were also analyzed for lignin, cellulose and polyphenolics and compared with the litter samples. Core samples were also analyzed for N and P these results are presented in Chapter 6.

5.2.4 Soil microbial and soil faunal populations

Core samples collected from the two sites (Table 3.2; marked ◇), were divided into their litter/soil components. To enumerate the soil microbial population the agar plate method for total microbial counts was used (Wollum II, 1982). In this method appropriately diluted sample suspensions were placed on suitable agar plates and after incubation colonies growing on the agar plates were counted. Litter/soil samples were examined to determine the relative proportions of the functional groups, bacterial and fungal, at the two sites. To determine total bacterial counts, sample suspensions were plated onto nutrient agar (pH 4.5), while Martins-Rose Bengal agar (pH 4.5) was used to determine fungal counts (Wollum II, 1982). The bacterial and fungal populations were recorded as colony forming units (cfu) per gram sample.

The remaining litter/soil core samples were examined for soil fauna (body length greater than 2mm). A handful of a sample was placed on one side of a tray and progressively spread out across the tray and any soil fauna observed were removed. This process was repeated until the entire sample had been examined (Anderson & Ingram, 1989).

Polyphenolics

Total extractable polyphenolics, including hydrolysable tannins and condensed tannins, were extracted using 50% methanol and heating at 77°C (Sauvestry, Page & Huot, 1992). Using the Folin-Denis method polyphenolic concentrations were colorimetrically determined at 760nm, using a visible light spectrophotometer (Allen, 1974; Anderson & Ingram, 1989).

Tannins

Tannins were extracted from samples using 50% methanol (Sauvestry, Page & Huot, 1992), and were quantified using a radial diffusion assay technique (Hagerman, 1987). In this method the tannin containing extracts are placed in wells of a protein containing agar gel. Bovine serum albumen (BSA), was used as the protein. As the tannins diffuse into the gel, they react with the BSA and a visible ring of precipitation develops. The square of the ring diameter is proportional to the quantity of tannin in the extract, these values being compared with a tannic acid standard (Hagerman, 1987).

5.2.3 Litter decomposition

For the litter decomposition experiment, 15 litterbags (15cm x 20cm) were buried under the litter layer, in each of the selected sites. The depth at which each bag was buried was dependent on the litter depth at each site (Table 5.5). Litterbags were constructed from PVC shadecloth which had a mesh size of approximately 3mm^2 (Singh & Gupta, 1977; Woods & Raison, 1982). Each litterbag contained 10g of dried needle litter taken from the respective compartments. Three litterbags were sampled from each compartment, at intervals of 6wks, 13wks, 23wks, 39wks and 52wks. The litterbags once removed from the litter were placed in paper packets to prevent loss of material. On return to the laboratory weight loss was recorded after drying and chemical analyses were conducted, to monitor changes in litter quality; these included total N and total P (Section 5.2.2). Dry mass measurements were not converted to ash free dry mass as it was found that soil did not adhere to the dried bags or contaminate the litter. Data are presented as percentage mass or nutrients remaining. The rate at which mass and nutrient loss occurred was determined by obtaining the slope between two time periods this was calculated as a percentage loss per week.

Determination of Nitrogen

During digestion the N in the sample was converted to ammonium sulphate $(NH_4^+)_2SO_4$. The NH_4^+-N concentration in the samples was then determined colorimetrically at 655nm, using a Sequoia-Turner visible light spectrophotometer. The colorimetric procedure used a solution containing sodium citrate, salicylate, sodium tartrate and sodium nitroprusside mixed with sodium hydroxide and sodium hypochlorite solutions. The mixed reagent, reacts with the NH_4^+-N to give a green colour, the intensity of which is proportional to the N concentration (Anderson & Ingram, 1989).

Determination of Phosphorus

The concentration of P in digested samples was colorimetrically determined using the ammonium molybdate/ascorbic acid procedure. This method is very sensitive and is widely used for determination of P. In an acid molybdate solution containing orthophosphate ions, a phosphomolybdate complex forms that can be reduced by reducing agents to a molybdenum blue colour. The intensity of the colour is proportional to the P concentrations, which was determined on a visible light spectrophotometer at 700nm (Anderson & Ingram, 1989; Olsen & Sommers, 1982).

Lignin and cellulose

The percentage lignin and cellulose of the litter samples was determined using the Acid Detergent Fibre (ADF) technique (Van Soest, 1966; Van Soest & Wine, 1968). Dried litter samples were finely crushed using a pestle and mortar and sieved through a 430μm sieve. The ADF was prepared from the samples by boiling with a H_2SO_4 solution of cetyltrimethyl ammonium bromide (CTAB). The CTAB dissolves the nitrogenous constituents and the acid hydrolyses the starch to leave a residue of lignin, cellulose and ash. Samples were then oxidized using a buffered potassium permanganate solution to remove the lignin. The percentage of lignin was determined by weight loss on drying. The percentage cellulose was determined by weight loss after ashing (Van Soest, 1966; Van Soest & Wine, 1968).

and not stand age; there was an overall decrease in remaining N in low altitude 15 year-old stands, when compared to an increase in remaining N in high altitude 15 year old-stands.

Analysis of (vi) indicated that there were significant differences in the rate of P loss amongst the different age/altitude groupings during phase P1 ($F_{11,24} = 7.44$; $p \leq 0.0001$) and phase P2 ($F_{11,24} = 2.72$; $p \leq 0.02$). Differences during phase P1 were attributable to the faster loss of P from 25 and 30 year-old stands when compared with P loss rates of 5 and 15 year-old stands and to slower P loss from low altitude 10, 25 and 30 year-old stands when compared with their corresponding stands in high altitudes, and faster P loss rates from the low altitude 15 year-old stand when compared with it's corresponding high altitude stand. Differences during phase P2 were attributable only to stand age, with a faster loss of P from 30 year-old stands when compared with the other stands.

Simple linear regression models were used to test the relationships between (vii) mass loss rates and stand age, mass loss rates and altitude (viii) N loss rates and stand age and, N loss rates and altitude, and (ix) P loss rates and stand age and, P loss rates and altitude. Regressions on the N and P loss rates were conducted on the full decomposition period (0-52 weeks) as well as the two phases of decomposition.

Regression analysis of (vii) revealed that there was no significant relationships between the mass loss rate (Phase M1) and stand age, and mass loss rate and altitude. No relationship was evident between mass loss rate (Phase M2) and stand age. However, there was a significant relationship between the mass loss rate (Phase M2) and altitude, indicating a decrease in mass loss rate with increasing altitude (Fig. 5.16).

Regression analysis of (viii) indicated that there were significant positive relationships between the overall N loss rate and stand age (Fig. 5.17), as well as the N loss rate during phases N1 and stand age (Fig. 5.18), indicating an increase in N loss rate with increasing stand age. Significant negative relationships between the overall N loss rate and altitude (Fig. 5.19), and the N loss rate during phase N2 and altitude (Fig. 5.20) were evident, indicating decreasing N loss rates with increasing altitude.

Loss rate values were determined by using the slope or change in mass or nutrient, over time $\frac{dx}{dt}$. These data are expressed as the percentage mass or nutrient lost per week. Using the percentage lose per week or loss rate data the following statistical analyses were conducted. One-way ANOVA was used to determine the effects of stand age and altitude on (iv) rate of mass loss data during phases M1 and M2, (v) rate of N loss, during phases N1 and N2 and (vi) rate of P loss, during phases P1 and P2. To determine the effect of stand age and altitude, the data were grouped as low altitude 5 years, high altitude 5 years, low altitude 10 years, high altitude 10 years etc. Where significant differences ($p \leq 0.10$) were evident multiple comparisons, using least significant differences ($p \leq 0.05$), were used to determine whether differences in the rate of loss were attributable to stand age or altitude.

Analysis of (iv) indicated that there were significant differences in the rate of mass loss (Phase M1) amongst the different age/altitude groupings ($F_{11,24} = 2.08$; $p \leq 0.06$). These differences in mass loss rate (Phase M1) were not attributable to stand age, but rather to differences in altitude. More specifically, there was a faster loss of mass from low altitude 15 year-old stands when compared with the corresponding high altitude stand, and slower mass loss from low altitude 30 year-old stands when compared with its corresponding high altitude stand. During phase M2, significant differences in mass loss rate, amongst the different altitude/age groupings were evident ($F_{11,24} = 2.90$; $p \leq 0.01$). These differences were attributable again to altitude differences, with a faster loss of mass from low altitude 10 year-old stands when compared with the corresponding high altitude stand, and slower mass loss from low altitude 15 year-old stands when compared with its corresponding high altitude stand.

Analysis of (v) indicated that there were significant differences in the rate of N loss amongst the different age/altitude groupings during phase N1 ($F_{11,24} = 18.62$; $p \leq 0.0001$) and phase N2 ($F_{11,24} = 2.44$; $p \leq 0.03$). Differences during phase N1 were attributable to faster loss of N from 25 year-old stands when compared with 5 and 10 year-old stands, and to differences in N loss rates in all the direct altitude comparisons. Thus there were faster N loss rates in low altitude 5, 15 and 25 year-old stands when compared with their corresponding high altitude stands, and slower N loss rates in low altitude 10, 20 and 30 year-old stands when compared with their corresponding high altitude stands. Differences in N loss rate during phase N2 were attributable to altitude

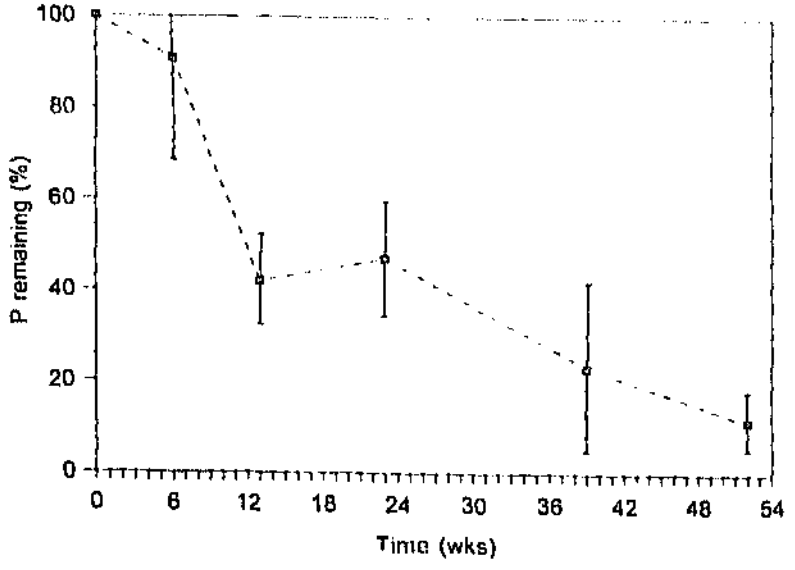


Figure 5.14 Mean phosphorus loss from *P. patula* litter over time in low altitude sites. P loss data was averaged over all stand ages. Standard deviations shown as bars.

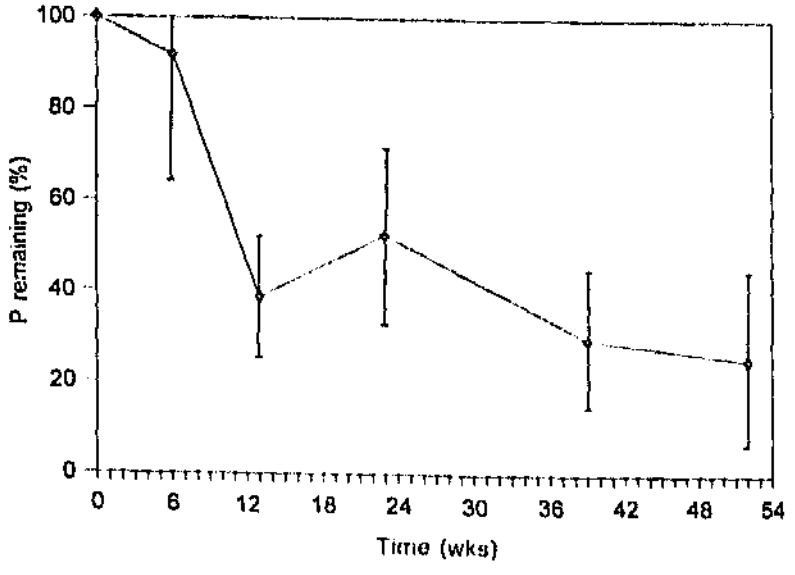


Figure 5.15 Mean phosphorus loss from *P. patula* litter over time in high altitude sites. P loss data was averaged over all stand ages. Standard deviations shown as bars.

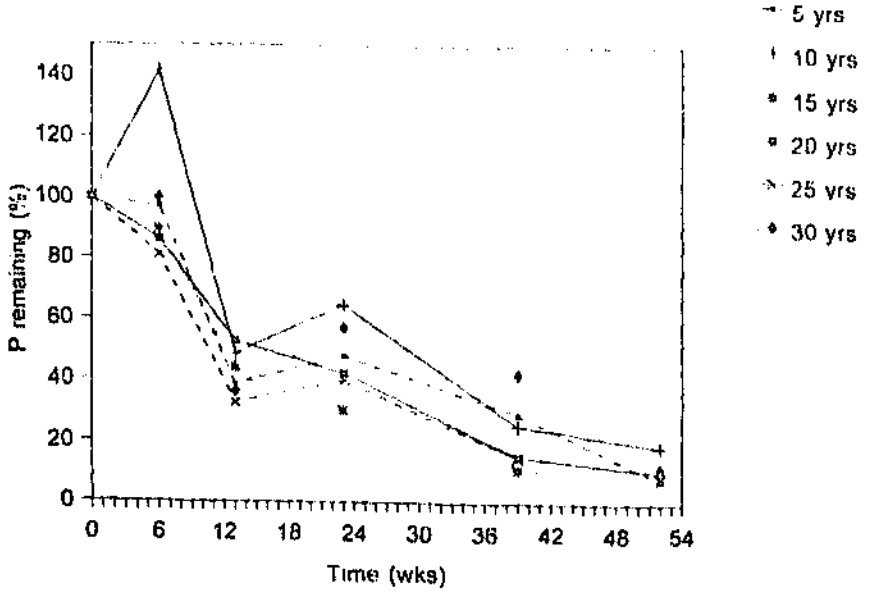


Figure 5.12 Phosphorus loss from *P. patula* litter over time in low altitude sites at various stand ages.

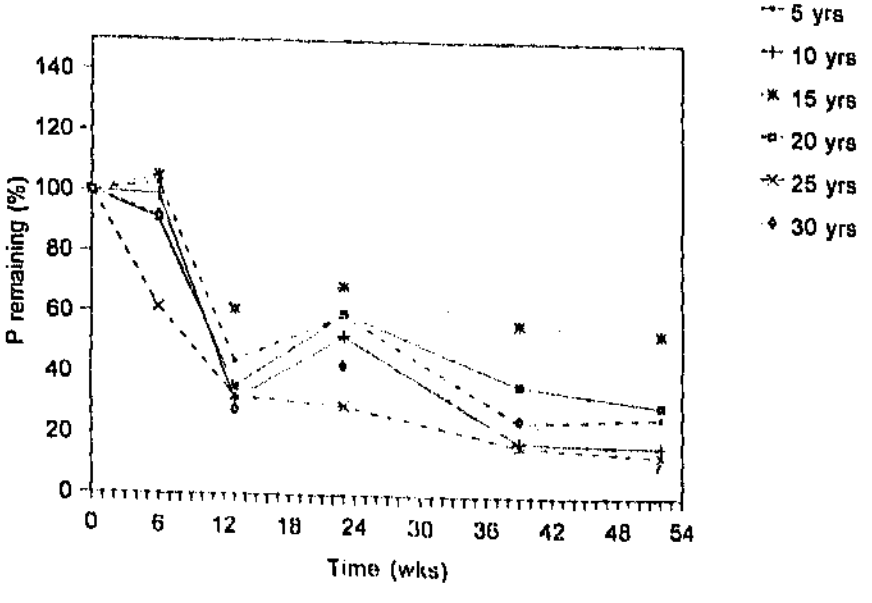


Figure 5.13 Phosphorus loss from *P. patula* litter over time in high altitude sites at various stand ages.

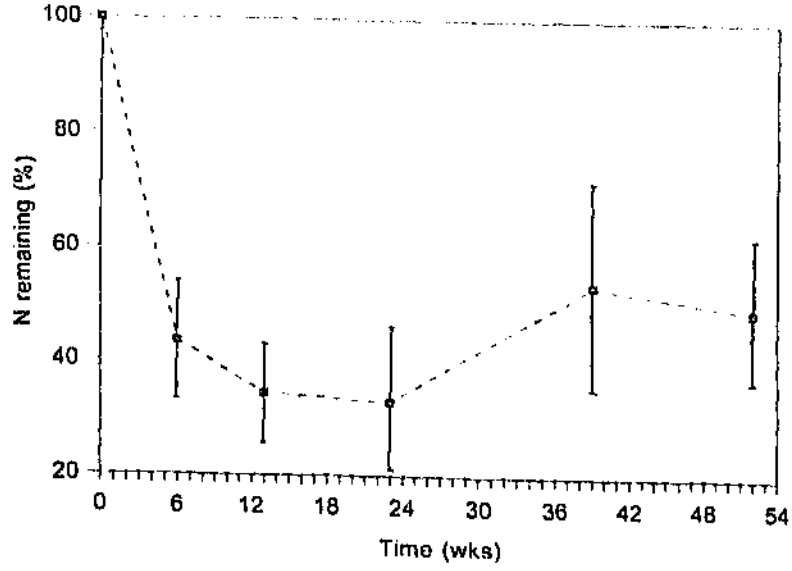


Figure 5.10 Mean nitrogen loss from *P. patula* litter over time in low altitude sites. N loss data was averaged over all stand ages. Standard deviations shown as bars.

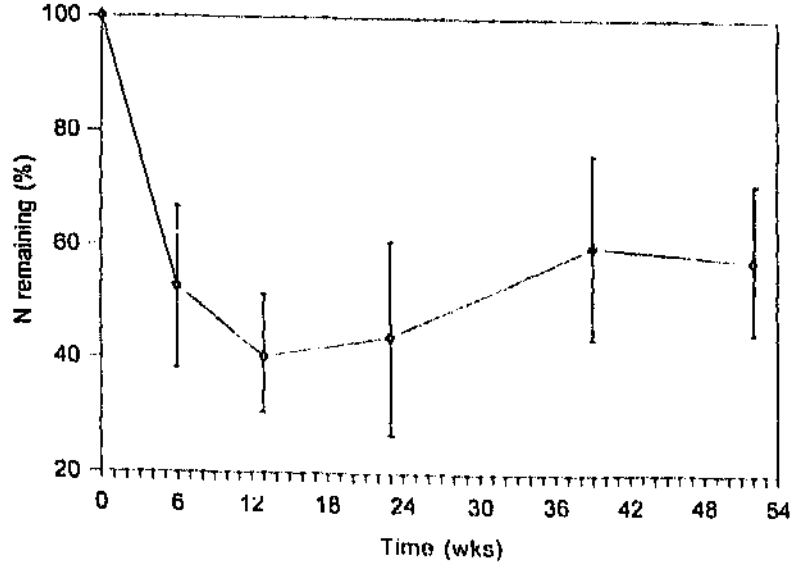


Figure 5.11 Mean nitrogen loss from *P. patula* litter over time in high altitude sites. N loss data was averaged over all stand ages. Standard deviations shown as bars.

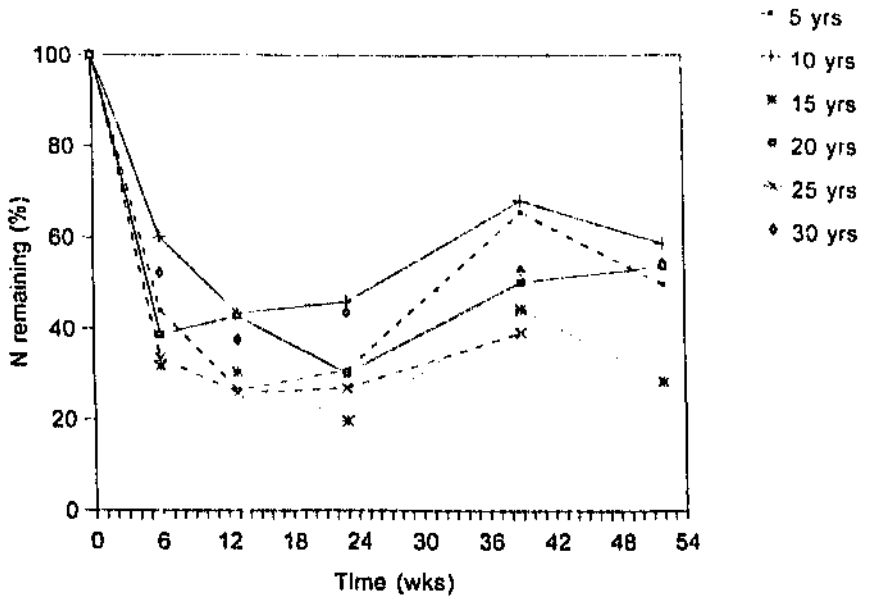


Figure 5.8 Nitrogen loss from *P. patula* litter over time in low altitude sites at various stand ages.

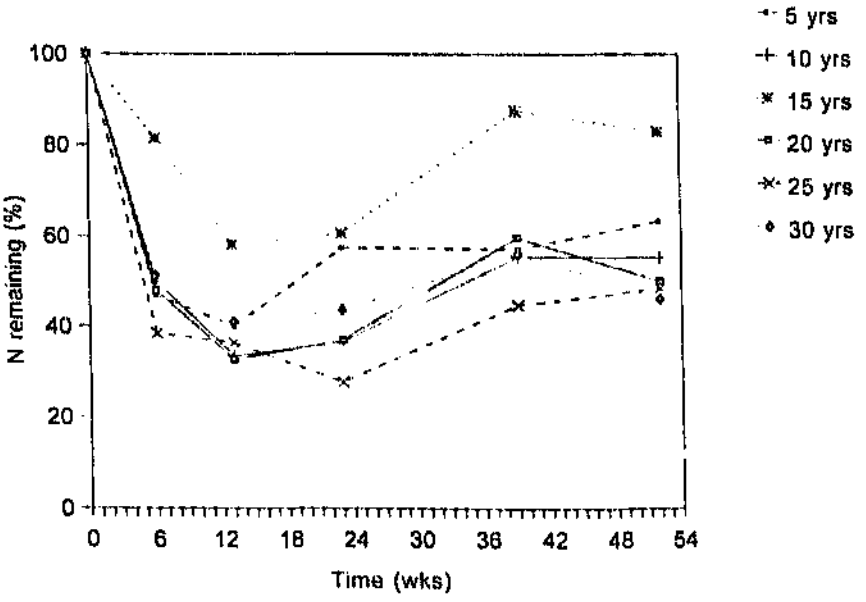


Figure 5.9 Nitrogen loss from *P. patula* litter over time in high altitude sites at various stand ages.

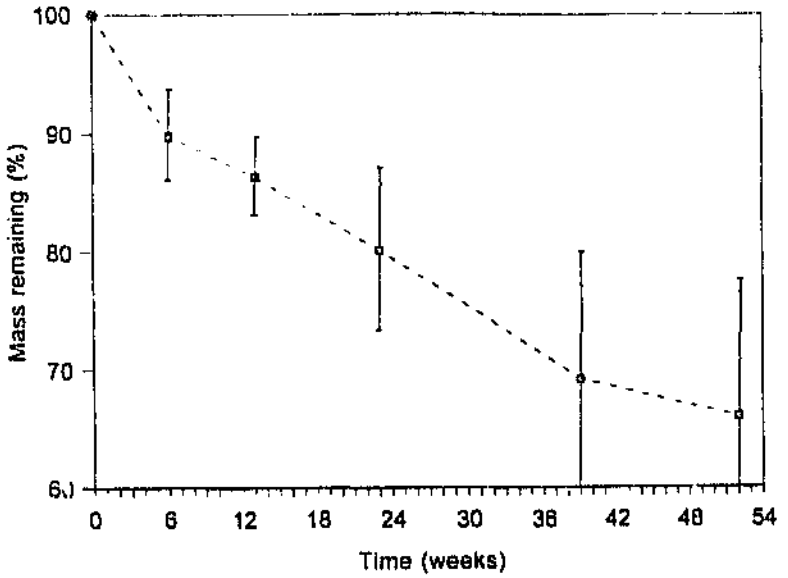


Figure 5.6 Mean mass loss from *P. patula* litter over time in low altitude sites at various stand ages. Mass loss data averaged over all stand ages. Standard deviations shown as bars.

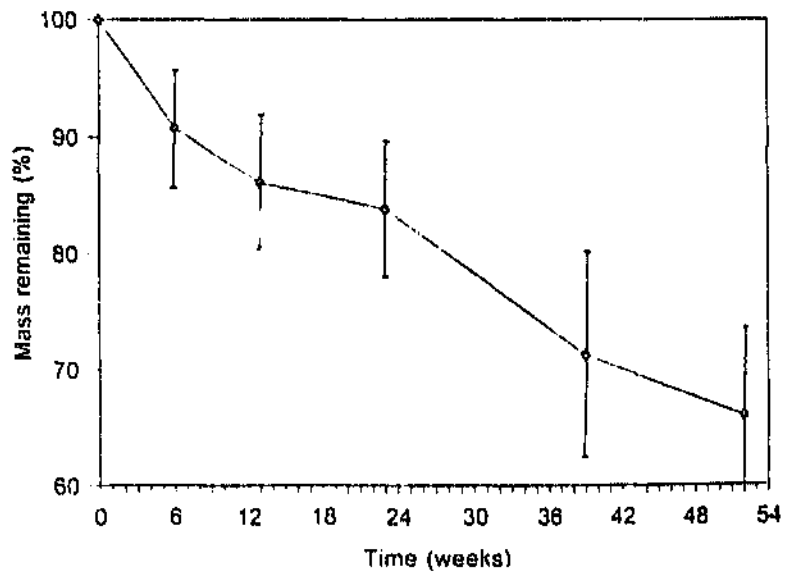


Figure 5.7 Mean mass loss from *P. patula* litter over time in high altitude sites at various stand ages. Mass loss data averaged over all stand ages. Standard deviations shown as bars.

Table 5.6 Litter decomposition rate constants (k) for all stand ages at low and high altitude sites; t_{50} is the time (days) required for 50% of the litter to decompose.

Stand age (yrs)	Low altitude		High altitude	
5	k	-0.51	k	-0.49
	R^2	0.87	R^2	0.90
	t_{50}	493	t_{50}	514
10	k	-0.66	k	-0.40
	R^2	0.82	R^2	0.76
	t_{50}	380	t_{50}	636
15	k	-0.43	k	-0.51
	R^2	0.69	R^2	0.81
	t_{50}	584	t_{50}	492
20	k	-0.44	k	-0.34
	R^2	0.66	R^2	0.72
	t_{50}	571	t_{50}	744
25	k	-0.54	k	-0.34
	R^2	0.78	R^2	0.82
	t_{50}	470	t_{50}	742
30	k	-0.35	k	-0.54
	R^2	0.66	R^2	0.71
	t_{50}	731	t_{50}	469

Simple linear regression analyses were conducted to determine whether any relationships existed between the decomposition constants and either stand age or altitude. However, no significant relationships were evident. Trends may be masked as a result of the high variability of the data which is indicated by the standard deviations shown in figure 5.6, for low altitude sites and figure 5.7 for high altitude sites. These graphs represent the mean percentage mass remaining, calculated for all samples regardless of stand age.

The N and P lost from the litter was monitored over a 52 week period and can also be divided into 2 phases (Figs. 5.8, 5.9, 5.12 & 5.13). Phase N1 and P1, occurred during the first thirteen weeks, where the N and P loss rate was rapid. During phase N2, from 13 to 52 weeks, N was no longer lost from the litter, but rather showed an increase. In contrast during phase P2, from 13 to 52 weeks, P continued to be lost from the litter but more gradually. The variability of the N and P loss data is shown as standard deviations on figure 5.10 (low altitude sites) and figure 5.11 (high altitude sites) for the N data and figure 5.14 (low altitude sites) and figure 5.15 (high altitude sites) for the P data.

model, the decomposition rate constant (k), for each stand age at both low and high altitude sites, was determined (Table 5.6). The equation used was as follows;

$$\frac{Lr}{Li} = EXP^{(k*t)} \qquad \text{(Equation 5.2)}$$

where, Lr = litter mass remaining

Li = initial litter mass

k = decomposition rate constant

t = time

(Weider & Lang, 1982; Woomer, *pers. comm.*).

From the decomposition constants presented in Table 5.6 it is evident that litter in the high altitude 5, 10, 20 and 25 year old stands decomposes slower than the low altitude stands of the same age. However, the litter in the low altitude 15 and 30 year old stands decomposes slower when compared with their corresponding high altitude stands. This is further emphasised when comparing the t_{50} i.e. the time required for 50% decomposition (Table 5.6). The t_{50} was calculated using the following equation;

$$t(0.5) = \frac{[\log_n(\frac{Lr}{Li})]}{k} \qquad \text{(Equation 5.3)}$$

where, t(0.5) = time required for 50% decomposition

Lr = litter mass remaining

Li = initial litter mass

k = decomposition constant

(Weider & Lang, 1982; Woomer, *pers. comm.*).

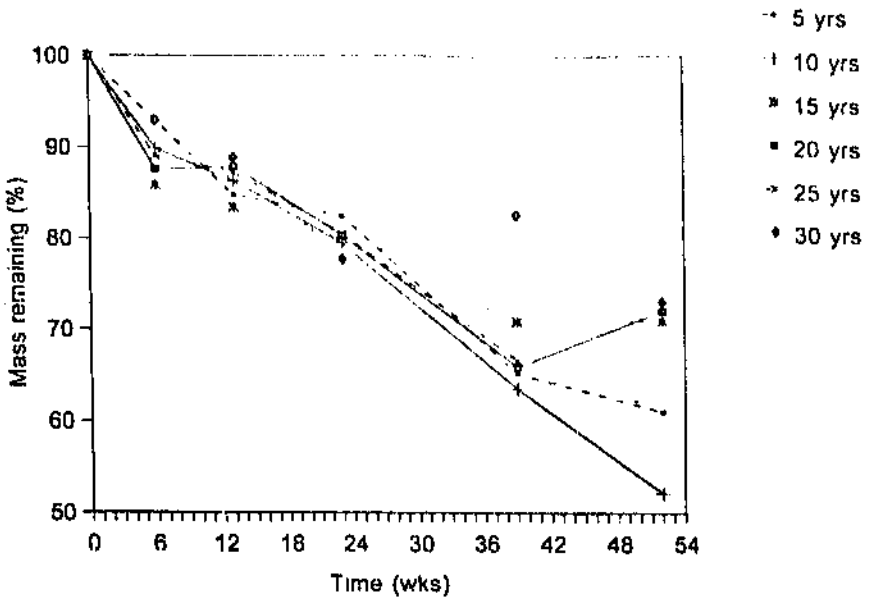


Figure 5.4 Mass loss from *P. patula* litter over time in low altitude sites at various stand ages.

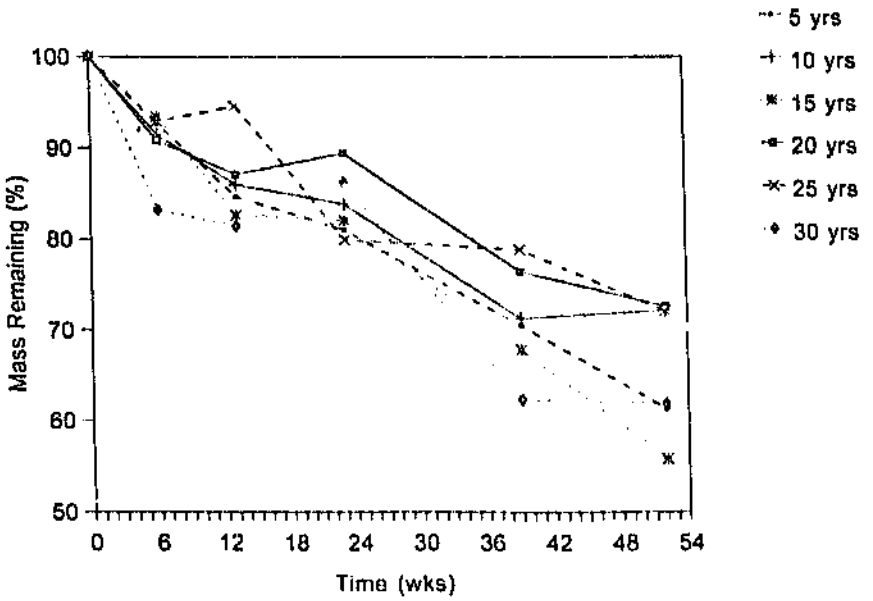


Figure 5.5 Mass loss from *P. patula* litter over time in high altitude sites at various stand ages.

Table 5.5 Mean litter depth, litter N and P values for sites of various stand ages at both low and high altitudes selected to be used in the decomposition study. Standard deviations and number of replicates are presented.

Stand age (yrs)	Litter depth (mm)	N (%)	P (%)
Low altitude			
5	38±19 n=15	1.49±0.48 n=3	0.07±0.02 n=3
10	42±14 n=15	1.22±0.14 n=3	0.06±0.01 n=3
15	33±20 n=15	2.04±0.61 n=3	0.10±0.03 n=3
20	50±18 n=15	1.48±0.04 n=3	0.08±0.02 n=3
25	56±14 n=15	1.96±0.03 n=3	0.08±0.02 n=3
30	52±15 n=15	1.43±0.14 n=3	0.07±0.02 n=3
High altitude			
5	95±28 n=15	1.31±0.18 n=3	0.07±0.01 n=3
10	92±44 n=15	1.58±0.29 n=3	0.09±0.04 n=3
15	76±26 n=15	1.51±0.27 n=3	0.08±0.02 n=3
20	85±27 n=15	1.82±0.44 n=3	0.09±0.04 n=3
25	95±28 n=15	1.92±0.26 n=3	0.12±0.04 n=3
30	71±21 n=15	2.11±0.26 n=3	0.10±0.02 n=3

To determine additional models that can be used to predict either litter depth or litter mass, simple linear regression models were used to examine the relationship between ratios of litter quality variables to litter accumulation. Analysis showed significant but weak negative relationships between litter depth and N:tannins (y=107.12-12.02x; $F_{1,31}$=4.58; $p \leq 0.04$; R^2 0.13), litter mass and N:tannins (y=77.733-9.832x; $F_{1,31}$=5.567; $p \leq 0.025$; R^2 0.16), litter depth and N:polyphenolics (y=117.19-2.57x; $F_{1,31}$=6.87; $p \leq 0.01$; R^2 0.19), litter mass and N:polyphenolics (y=84.92-2.06x; $F_{1,31}$=7.69; $p \leq 0.01$; R^2 0.21), litter depth and N:polyphenolics + tannins (y=118.43-2.24x; $F_{1,31}$=6.91; $p \leq 0.01$; R^2 0.19), litter mass and N:polyphenolics + tannins (y=86.18-1.81x; $F_{1,31}$=7.88; $p \leq 0.01$; R^2 0.21), litter depth and lignin + cellulose + polyphenolics:N (y=109.52-0.88x; $F_{1,31}$=4.46; $p \leq 0.04$; R^2 0.13) and litter mass and lignin + cellulose + polyphenolics:N (y=77.16-0.67x; $F_{1,31}$=4.55; $p \leq 0.04$; R^2 0.14).

5.3.3 Litter decomposition

Litter decomposition in a stand of each age group at both low and high altitudes was monitored, litter depth and litter N and P data for the specific sites used in this study are provided in Table 5.5.

Decomposition was measured as mass lost from the litter in buried litterbags at intervals over a 52 week time period. The data, expressed as percentage litter mass remaining, at both low and high altitudes are presented graphically in figures 5.4 and 5.5, respectively. Decomposition can be divided into two phases. Phase M1, occurring during the first six weeks, where mass loss was rapid and phase M2, from 6 to 52 weeks, where mass loss occurred more gradually. Using the following decomposition rate equation in a non-linear regression

of the litter quality variables and altitude. **Hypothesis** 3 can therefore be rejected as litter quality does not decrease with increasing altitude. *P. patula* growing in high altitude sites does not have a poorer litter quality than trees growing at low altitudes. Although litter quality does influence the rate of decomposition, the litter quality of *P. patula*, examined in this study does not account for the observed litter accumulation in high altitude sites.

5.4.2 Litter decomposition

The litter bag technique is commonly used in decomposition studies despite concerns regarding the exclusion of soil fauna, changes in the micro-climate and compression of material (Woods & Raison, 1982). Initially, litter decomposition was to be determined using the litterfall method, which compares the amount of litter accumulated over a specific period with the amount of litterfall collected during the same period (Singh & Gupta, 1977; Woods & Raison, 1982). However, as a result of problems experienced with the collection of litterfall data, this method was abandoned. The litterbag method was subsequently selected to determine decomposition rates, and afforded the opportunity of following the changes in litter quality during decomposition, an exercise that would not have been otherwise achieved. Litterbags were placed under the L layer in the region of the F layer, which is regarded as the most microbiologically active area (Woods & Raison, 1982).

The overall percentage mass lost from both low and high altitude sites (Figs. 5.4 & 5.5), was 34 ± 10%; this represents the mean mass lost over 52 weeks, calculated regardless of stand age and can be compared with other mass loss figures obtained in previous studies for other *Pinus* ecosystems (Table 5.10). Decomposition was divided into two phases, M1 and M2. A rapid loss in mass occurred during the first six weeks (Phase M1). This phase can be attributed to the initial leaching phase of decomposition when water soluble compounds are released from the litter (Ibrahima, Joffre & Gilion, 1995). The biological phase of decomposition would have occurred concurrently with the initial leaching phase and continued thereafter, continuing into phase M2, during which a more gradual loss in mass occurred (Parsons, Taylor & Parkinson, 1990; Stump & Binkley, 1993). This two phase pattern in mass loss is similar to that observed in other studies (Smolander *et al.*, 1996) although

Table 5.9 Comparison of *P. patula* litter quality with other *Pinus* species.

Species	Litter quality Component	(%)	Reference
P. patula	N	1.65 ± 0.18	This study
	P	0.08 ± 0.01	
	Lignin	20.84 ±0.89	
	Cellulose	28.47 ± 1.34	
	Polyphenolics	31.00 ± 4.96	
	Tannins	5.65 ± 0.19	
P. patula	N	0.57	Freumond, 1991
P. elliottii	N	0.35	Gholz *et al.*, 1985
	P	0.02	
	Lignin	23.70	
	Cellulose	20.93	
P. sylvestris	N	0.41	Berg & Wessén, 1984
	P	0.02	
	Lignin	22.00	
	Cellulose	29.00	
P. resinosa	N	0.43	Aber, Melillo & Mc Clougherty, 1990
	Lignin	27.70	
	Cellulose	46.50	
P. alba	N	0.44	Aber, Melillo & Mc Clougherty, 1990
	Lignin	22.50	
	Cellulose	44.70	
P. kesiya	Lignin	35.00	Kshattriya, Sharma & Mishra, 1992
	Cellulose	26.00	
P. contorta	N	0.45	Stump & Binkley, 1993
	Lignin	25.29	
	Cellulose	36.99	

unsaturated substances which may appear as lignin, and which is also sensitive to heat, these factors may result in increased lignin values. Permanganate lignin represents a true theoretical lignin value, the ADF method being less sensitive to the above mentioned factors (Van Soest, 1966). However, *P. patula* litter can be regarded as a high lignin substrate with the lignin concentration falling within the 17-29% range (Stump & Binkley, 1993).

An increase in litter N and P was evident as the stands aged, indicating that older trees retranslocated less N and P from the needles prior to needle fall. However, very little change was evident in the other litter quality variables as the stands aged. Studies on *P. kesiya* also showed that there were no differences in both lignin and cellulose concentrations of litter between 5 and 15 year old stands (Kshattriya, Sharma & Mishra, 1992). This can be attributed to the uniformity of senescent needles in age, shape and size, so that structural compounds such as lignin and cellulose would show little variability as the tree aged (Berg & Wessén, 1984). Several

9) The loss of N from the litter increased with increasing stand age (Phase N1). Overall N loss decreased with increasing altitude, indicating an immobilization of N within the litter at high altitudes.

10) During decomposition, changes in litter P occurred in two phases, phase P1 (0-13 weeks) and phase P2 (13-52 weeks). Litter P decreased during both phases.

11) The loss of P from the litter increased with increasing stand age. A rapid loss of P was evident from high altitude sites during phase P1; this rate slowed down during phase P2.

12) The enumeration of the soil microbial populations, at a low and high altitude site, indicated that there were no significant differences between the bacterial and fungal functional groups.

13) Microbial activity and biomass was reduced in trenched plots.

5.4 DISCUSSION

5.4.1 Litter quality

The litter quality study used litter collected from litter traps which had fallen over a three month period. This raised some concerns regarding the effects of leaching and onset of decomposition, by both epiphytic and endophytic organisms, on the litter quality (Dickinson & Pugh, 1974). However, as the three month collection period was during the drier, cooler months of winter, it was assumed that any loss of nutrients from the litter was minimal. The average litter quality of *P. patula* (Table 5.1) can be compared with other reports for *Pinus* spp. in the literature (Table 5.9). Litter N and P was higher in this study than that reported for other *Pinus* spp. and for *P. patula* litter studied by Freimond (1993) Variability both within and between species has been reported previously and may be attributable to site factors (Dickinson & Pugh, 1974). Seasonality in litter N concentrations has also been reported (Versfeld & Donald, 1991) although seasonal changes in litter quality was not monitored in this study. Litter lignin and cellulose percentages were lower in this study than those reported in some of the other species and may be attributed to variability between species (Mecontemeyer, 1978) and the analytical method employed (Van Soest, 1966). Most studies cited in table 5.9 have used the 72% sulphuric acid method for quantifying lignin. A method which is susceptible to the presence of polyphenolic and other

Mycorrhizal colonization

The CON plots were significantly drier than the T plots in both the L (t_n=1.40; $p \leq 0.1$) and F layers (t_n=3.20; $p \leq 0.05$), with the F (CON plots 6.9%; T plots 10.9%) layers being moister than the L layer (CON plots 1.6%; T plots 2.5%). With regard to mycorrhizal colonization CON plots had significantly higher levels of mycorrhizal roots at 32 ± 11%, than T plots where colonization was only 3 ± 1% (t_n=6.43; $p \leq 0.0001$). In T plot samples roots were dying or dead with cortical tissue sloughing off the root axis, indicating that the majority of active mycorrhizal roots were successfully excluded from the trenched plots.

5.3.6 Summary of results

To clarify the major findings of this section a summary of the results is provided.

1) Litter N and P concentrations were shown to increase with increasing stand age.

2) The litter quality ratios, C:N and lignin + cellulose + polyphenolics:N, gave the most significant negative linear relationships with increasing stand age.

3) No relationships were evident between any of the litter quality variables and the altitude of the site.

4) A model predicting litter depth using the litter quality variables, N and tannins, and the site characteristic, altitude accounted for 56% of the variability observed in litter depth.

5) Litter decomposition, as measured by mass loss, occurred in two phases. During phase M1 (0-6 weeks) mass loss was rapid. Mass loss continued during phase M2 (6-52 weeks) but more gradually.

6) The litter decomposition rate constants were lower in high altitude 20 and 25 year old stands when compared with the corresponding low altitude sites, while the opposite held for 10, 15 and 30 year old stands. In the high altitude 20 and 25 year old stands a period of two years would be required to decompose 50% of the litter.

7) The rate of decomposition during phase M1 showed no significant relationships with either stand age or altitude, while regression analysis of phase M2 data indicated that the rate of decomposition decreased with increasing altitude.

8) During decomposition, changes in litter N occurred in two phases. Phase N1 (0-13 weeks) showed a loss of N from the litter, while phase N2 (13-52 weeks) showed an accumulation of N.

5.3.5 Microbial activity and biomass

Respiration

The quantity of respired CO_2 absorbed by the soda lime from the CON and T plots in the high altitude 42 year old site are presented in Table 5.8. Although the respiration from the forest floor was slightly lower in T plots this difference was not significant. Respiration results, measured using the SIR technique, for both the L and F litter layers are presented in Table 5.8. Using two-sample t-tests a significant difference at the 10 % level was recorded for samples from the F layer, with CON plots respiring more than T plots ($t_0 = 1.57$; $p \leq 0.1$). Although the L layer samples from the CON plots respired more than the corresponding sample in the T plots, significant differences were masked as a result of the variability in the data.

Table 5.8 Respiration and microbial C values for control and trenched plots as measured by the soda lime, SIR and microbial C techniques. Mean values of six replicate samples and standard deviations are presented.

	Control plots		Trenched plots	
Method	L layer	F layer	L layer	F layer
Respiration				
Soda lime ($mgCO_2\ hr^{-1}$)	8.86 ± 7.59	-	7.93 ± 6.95	-
SIR ($mgCO_2\ g^{-1}\ hr^{-1}$)	0.70 ± 0.49	0.72 ± 0.20	0.54 ± 0.36	0.40 ± 0.19
Microbial C				
C_{micro} ($mgC\ g^{-1}$)	0.004 ± 0.001	0.002 ± 0.001	0.003 ± 0.002	0.001 ± 0.001

Microbial C

Results of the microbial C measured in CON and T plots for the L and F layers are presented in Table 5.8. Using two sample t-test, the CON plots, in both the L and F layers, showed significantly higher C_{micro} concentrations than the T plots (L layer - t_0 1.49; $p \leq 0.1$ and F layer - t_0 1.50; $p \leq 0.1$).

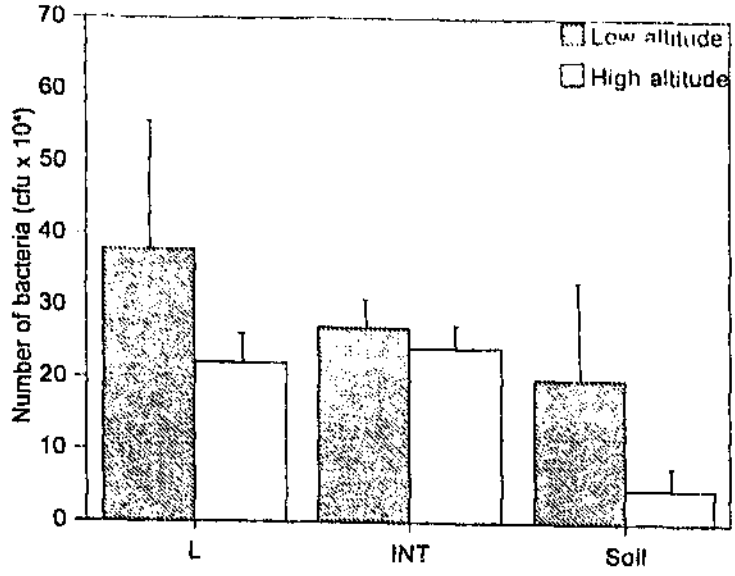


Figure 5.25 Bacterial counts of the litter (L), interface (INT) and soil layers of the forest floor at a low and high altitude site. Standard deviations (+) shown on bars; *cfu* - colony forming units.

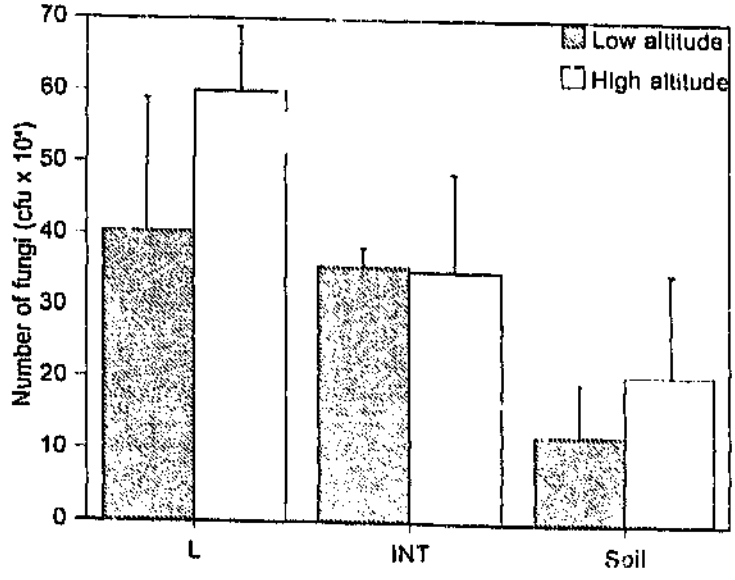


Figure 5.26 Fungal counts of the litter (L), interface (INT) and soil layers of the forest floor at a low and high altitude site. Standard deviations (+) shown on bars; *cfu* - colony forming units.

Table 5.7 Changes in percentage lignin, cellulose and polyphenolics through a litter profile, compared with foliar samples. Mean values of three replicate samples and standard deviations are presented.

Sample	Lignin (%)	Cellulose (%)	Polyphenolics (%)
Foliar	15.30±0.91	25.07±1.16	27.37±0.35
L1	29.86±2.47	28.61±2.77	11.17±0.85
L2	31.17±7.39	24.48±2.77	9.57±0.51
F	29.85±0.33	17.94±2.85	6.30±1.25

Using one-way ANOVA and multiple comparisons, analyses indicated that the foliar lignin concentration was significantly lower than lignin in the litter layers ($F_{3,8}=11.01$; $p\leq 0.003$) and cellulose concentrations were similar in foliar and litter samples but becoming significantly lower in the F layer ($F_{3,8}=4.71$; $p\leq 0.93$). Polyphenolics in the foliar samples were highest and then decreased throughout the litter profile ($F_{3,8}=397.00$; $p\leq 0.000001$).

5.3.4 Soil microbial and soil faunal populations

Examination of litter/soil core samples indicated an absence of soil fauna ≥ 2mm. Results for the microbial plate counts of the various litter/soil layers in a low and a high altitude 25 year-old site are presented in figures 5.25 and 5.26, respectively. ANOVA showed no significant differences between low and high altitude sites with regard to either bacterial or fungal populations. However, generally bacterial numbers decreased down the litter/soil profile and numbers of bacteria tended to be higher in low altitude sites. Fungi showed a general decrease down the litter/soil profile with fungal numbers being higher in high altitude L and soil layers.

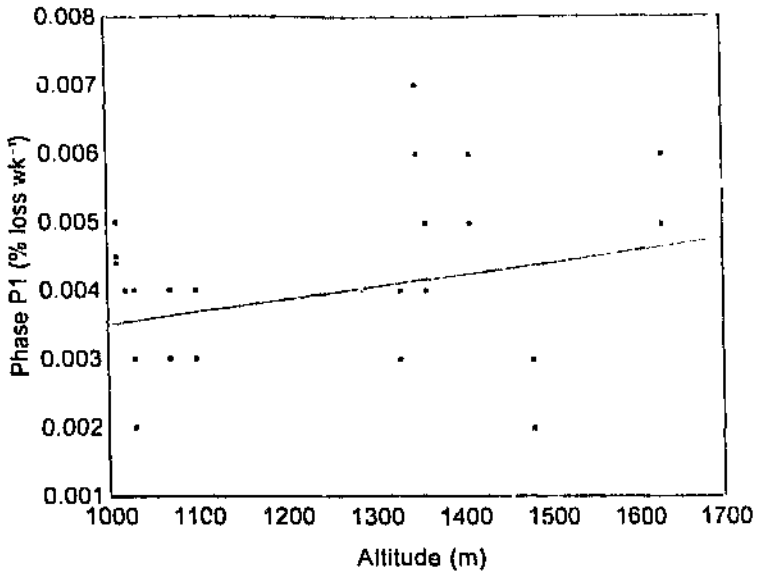


Figure 5.23 Linear regression of P1 loss rate and altitude. $y=0.002+0.000002x$; $F_{1,33}=3.97$; $p\leq 0.05$; $R^2=0.11$.

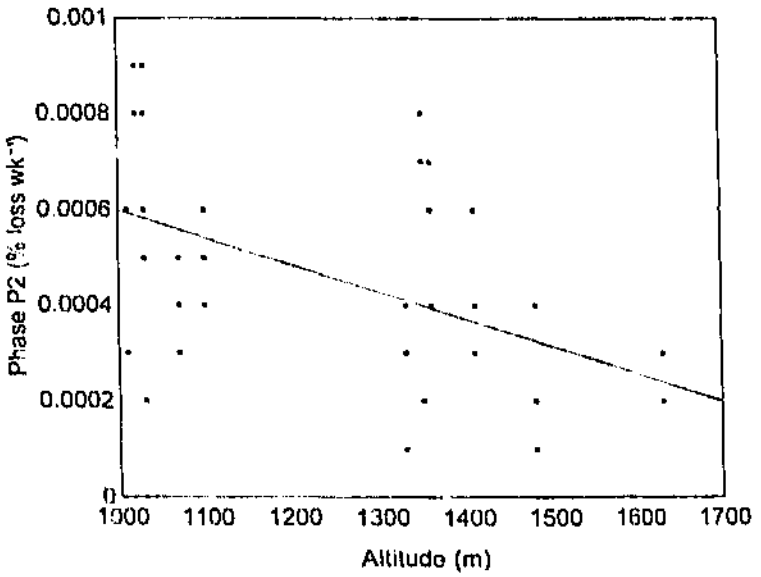


Figure 5.24 Linear regression of P2 loss rate and altitude. $y=0.001-0.000001x$; $F_{1,34}=11.94$; $p\leq 0.002$; $R^2=0.26$.

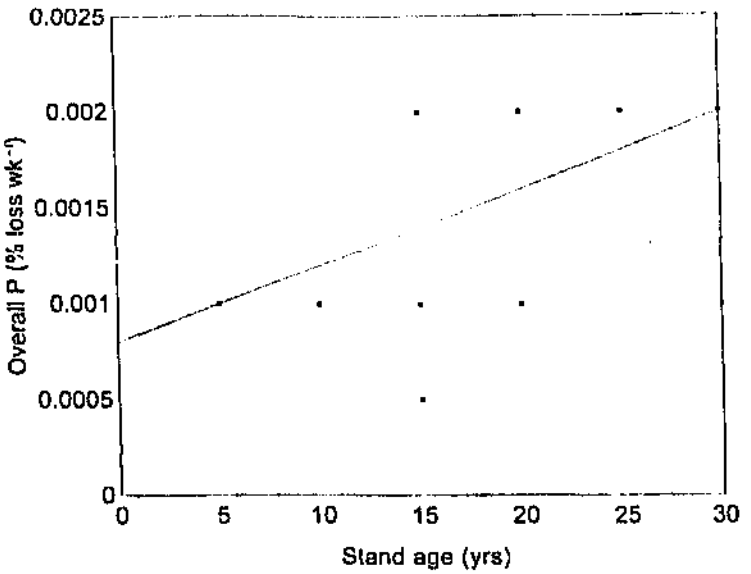


Figure 5.21 Linear regression of overall P loss rate and stand age, several points on the graph coincide. $y=0.001+0.00003x$; $F_{1,33}=10.89$; $p\leq 0.002$; $R^2=0.25$.

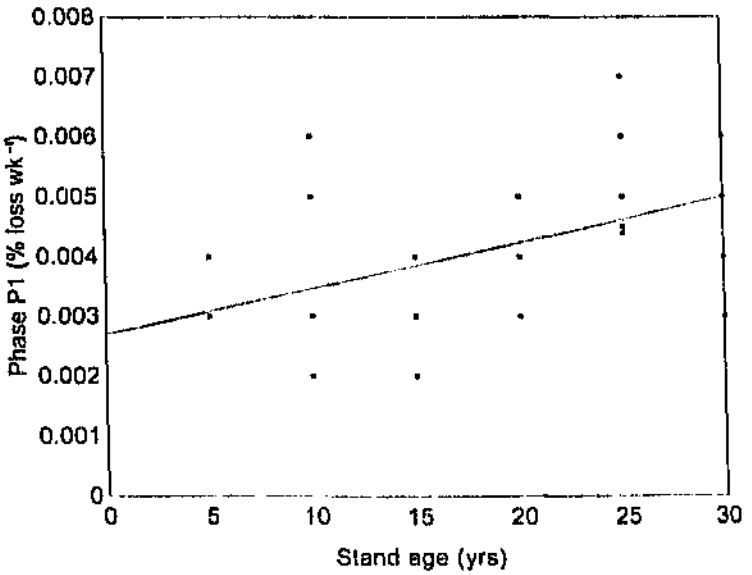


Figure 5.22 Linear regression of P1 loss rate and stand age. $y=0.003+0.00009x$; $F_{1,33}=9.89$; $p\leq 0.003$; $R^2=0.22$.

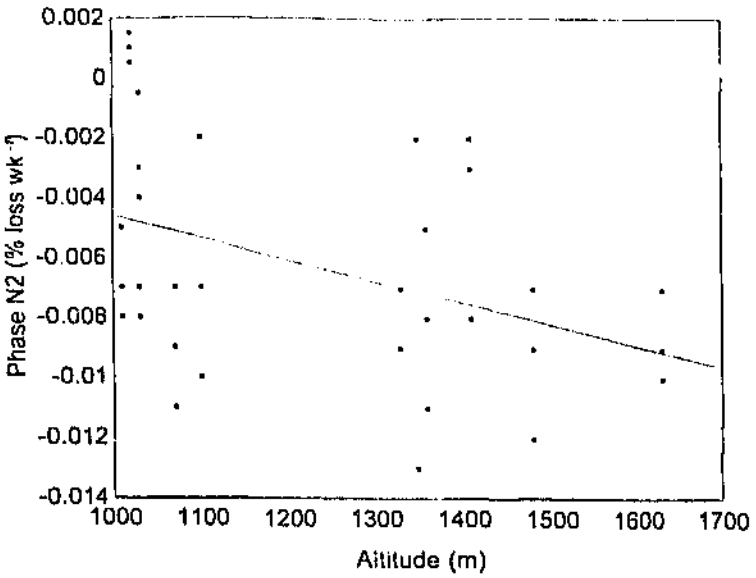


Figure 5.20 Linear regression of N2 loss rate and altitude. $y=0.03-0.00001x$; $F_{1,32}=6.25$; $p\leq 0.02$; $R^2=0.16$.

Regression analysis of (ix) indicated that there were significant positive relationships between the overall P loss rate and stand age (Fig. 5.21), and P loss rate (phase P1) and stand age (Fig. 5.22). This indicates increasing P loss rates with increasing stand age. A significant positive relationship was evident between P loss rate (Phase P1) and altitude (Fig. 5.23), while during phase P2 a negative relationship was evident (Fig. 5.24) which indicates a more rapid loss of P from high altitude sites during the first phase of decomposition which slowed down during the second phase. No relationship was evident when examining overall P loss rate (0-52 weeks) probably due to the masking of the phase P1 and P2 relationships.

The effect of decomposition on lignin, cellulose and polyphenolic concentrations can be seen down a litter profile which represents change over time, litter at the bottom of the profile being older than newly fallen litter at the top of the forest floor. Results from these analyses compared with the concentrations in foliar samples are presented in Table 5.7.

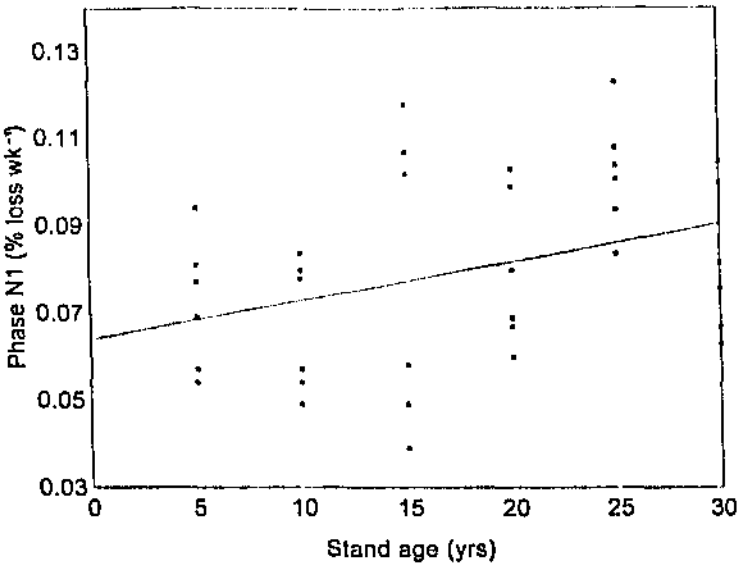


Figure 5.18 Linear regression of N1 loss rate and stand age. $y=0.06+0.0009x$; $F_{1,34}=5.44$; $p \leq 0.02$; $R^2=0.14$.

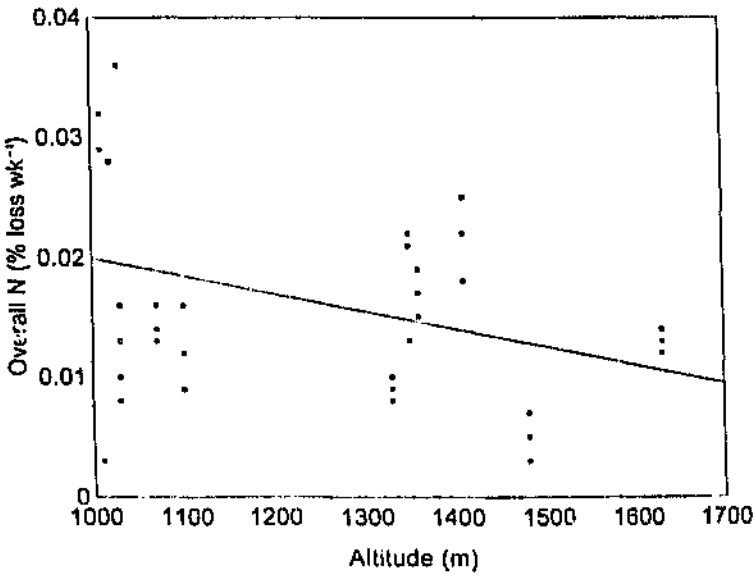


Figure 5.19 Linear regression of overall N loss rate and altitude. $y=0.03-0.00001x$; $F_{1,33}=6.04$; $p \leq 0.02$; $R^2=0.15$.

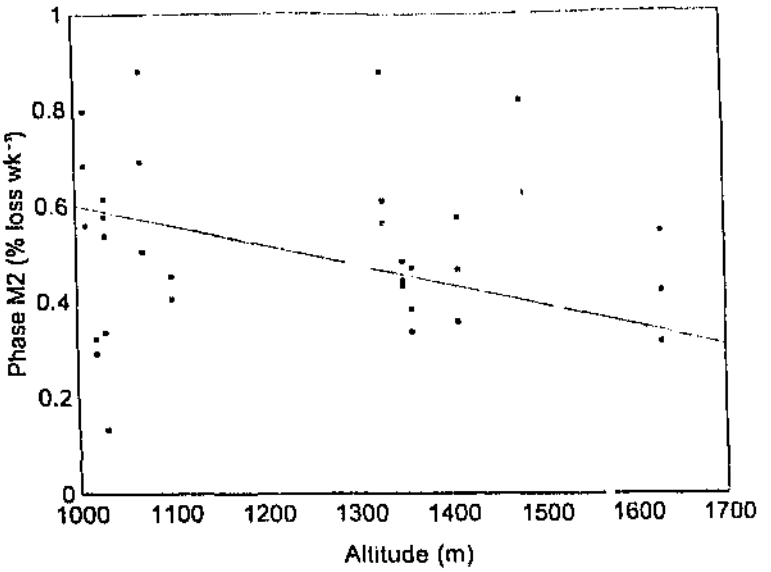


Figure 5.16 Linear regression of M2 loss rate and altitude. $y = 1.02\text{-}0.0004x$; $F_{1,30} = 8.84$; $p \leq 0.01$; $R^2 = 0.22$.

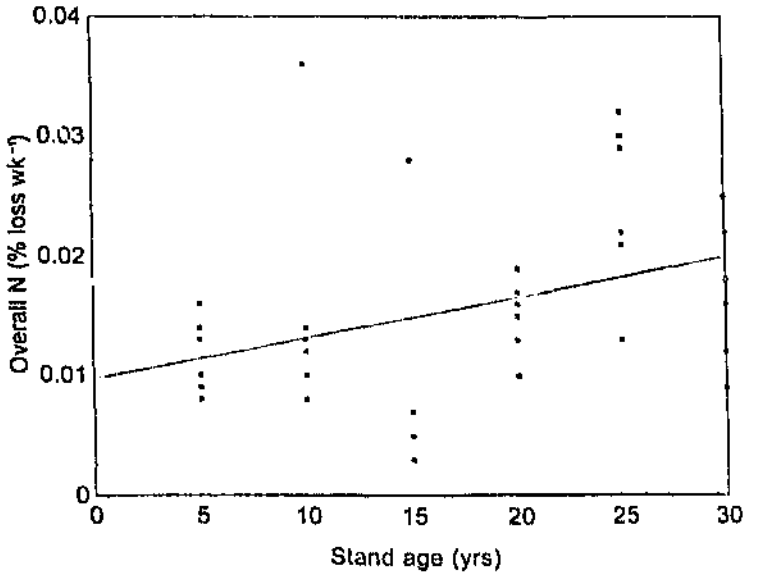


Figure 5.17 Linear regression of overall N loss rate and stand age. $y = 0.01 + 0.0004x$; $F_{1,33} = 6.73$; $p \leq 0.01$; $R^2 = 0.17$.

pH and exchangeable acidity

Soil pH has a controlling influence on the availability of nutrients, as well as the cation exchange capacity and aluminium toxicity. Measurements of pH were taken of the litter and soil samples using a 1M KCl solution (1:5 dilution).

Exchangeable aluminium is the dominant cation associated with soil acidity (pH < 4.5). At higher pH values Al^{3+} is not found in solution but is bonded to clay particles, at lower pH values Al comes off the exchange complexes, going into solution which results in aluminium toxicity (Barnhisel & Bertsch, 1982; Sanchez, 1976).

There is a strong correlation between exchangeable Al^{3+}, pH and decreased plant productivity (Okalebo, Gathua & Woomer, 1993), for this reason exchangeable acidity was determined for the INT and soil layers. Samples were extracted in a 1M KCl solution. Extracts were subsequently titrated with a 0.1M solution of NaOH using phenolphthalein as the indicator (Anderson & Ingram. 1989; Sanchez, 1976).

N mineralization

The potential N mineralization rates of the INT and soil layers were determined using the anaerobic incubation technique. This method provides a rapid index of the labile pool of plant available N, although it does not adequately reflect field mineralization. Fresh INT and soil samples were incubated under waterlogged conditions, for 7 days at 40°C (Time 1) (Waring & Bremner, 1964). Under these conditions organic N is mineralised to NH_4^+-N (Alexander, 1977). The NH_4^+-N produced was then extracted using 0.5M K_2SO_4; this extraction was also conducted on unincubated samples (Time 0). Concentrations of NH_4^+-N, in both Time 1 and Time 0 sample extracts were determined colorimetrically at 620nm using the indophenol method (Dorich & Nelson, 1983).

The indophenol blue reaction, occurs between ammonium, phenol and hypochlorite in an alkaline medium. Indophenol is an amino derivative of phenylquinone-monoamine. The resulting blue colour is similar to that of the indophenol dyes, and its intensity is proportional to the ammonium concentration. The indophenol

6.2.4 Chemical analysis of forest floor and soil components

Total N and P

All litter and soil samples were chemically analyzed for total N and P, using the Kjeldahl procedure followed by specific colorimetric analysis (Anderson & Ingram, 1989; Section 5.2.2; chapter 5).

Resin-extractable available P

Phosphorus occurs in many different forms in the soil, each with different solubilities. The form of P extracted is dependent on the extraction medium. The rate of extraction has been shown to be dependent on the rate of release of P from the soil into an aqueous medium. Studies have shown that anion exchange resin bags behave in a similiar manner to roots and that resin extractable P correlates well with plant P uptake. The method used in this study was one modified by Sibbensen, (1978).

Resin extractable P was determined for litter and soil samples. The concentrations of the extracted P were determined colorimetrically at 700nm, using a visible light spectrophotometer (Okalebo, Gathua & Woomer, 1993). The colorimetric technique used was the ammonium molybdate, ascorbic acid technique where samples are allowed to react with a composite reagent of molybdate, ascorbic acid and trivalent antimony. The molybdic acids formed are converted by reducing agents to a blue-coloured complex, the intensity of which was proportional to the P concentration in the sample extract (Anderson & Ingram, 1989; Murphy & Riley, 1962).

Cations

All litter and soil samples were chemically analyzed for the major cations K^+, Mg^{2+} and Ca^{2+}. Litter samples were digested using a modified Kjeldahl procedure (Anderson & Ingram, 1989; Section 5.2.2; chapter 5). Cations from soil samples were extracted in a 1M NH_4OAc solution which ensured that the maximum exchange occurred between the NH_4^+ and the cations originally occupying the exchange sites on the soil surface. The soil extract and the digested litter samples were diluted with lanthanum chloride and cation determinations were made following the procedure outlined in section 6.2.3 (Knudsen, Petersen & Pratt, 1982; Thomas, 1982).

natural leachate collection. Ten plastic leachate collection trays (0.9m x 0.5m) were placed under the litter layer at the interface of the litter and soil. A section of litter, 1.2m x 1.0m, was cut through with a sharp knife and lifted to facilitate placement of the leachate trays. The trays were positioned at a slight angle to ensure runoff of leachate into plastic collection tubes. The collection tube drained into a dark, 2l plastic collection bottle which was placed below the leachate tray (Anderson & Ingram, 1989). On collection the samples were frozen and returned to the laboratory for analysis. The amount of leachate collected in each bottle and the rainfall during the collection period was recorded.

6.2.3 Chemical analysis of vegetation components

Total N and P

Needle, branchwood, stemwood, bark and root samples were analyzed for total N and P using a modified Kjeldahl procedure followed by specific colorimetric analysis (Anderson & Ingram, 1989; Section 5.2.2; chapter 5).

Cations

Needle, branchwood, stemwood and bark samples were analyzed for the major cations K^+, Ca^{2+}, and Mg^{2+}. To ensure that all the nutrients were brought into solution, samples were first digested using wet oxidation based on the modified Kjeldahl procedure (Anderson & Ingram, 1989; Section 5.2.2; chapter 5).

Digested solutions were diluted with lanthanum chloride. This was added as a releasing agent to prevent the formation of refractory compounds, which could interfere with the determination (Okalebo, Gathua & Woomer, 1993). Cation concentrations were determined using an atomic absorption spectrophotometer (Knudsen, Petersen & Pratt, 1982; Thomas, 1982).

these branches mature needles from the previous seasons growth were collected. Cross sections of the branches were taken, for branchwood samples Stemwood samples were taken using an increment borer (diameter 10mm) and bark samples were also collected from the sample trees. All samples were oven dried at 60°C for 72hr. Needle samples were crushed and put through a 2mm sieve. Branchwood, stemwood and bark samples were ground in a Fritsch sample mill.

Forest floor and soil components

Thirty litter/soil core samples were collected from the forest floor using a stainless steel, 1m long, soil corer (internal diameter, 8cm). Three samples were taken from underneath each sample tree approximately 1.0m from the base of the tree. The soil corer was hammered through the litter into the soil, and down to the shale bedrock. Upon removal of the corer, samples were placed in plastic sleeves and sealed. Cores were returned to the laboratory and divided into litter and soil layers. The depth of each layer was recorded. The litter/soil layers were as follows;

- L1, newly fallen litter lying loosely on the forest floor;
- L2, thick mat of litter, tightly interwoven with ectomycorrhizal (ECM) fine roots;
- F, fermentation layer, interwoven with ECM roots but being looser than the previous layer;
- INT, the interface layer between litter and soil;
- Soil layer to the shale.

The respective layers of the three litter/soil samples, for each tree, were bulked to provide sufficient material for analysis. Fine roots, with a diameter of $\leq$2mm, were separated from the litter by hand, and from the soil samples by sieving (Nadelhoffer & Raich, 1992). All litter, soil and a portion of the root samples were oven dried at 60°C for 72 hrs. After drying, litter samples were crushed and put through a 2mm sieve. Where finer samples were required they were ground further, using a pestle and mortar, and sieved through a 430μm sieve.

Litter leachate

Leachate from the litter was collected to determine whether nutrients were transported from the litter into the soil. Litter leachate was collected during a five day period in December 1993, the rainfall season, ensuring

nutrient are cycled the following hypotheses were tested:

Hypothesis 6: The fine feeder roots of *P. patula* are distributed mainly within the litter layer (Fig. 2.1; chapter 2).

Hypothesis 7: *P. patula* acquires the majority of its nutrients from the litter layer (Fig. 2.1; chapter 2).

Studies by Morris (1996) indicated that there was a relationship between litter accumulation and decreasing topsoil Ca^{2+} concentrations. The decrease in base cations may have occurred as a result of a greater degree of leaching due to increased rainfall at higher altitudes and/or the inability of the soil to supply cations. Because cations influence microbial activity (Alexander, 1977) and concerns that acid rain in the Mpumalanga escarpment may increase the leaching potential of cations (Carlson, 1992; Olbrich, 1995) the status of the cations, K^+, Ca^{2+} and Mg^{2+}, was examined in the high-litter, high altitude site.

6.2 METHODS

6.2.1 Study site

Nutrient cycling studies were conducted in a 42 year-old *P. patula* compartment in the Brooklands plantation (Table 3.2; chapter 3). The 36ha compartment was planted in 1951 and the trees were last thinned in 1975 to 274 stems per hectare (sph). Studies were conducted within a 1ha area which had a gentle slope and was situated at an altitude of 1350m. The geology of the site was shale, with shallow soils.

6.2.2 Sampling

Vegetation components

Ten trees were randomly selected from within the study area. Sampling was conducted during June as seasonal variation of foliar nutrient concentrations has been shown to be minimized by winter sampling (Payn, Schatz & Clough, 1989). Branches from the upper third of the canopy were selected from each tree and cut. From

6 NUTRIENT CYCLING IN A LITTER ACCUMULATION SITE

6.1 INTRODUCTION

The nutrient supply to an ecosystem is balanced by both inputs and outputs and involves the interaction of the plant community with its environment (Hüttl & Schaaf, 1995; Vitousek, 1984). Nutrients within the system are utilized to increase biomass i.e. net primary production. Nutrient supply may increase as a result of deposition and fertilization or it may decrease as a result of nutrient immobilization, harvesting and increased leaching. Therefore, the conservative use of nutrients is important to ensure that there is sufficient nutrient reserves to allow for the growth of succeeding plant communities (Hüttl & Schaaf, 1995; Olson, 1963).

In managed, even-aged plantations nutrient cycling studies are important in order to ensure sustained productivity of the afforested sites, over successive rotations. Studies by Morris (1986; 1995) have shown reduced growth in second rotation, high altitude *P. patula* plantations when compared with first rotation growth and proposed that this was a result of N deficiencies induced by continued litter accumulation. This raised concerns that litter accumulation in *P. patula* plantations may have adverse affects on the cycling of nutrients and may result in decreased long-term site productivity. This study was designed to determine the nutrient dynamics of a *P. patula* site in which litter has accumulated.

The nutrient dynamics of N and P within the system were examined, as N is regarded as most limiting in forest ecosystems, in addition there is increasing evidence that for a number of forests P is immobilized in the first stages of decomposition to a significantly greater extent than is N (Attiwill & Adams, 1993). Phosphorus levels are also thought to control the economy of other nutrients in the ecosystem. Reports have also suggested that there is a high level of interaction between the N and P cycles (Attwill & Adams, 1993).

To establish a complete nutrient budget for a high litter, high altitude, 42 year-old site the nutrient levels of the forest floor, roots and above-ground components were measured. To further elucidate the route by which

activity. Therefore their presences is not responsible for retarded decomposition rates and ultimately accumulation of litter. **Hypothesis 5** can therefore be rejected. This conclusion confirms the findings of other similar studies which concluded that exclusion of ECM roots did not increase microbial activity or biomass (Dighton, Thomas & Latter, 1987; Harmer & Alexander, 1985). A recent study by Zhu & Ehrenfeld (1996) showed that the presence of *P. rigida* mycorrhizal roots stimulated litter decomposition.

5.5 CONCLUSIONS

The output of nutrients from the litter i.e. the decomposition process is influenced by litter quality. Litter quality studies of *P. patula* have shown that litter N and P increases with increasing stand age, thereby improving litter quality (rejection of **Hypothesis 2**). However, increasing altitude was not accompanied by a change in litter quality (**Hypothesis 3** was rejected). The rate of decomposition, as measured by mass loss, during phase M2, indicated that there is a general decrease in decomposition rate with increasing altitude (accept **Hypothesis 4**). From these results it is evident that in high altitude sites the decomposition process is compromised, resulting in an imbalance between the input and output processes, which is manifested by the accumulation of litter on the forest floor. This decrease in decomposition cannot be attributed to any significant differences in the litter/soil microbial populations between low and high altitude sites, although there are indications that bacterial counts are lower and fungal counts are higher in high altitude sites. This area would require further investigation. There is also no evidence to suggest that the presence of active ectomycorrhizal roots suppresses the decomposition of litter in high altitude sites as a result of competition between saprotrophic micro-organisms and mycorrhizal fungi. It is therefore, probable that abiotic environmental factors such as temperature, rainfall and pH are responsible for the decrease in decomposition observed in the high altitude sites, by way of their influence on microbial activity. These interactions will be discussed further in chapters 6 and 9.

litter in low altitude sites when compared with high altitude sites. However, the present study examined the population at only one point in time and significant differences may become evident if the population were to be monitored regularly over a longer time period. Seasonal differences may also be important as during the drier, winter months or under drought conditions the composition of the microbial community may change with fungi becoming more dominant. Under these conditions the differences between low and high altitude sites may become apparent. The population diversity was not examined in this study and is an area requiring further study.

5.4.4 Microbial activity and biomass

The high variability observed in the soda lime samples (Table 5.8) possibly reflects localised pockets of microbial activity which respond to moisture availability in the litter. To reduce variability would require a bigger sample size. The quantity of CO_2 absorbed by the soda lime in the CON samples (Table 5.8) was higher than values recorded during a pilot study ($5.48 \pm 1.80 mgCO_2\ hr^{-1}$) which was conducted in July 1992. The variability observed during the pilot study was lower, which could be attributed to the drier, winter conditions during which the study was conducted. In this case localised moisture pockets would be less influential.

It was evident from the SIR and C_{micro} experiments that the elimination of active ECM roots resulted in reduced microbial activity and biomass (Table 5.8) which is contrary to the results obtained by Gadgil & Gadgil (1971 & 1975). This decrease may have resulted from the increased moisture content of the trenched plots. Several studies using trenching methods (Gadgil & Gadgil, 1975; Harmer & Alexander, 1985; Staaf, 1986) or microcosm experiments in the absence of roots (Dighton, Thomas & Latter, 1987; Zhu & Ehrenfeld, 1996) have reported an increase in moisture emphasizing the important role of roots in the uptake of water. The increased moisture may have resulted in anaerobic conditions becoming prevalent in the litter layer which are inhibitory to microbial (especially fungal) respiration. Reduced microbial activity may also have resulted from C limitations due to the absence of root exudates and the loss of micro-organisms usually associated with the 'mycorrhizosphere'. Although it is certainly true that there are many competitive and synergistic interactions between saprotrophic micro-organisms and mycorrhizas, the presence of ECM does not suppress microbial

concentrations were similar in foliar, L1 and L2 samples (39%), decreasing to 18% in the F layer (Table 5.7); indicating the presence of cellulolytic activity in this layer. Cellulose concentrations in the litterfall (Table 5.1) were lower (28%) than those measured through the profile but the reason for this difference is unknown. Polyphenolics decreased from 27% in foliar samples to only 6% in the F layer, showing a continual decrease throughout the litter profile (Table 5.6). Polyphenolic concentrations in the litterfall (Table 5.1) were similar (31%) to those measured in foliar samples, the reduction of polyphenols in the litter probably reflects changes in the extractable nature of the phenolic molecules resulting from their polymerization during decomposition (Mc Key, 1979). Many studies have shown that after an initial period during which various compounds are lost, the rate of decomposition decreases and the litter becomes more resistant to breakdown. This is caused by the accumulation of condensed or polymerised polyphenols which may originate from the litter itself or be synthesised by micro-organisms (Singh & Gupta, 1977).

5.4.3 Soil microbial and soil faunal populations

No soil fauna, ≥ 2mm, was recorded in the litter/soil samples. However, this is not conclusive as microscopic fauna *e.g.* Acari (mites) were not examined. Studies by Setälä *et al.*, (1991) and Shaw *et al.*, (1991) have shown that coniferous forests have low total soil faunal biomass when compared with other ecosystem types. The differences in biomass is related to the type of litter layers found. Mull layers are characterised by having a high soil faunal biomass, of which Oligochaetes (earthworms) are dominant. Mor layers have low soil fauna biomass, dominated by Acari (mites) and Enchytraeida (potworms) (Setälä, *et al.*, 1991; Shaw *et al.*, 1991).

From the soil microbial study no significant differences in the levels of bacterial and fungal populations were apparent between the low (Fig. 5.25) and the high altitude sites (Fig. 5.26) examined although several trends were apparent. These trends included a general decrease in both bacterial and fungal numbers down the litter/soil profile with higher bacterial counts occurring in low altitude sites, and higher fungal counts being measured in the L and soil layers of the high altitude sites. The lack of significance in these differences may be a result of the high variability observed. Studies conducted in India by Kshattriya, Sharma & Mishra (1992) found that fungal and bacterial counts and invertase, amylase and cellulase enzyme activity were greatest on

old low altitude stands and in the 10, 20 and 30 year old, high altitude stands. During Phase N2, N was immobilized at approximately the same rate in all stands, in low altitude 15 year old stands an overall loss of N was apparent, although this loss was very small.

Trends were evident between the rate of N loss and stand age showing an increase in N loss as the stand ages, when examining both overall N loss (Fig. 5.17) and N loss during phase N1 (Fig. 5.18). Therefore, as the stand ages, N is lost faster during the first 13 weeks due to the higher initial N concentrations (Fig. 5.2). The immobilization of N both overall and during phase N2 was also shown to be related to altitude, with N becoming increasingly immobilized in the higher altitude sites (Figs. 5.19 & 5.20). Immobilization of N, indicates a N deficiency which ultimately affects microbial activity, resulting in reduced decomposition of litter. The majority N would remain bound in organic form with only a limited amount of N being made available for plant uptake.

The changes in litter P were also monitored during decomposition and two phases, phase P1 and P2, representing a continual loss in P were evident (Figs. 5.12 & 5.13). The mean percentage P lost during the 52 week period, calculated regardless of stand age in low and high altitude sites, was $88 \pm 4\%$ and $75 \pm 16\%$, respectively. Analysis revealed that the rate of P loss (Phase P1) was faster from older stands, this may be attributed to the increase in initial litter P concentrations with stand age (Fig. 5.3), this was further substantiated by the regression analysis (Fig. 5.22). Differences in P loss related to altitude can also be linked to higher initial P concentrations (Table 5.5) in the high altitude 10, 25 and 30 year-old stands. Regression analysis indicated a more rapid loss of P from high altitude sites during phase P1 (Fig. 5.23) which slowed down during the second phase (Fig. 5.24), as a result of P becoming limiting.

The litter core samples collected were assumed to represent current litterfall at the top of the profile, ageing down the profile to ± 20 years (half the stand age; Chapter 6). Lignin concentrations in the litter layers (30%) was twice that of the foliar lignin (15%) (Table 5.7) with the concentration in the litterfall being 21% (Table 5.1). These trends highlight the recalcitrant nature of lignin which would adversely affect the production of microbial enzymes, resulting in reduced decomposition rates (Kshattriya, Sharma & Mishra, 1992). Cellulose

the low altitude site which may have contributed to faster mass loss during Phase M1, resulting in a N deficiency during phase M2, hence the slower mass loss rates. Faster mass loss rates from 10 year old stands in low altitude sites (Phase M1) may be attributed to pruning and thinning operations. These operations would open up the forest canopy resulting in improved air circulation and warmer temperatures on the forest floor, which would contribute to increased decomposition. Slower mass loss rates were evident in the low altitude 30 year-old stand (Phase M1) which can also be attributed to the higher initial litter N concentrations in the corresponding high altitude stand (Table 5.5).

No significant relationships were evident between the decomposition rate constants or mass loss rate (Phase M1) and either stand age or altitude. Large variability in the mass loss data mask any significant trends (Figs. 5.6 & 5.7). However, the mass loss rate during phase M2 was shown to decrease with increasing altitude (Fig. 5.16). This relationship is significant when considering that litter accumulation has been shown to increase with increasing altitude (Fig. 4.10; chapter 4). From this relationship which shows a decrease in decomposition with increasing altitude and from the generally lower decomposition constants in high altitude sites presented in Table 5.6, **hypothesis 4** can be accepted, the litter decomposition rate does show a general decrease with increasing altitude.

The changes in litter N were monitored during decomposition and two phases were evident, phase N1 represented a loss in N while phase N2 showed an accumulation of N in the litter, indicating that N becomes immobilized (Figs. 5.8 & 5.9). The mean percentage N lost during the 52 week period, calculated regardless of stand age in low and high altitude sites, was $51 \pm 12\%$ and $40 \pm 14\%$, respectively. ANOVA and multiple comparisons of the N loss rate data (Phase N1) revealed that N was lost faster from older stands which may be attributed to the increase in litter N concentrations as the stand ages (Fig. 5.2). Comparisons between low and high altitude stands revealed significant differences with faster N loss rates from 5, 15 and 25 year old low altitude stands and slower rates in the 10, 20 and 30 year old low altitude stands. These differences could be linked to higher initial litter N concentrations (Table 5.5) in the 5, 15 and 25 year

the duration of phase 1 is variable, a factor which is dependent on the frequency of sample collections. The overall loss of mass was further emphasized when examining the decomposition rate constants (k) which were all negative (Table 5.5). Slower decomposition rates were evident in the 5, 10, 20 and 25 year old, high altitude sites when compared with the corresponding low altitude sites. The reason for the slow decomposition rate in the low altitude, 30 year old stand is not clear, but may be related to the sites south-east aspect.

Table 5.10 Annual percentage mass loss from litter for various *Pinus* species.

Species / Location	Mass loss ($\% yr^{-1}$)	Reference
P. patula/ Mpumalanga	34	This study
P. patula / India	13	Singh, 1982
P. sylvestris / England	12	Singh & Gupta, 1977
P. sylvestris / Sweden	20-36	Berg & Staaf, 1980
P. sylvestris / Finland	34	Smolander *et al.*, 1996
P. alba / Tennessee, USA	40	Singh & Gupta, 1977
P. taeda / Tennessee, USA	44	Singh & Gupta, 1977
P. radiata / New Zealand	50	Gholz *et al.*, 1985
P. contorta / Wyoming, USA	18-23	Fahey, 1983
P. elliottii / Florida, USA	13-17	Gholz *et al.*, 1985

ANOVA and multiple comparisons conducted on mass loss rate data (Phases M1 & M2) revealed that rate of mass loss was not linked to stand age, confirming studies by Berg & Staaf (1980). However, significant differences in the rate of mass loss were evident between some stands of the same age at low and high altitudes. Faster mass loss rates were evident in 15 year old stands (Phase M1) at low altitudes followed by slower mass loss rates during Phase M2. This can be attributed to the higher initial litter N concentrations (Table 5.5) in

The stemwood biomass was the largest component in the aboveground component (excluding the forest floor), followed by branchwood, needle and bark biomass. The average litter depth was 217.00 ± 3.41mm, which was converted to a litter mass of 143 000kg ha^{-1} (Fig. 4.11; chapter 4). This figure would include the root biomass, as the majority of roots are distributed in the litter. The biomass of the litter minus the root biomass (125 475kg ha^{-1}) therefore forms a significant portion of the forest ecosystem, equal to that of the stemwood.

6.3.7 The nutrient cycle

Using the mean nutrient values from tables 6.1, 6.2 and 6.3 and the biomass figures presented in table 6.5, it was possible to determine the size of the nutrient pools. The soil nutrient pools were calculated using the formula;

$$\frac{NC}{100} \times BD \times D \qquad \text{(Equation 6.9)}$$

where, NC = nutrient concentration converted to mg kg^{-1}

BD = bulk density in kg m^{-3}

D = soil depth in m

(Schutz, 1990).

The bulk density of the soil was taken to be 1.18g cm^{-3} (1180kg m^{-3}) (Morris, 1986). The soil depth measured at this site was 9cm, however, because of the high exchangeable acidity, low pH and limited distribution of roots within the soil, nutrient pool figures are presented only for the top 1cm.

Nutrient cycle diagrams are presented for the N (Fig. 6.1) and P (Fig. 6.2) cycles. Percentages shown respresent the contribution made to a particular nutrient pool (i.e. 48% of the tree N pool is found in the needle component (Fig. 6.1). The distribution of the cations, K^+, Mg^{2+} and Ca^{2+}, in the biomass components are presented in Table 6.6. The highest K^+ content is found in the L2 layer. Stemwood followed by the L2 layer had the highest Mg^{2+} content, with Ca^{2+} being highest in the soil layer. Of the cations, in the tree pool, Mg^{2+}

6.3.5 Root distribution and mycorrhizal percentage

No roots were found in the L1 layer. The majority (40%) of the fine roots occur in the L2 layer, followed by 31% in the F layer, 26% in the INT and 3% in the soil. There were significantly less roots within the soil layer ($F_{3,8}$=6.40, $p \leq 0.02$). The highest percentage of mycorrhizal roots occurred in the L2 layer, although differences in the mycorrhizal percentage between the L2, F, INT and soil layers were not significant. Approximately, half of the roots occurring in the L2 (53%) and F (48%) layers and a significant proportion of roots occurring in the INT (41%) and soil (36%) layers were ectomycorrhizal.

6.3.6 Above- and belowground biomass

The DBH, height and crown height of the study site were measured as 40.73 ± 5.23cm, 24.6 ± 2.28m and 9.61 ± 2.06m, respectively. The biomass of the above- and belowground components are presented in Table 6.5.

Table 6.5 Biomass of the various above- and belowground components including the forest floor.

Component	Biomass (kg ha^{-1})
Needle	14 250
Stemwood	125 000
Branchwood	21 000
Bark	6 940
Litter	143 000
Roots	17 525

6.3.4 Nutrient content of the leachate

The average amount of leachate collected was 1717ml, during a period which received 37.1mm of rainfall. The nutrient content of the leachate is presented in Table 6.4. The cation K^+, was found in the highest concentration, followed by Mg^{2+} and Ca^{2+}. This confirms the highly mobile nature of K^+, which is readily leached from plant material (Olbrich, 1995; Zamierowski, 1975). Total and inorganic NH_4^+-N were found in higher concentrations than the total and inorganic P. The pH of litter leachate was found to be more acidic than the underlying soil layer (Table 6.2).

Table 6.4 Nutrient content of the leachate. Mean values of 10 replicates and standard deviations are presented.

Leachate analysis	$\mu g\ ml^{-1}$
N	0.79 ± 0.17
P	0.23 ± 0.12
Inorg. NH_4^+-N	0.24 ± 0.17
Inorg. P	0.18 ± 0.12
K^+	31.60 ± 6.53
Ca^{2+}	6.05 ± 1.87
Mg^{2+}	9.88 ± 1.39
pH	3.88 ± 0.15

Litter pH was found to be 3.26 in the L1 layer, decreasing to 2.16 in the F layer (Table 6.2). Significant differences in the pH between the litter/soil layers were evident ($F_{4,38}$ 135.07, $p \leq 0.00001$), with pH in the L1 layer being higher than that of the L2 and F layers. The pH of the INT layer was similar to that of the L1 layer, with the pH of the soil being higher. The litter layers were more acidic than the soil. The high exchangeable acidity of the INT and soil layers (Table 6.2) indicates the presence of aluminium in the soil solution.

6.3.3 Nutrient content of the root component

No roots were found in the L1 layer, i.e. newly fallen litter. The N and P values for roots within the litter and soil layers are presented in Table 6.3. No significant differences were evident between the roots occurring in the L2, F, INT and soil layers with regard to either N or P concentrations.

Table 6.3 Nutrient content of the root component. Mean values of 10 replicates and standard deviations are presented.

Component	N (%)	P (%)
L1	-	-
L2	1.13 ± 0.28	0.12 ± 0.03
F	1.08 ± 0.16	0.08 ± 0.004
INT	1.04 ± 0.28	0.09 ± 0.01
Soil	0.90 ± 0.22	0.09 ± 0.02

Table 6.2 Nutrient content of the litter and soil components. Mean values of 10 replicates and standard deviations are presented.

Component	N (%)	N-min $\mu gN\ g^{-1}d^{-1}$	P (%)	Res.Ex.P (%)	K^+ (%)	Ca^{2+} (%)	Mg^{2+} (%)	pH	Ex. acid. $cmol^+\ kg^{-1}$
L1	1.12 ±		0.07 ±	0.006 ±	0.70 ±	ud*	0.62 ±	3.26 ±	
	0.53		0.01	0.001	0.30		0.14	0.09	
L2	1.32 ±		0.09 ±	0.006 ±	0.99 ±	ud	0.48 ±	2.30 ±	
	0.23		0.01	0.001	0.32		0.03	0.04	
F	1.24 ±		0.08 ±	0.003 ±	0.09 ±	ud	0.29 ±	2.16 ±	
	0.20		0.01	0.001	0.03		0.09	0.08	
INT	0.67 ±	4.35 ±	0.07 ±	0.001 ±	0.21 ±	0.07 ±	0.04 ±	3.29 ±	11.22 ±
	0.42	1.68	0.02	0.001	0.05	0.02	0.01	0.36	2.82
Soil	0.49 ±	1.66 ±	0.08 ±	0.001 ±	0.19 ±	0.04 ±	0 03 ±	4.08 ±	5.70 ±
	0.30	0.94	0.01	0.0001	0.05	0.01	0.004	0.23	2.58

* ud, undedected

6.3.2 Nutrient content of the litter and soil components

The nutrient contents of the litter/soil components are presented in Table 6.2. The litter generally had a higher nutrient content than the soil. Litter N was found in higher concentrations than litter P, and of the cations, Mg^{2+} and K^{+} were found in fairly large quantities while Ca^{2+} was undetectable within the litter layers. Significant differences in the distribution of N throughout the litter/soil layers were evident ($F_{8,81}=42.54$, $p \leq 0.0001$), which can be attributed to the significantly lower N content in the INT and soil layers when compared with those found in the litter layers above. The percentage N content of the L1, L2 and F layers were not significantly different from that found in the needles (Table 6.1).

Significant differences were evident in the distribution of P in the litter/soil layers and when compared to needle P ($F_{8,81}=110.74$, $p \leq 0.0001$). The percentage P in the L1 layer was significantly lower than the needle P content (Table 6.1), indicating a retranslocation of P prior to litterfall. The percentage litter P of the F layer was not significantly different from that of the INT and soil layers. Significant differences were evident in the distribution of resin-extractable P in the litter/soil layers ($F_{4,44}=35.06$, $p \leq 0.00001$) with more available P in the L1 and L2 layers and half of this quantity in the F layer; this decreased further in the INT and soil layers. The ratios of total P to resin-extractable P throughout the litter/soil profile increased from 12:1 in the L1, 15:1 in the L2, 27:1 in the F, 70:1 in the INT and 80:1 in the soil layers. No significant differences were evident between the K^{+} concentrations of the needles and the litter layers, however; the Mg^{2+} concentration of the needles was significantly higher than that of the litter layers.

The levels of K^{+} were concentrated in the L1 and L2 layers while there was a progressive decrease in Mg^{2+} from the L1 layer through to the soil.

The concentration of soil N was greater than soil P, and of the cations, K^{+} was found in higher concentrations than either Ca^{2+} or Mg^{2+}. Using a two sample t-test, no significant differences were found between the N mineralization rates of the INT and soil layers ($t_4=0.51$, $p \leq 0.62$).

6.3 RESULTS

6.3.1 Nutrient content of the vegetation components

Of the vegetation components needle foliage had the highest percentage concentration of all the nutrients with N being the major nutrient (Table 6.1). This component, being photosynthetically active, requires an adequate supply of nutrients, especially N, to be effective. Bark samples had higher N concentrations than either branchwood or stemwood, although this difference was not statistically significant. This difference may be attributed to the microbial population, including lichens, supported on the bark surfaces. There were no significant differences in the distribution of either P, or the cations Mg^{2+} and Ca^{2+}, between the stemwood, branchwood and bark components. However, K^{+} concentrations in the stemwood were lower than in either the branchwood or bark.

Table 6.1 Nutrient content of the vegetation components. Mean values of 10 replicates and standard deviations are presented.

Component	N (%)	P (%)	K^{+} (%)	Ca^{2+} (%)	Mg^{2+} (%)
Needles	1.67 ± 0.17	0.15 ± 0.02	0.84 ± 0.17	0.21 ± 0.19	1.06 ± 0.09
Stemwood	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.01 ± 0.001	0.30 ± 0.08
Branchwood	0.05 ± 0.03	0.02 ± 0.01	0.28 ± 0.07	0.05 ± 0.03	0.34 ± 0.08
Bark	0.13 ± 0.05	0.02 ± 0.005	0.45 ± 0.26	0.04 ± 0.02	0.21 ± 0.04

Aboveground biomass is presented as kg ha^{-1} and was based on 274 stems per hectare (sph).

6.2.10 Belowground biomass

Root mass was determined using the following equation;

$$\text{Rootmass (kg)} = 2.75 + 0.0015x \qquad \text{(Equation 6.8)}$$

where, x = DBH^2 (cm) x height (m)

(Morris, 1986).

Belowground biomass is presented as kg ha^{-1} and was based on 274 sph.

6.2.11 Data analysis

Although the nutrient contents of the different components were statistically analyzed, using one-way ANOVA, it must be noted that all samples were collected from within one compartment therefore, samples were pseudo-replicated and were not independent from each other. ANOVA was conducted to determine whether there were any differences in nutrient content between the various components. Where significant differences were evident multiple range tests, using least significant differences, were conducted (Sokal & Rohlf, 1981; Zar, 1984). Data on N mineralization were analyzed using a two sample t-test. All analyses were carried out using Statgraphics version 5.0.

6.2.9 Aboveground biomass

Models predicting relationships between biomass components and tree dimensions were used to determine the aboveground biomass of the study site. The only models available for *P. patula* were those developed for the species growing in the Natal, Drakensberg (Bosch, 1985). Tree dimensions used in the models were diameter at breast height (DBH, measured in cm @ 1.3m), tree height (m) and crown height (m). Fifty trees within the study site were measured. Height measurements were taken with a Blum Liess Hypsometer and DBH measurements with a diameter tape. Height measurements were taken to the base of the crown and the top of the tree. The difference in height between the base of the crown and the full tree height was taken as the crown height.

Equations for biomass determinations were as follows;

$$\text{Stem mass (kg)} = 50.782 + 0.104x + 0.0003x^2 \qquad \text{(Equation 6.4)}$$

where, x = DBH (cm) x height (m)

$$\text{Branch mass (kg)} = 9.78 + 0.171x \qquad \text{(Equation 6.5)}$$

where, x = DBH (cm) x crown height (m)

$$\text{Needle mass (kg)} = 4.57 + 0.028x \qquad \text{(Equation 6.6)}$$

where, x = DBH (cm) x crown height (m)

$$\text{Bark mass (kg)} = -35.78 + 1.50x \qquad \text{(Equation 6.7)}$$

where, x = DBH (cm)

(Bosch, 1985).

Percentage ectomycorrhizal roots

While determining root length (Tennant, 1975), ECM colonization was assessed, by recording the number of mycorrhizal root tips intercepting a line. These data were converted to mycorrhizal root length, using equation 6.1. Percentage mycorrhizal root length within a particular layer was determined using the following equation;

$$MR = \frac{ML}{RL} \times 100 \qquad \text{(Equation 6.3)}$$

where, MR = mycorrhizal roots (%)

ML = mycorrhizal root length (cm) within a particular layer

RL = root length (cm) within a particular layer

(Schenck, 1982).

6.2.7 Litter depth

Fifteen randomly selected litter samples were cut from the forest floor, using a 30cmx30cm frame (Fig. 4.3; chapter 4). To convert litter depth measurements to litter mass (t ha^{-1}) the equation presented in figure 4.11 was used (Section 4.3.2; chapter 4).

6.2.8 Litterfall

Litterfall production has been shown not to increase with increasing altitudes, and to increase only gradually after 10 years (Section 4.3.1; chapter 4). To quantify litter production in the study site, the average litterfall data (1992-1994) for the 30 year old *P. patula* compartments was used (Table 4.4, chapter 4).

6.2.6 Determination of root distribution

A subsample of roots from each litter/soil layer was used to determine root length. Root length was determined by using a modified grid line intersect method (Tennant, 1975). Length estimates made by this method are less time consuming than direct measurements. For length estimates a 1cm x 1cm grid, drawn onto a transparency sheet, was placed into a shallow white plastic dish (25cm x 35cm). Water containing the roots was poured into the dish and roots were held in place over the grid with a piece of perspex. The intercepts of the roots, with both vertical and horizontal lines, were counted. A count of one was given if a root crossed a line, if the root end touched the line or if the curved portion of the root touched the line. A count of two was given if the curved portion of the root lay on or along a line. To determine root length the following equation was used;

$$RL = N \times 0.7857 \qquad \text{(Equation 6.1)}$$

where, RL = root length (cm)

N = number of intercepts

(Tennant, 1975).

To determine the percentage of roots distributed within the layers equation 6.2 was used;

$$R = \frac{RL}{RT} \times 100 \qquad \text{(Equation 6.2)}$$

where, R = percentage of roots (%)

RL = root length within a particular layer (cm)

RT = total root length (cm)

colorimetric reaction has been modified by the introduction of a catalyst, nitroprusside, which accentuates the blue colour at room temperature (Dorich & Nelson, 1983).

6.2.5 Chemical analysis of leachate

Total and inorganic N and P

The use of the alkaline persulphate digestion for total dissolved N in water samples was developed by Karoleff in 1969 (Langler & Hendrix, 1982). The method was later modified and combined with an acid persulphate digestion for determination of total dissolved P. The decomposition of persulphate proceeds via two processes. Firstly, by thermal decomposition and secondly by an acid catalyzed decomposition. Both pH and temperature are important factors in the recovery of N and P. The N is oxidized first in the alkaline medium, as digestion proceeds the resulting bisulphate ions lowers the pH, which permits the digestion of the P containing components (Langler & Hendrix, 1982).

Leachate samples were brought to room temperature, and 100ml aliquots were digested with 5 % $K_2S_2O_8$. Flasks were covered with foil and autoclaved at 121°C for 30min (Langler & Hendrix, 1982). Digested and undigested samples were colorimetrically analyzed for N using the indophenol method (Dorich & Nelson, 1983; Section 6.2.4). Digested samples provided determinations of total N and undigested samples, inorganic NH_4^+-N (Harwood & Kühn, 1970; Scheiner, 1976; Wetzel & Likens, 1979). Digested and undigested samples, were colorimetrically analyzed for P using the ammonium molybdate, ascorbic acid technique (Murphy & Riley, 1962; Section 6.2.4). Digested samples provided determinations of total P and undigested samples, inorganic P (Wetzel & Linkens, 1979).

Cations

The major cations K^+, Mg^{2+} and Ca^{2+} were determined by diluting leachate samples with lanthanum chloride and determining concentrations on the atomic absorption spectrophotometer (Knudsen, Petersen, Pratt, 1982; Thomas 1982; Wetzel & Likens, 1979; Section 6.2.3).

as is evident from the exchangeable acidity values (Table 6.2). P-fixing is a process by which available P in acid soils is transformed into relatively insoluble aluminium and iron phosphates thereby reducing the amount of P available for plant uptake (Sanchez, 1976). The P reserves in the 42 year-old, high-litter, high altitude *P. patula* compartment was 246.8kgP ha^{-1}.

Cations

The cation Mg^{2+} serves as constituents of organic structures, especially enzymes, K^+ functions mainly in osmoregulation and the regulation of enzymic reactions and Ca^{2+} provides reversible intermolecular linkages in the cell walls and at the plasma membrane a functions which acts as a control mechanism for growth and development (Marschner, 1995). Complete nutrient cycles for the cations, K^+, Mg^{2+} and Ca^{2+}, were not determined although their distribution throughout various biomass component were examined (Table 6.6). Of these cations, Mg^{2+}, had the highest concentration within the tree pool, accumulating mainly in the stemwood, K^+ accumulated mainly in the litter and soil pools with the highest concentration occurring in the L2 layer. The distribution of Ca^{2+} was low in the tree, litter and soil pools (Table 6.6). Of the total nutrient reserves K^+ was the highest with 1182kg ha^{-1}, followed by Mg^{2+} (996kg ha^{-1}) and Ca^{2+} (93kg ha^{-1}) (Table 6.6).

6.4.8 Nutrient allocation

Allocation of nutrients within the tree pool indicates that nutrients are stored in different components (Fig. 6.1, Fig. 6.2 & Table 6.6)). The major nutrient within the needle component was K^+, followed by N, Mg^{2+}, Ca^{2+} and P in decreasing order. The distribution of nutrients within the stemwood component was Mg^{2+} > N > K^+ > P > Ca^{2+} with maximum amounts of Mg^{2+} and P accumulating in this component. In branchwood the distribution of nutrients was Mg^{2+} > K^+ > Ca^{2+} > N > P with Ca^{2+} being preferentially stored in this component. The distribution of nutrients in the bark component was K^+ > Mg^{2+} > N > Ca^{2+} > P.

Within the forest floor pool, the distribution of nutrients within both the L1 and L2 layers follows the pattern, N > K^+ > Mg^{2+} > P. The pattern changes, with regard to the storage of cations, within the F layer with N > Mg^{2+} > K^+ > P, and the INT layer with N > K^+ > Ca^{2+} > P > Mg^{2+}. The main storage layer for

The litter P pool is approximately 3 times greater than the P pool in the trees (Fig. 6.2), 67% of the P occurring in the stemwood and only 22% occurring in the needle component. A significant proportion (43%) of P is retranslocated from the needles prior to needle fall indicating soil P limitation, leaving 57% of the P to be returned to the forest floor annually via the litterfall. This retranslocation of P indicates that soil P is limiting (Proe & Millard, 1995). The quantity of P retranslocated would be greater in younger stands of *P. patula* as is evident from the litter quality studies which show that litter P concentration increase with increasing stand age (Fig. 5.7, chapter 5). This internal cycling of P suggests a deficiency of P within the system. The pool of P stored in the litter layers represents an accumulation of 26 years of P input, this again assumes that the rate of litterfall is constant over the rotation. The root P pool is approximately half of the tree pool. In calculating the litter and root pools, some double accounting may again have occurred. Results from resin extractable P analyses showed that 0.006% of available P is present in the litter layers (L1 & L2), an amount which is halved in the F layer and decreases further in the INT and soil layers. The annual amount of P mineralized by microbial activity was difficult to determine from this study. It may be assumed that an amount of nutrients equivalent to one years litterfall is mineralized each year from the forest floor (Helmisaari, 1995) indicating a potential annual mineralization of 4kgP ha^{-1}. From litter decomposition studies it was shown that 75% of the litter P was lost from the litter in high altitude sites (Fig. 5.13; chapter 5) reducing the quantity of P mineralized from the litter to 3kgP ha^{-1}. The phosphorus content required for optimal plant growth is 0.3-0.5% of the biomass (Marschner, 1995). The total biomass (Table 6.5) was 184 715kg ha^{-1}, therefore plants would have required between 369-923kgP ha^{-1}. Given the stand age it is possible to calculate that a maximum of 126kgP ha^{-1} could be supplied by litter decomposition assuming that all the P mineralized would be taken up by the roots. This would be an over-estimation as it assumes that litter production is constant as the stand ages, whereas it has been shown that the relationship is curvilinear with a general increase with increasing stand age (Fig. 4.3; chapter 4). From figure 6.2 P reserves in the biomass were lower at 0.03%. Phosphorus, both organic and inorganic were shown to be lost from the litter as a result of leaching (Table 6.4), the annual magnitude of which was difficult to assess. It was estimated that the potential maximum amount of P, both organic and inorganic, lost from the litter would be 0.000003kgP ha^{-1}. This respresents a fraction of the P stored in the litter layers. It is assumed that P lost from the litter through leaching would be immediately taken up by the plant roots in the soil. The soils in the study area are known to be P-fixing soils

g^{-1} d^{-1}, respectively. This respresents N mineralized under optimum conditions. Some studies assume that an amount of nutrients equivalent to one years litterfall is mineralized each year from the forest floor (Helmisaari, 1995) which in this case would amount to 62kgN ha^{-1}. This assumes 100% loss of N from the litter during decomposition however, decomposition studies (Fig. 5.9; chapter 5) revealed that only 40% of the litter N was lost from the litter in high altitude sites. Therefore, it can be estimated that only 25kgN ha^{-1} is made available. The nitrogen content required for optimal plant growth is 2-5% of the biomass (Marschner, 1995). The total biomass i.e. addition of needle, stemwood, branchwood, bark and roots (Table 6.5) was 184 715kg ha^{-1}, therefore plants would have required between 3700-9200kgN ha^{-1}. Given a stand age of 42 years it is possible to calculate that a maximum of 1050kgN ha^{-1} could be supplied by litter decomposition assuming that all the N mineralized would be taken up by the roots. This would be an over-estimation as it assumes that litter production is constant as the stand ages, whereas it has been shown that the relationship is curvilinear with a general increase with increasing stand age (Fig. 4.3; chapter 4). From figure 6.1 the N reserves in the biomass were considerably lower at 0.18%. An average amount of 1717mls of leachate was collected from the leachate trays, during a period which received 37.1mm of rainfall. It is difficult to determine the annual amount of leachate as this would be dependent on the rainfall intensity and the moisture holding capacity of the forest floor at the time of the rain. Nitrogen, both organic and inorganic, was lost from the litter as a result of leaching (Table 6.4). Estimating that annual rainfall is approximately 32 times higher than that collected during the leachate collection period it is possible to calculate the maximum quantity of N lost from the litter. This indicates that there is a potential for 0.00002kgN ha^{-1} (total N) and 0.000004kgNH_4^+ ha^{-1} (inorganic N) to be lost from the litter annually. This represents a fraction of the N stored in the litter layers. It is assumed that N lost during leaching would be immediately taken up by the roots in the soil. The N reserves in the 42 year-old, high-litter, high altitude *P. patula* compartment were 2358kgN ha^{-1} (addition of all N pools).

The P cycle

Phosphate once taken up by plant roots remains in an oxidized form and is essential in the formation of organic compounds particularly nucleic acids, inorganic P is also involved in many enzyme reactions (Marschner, 1995). Because of the importance of this element to plant growth a study of the P cycle was considered essential.

6.4.7 Nutrient cycles

Singh (1982) reported that the nutrient capita of N and P, in *P. patula* stands in India, are distributed in descending order, among the soil, tree and forest floor pools. However, from this study it is evident that the nutrient capita is mainly distributed in the forest floor pool followed by the soil and tree pools.

The N cycle

There are two sources of inorganic N taken up by plant roots nitrate and ammonium. NO_3^- is mobile in the xylem and can be stored in vaculoes, NH_4^+ is immediately incorporated into organic compounds, such as amino acids and amides, in the roots (Marschner, 1995). Plants growing in acid soils *e.g. P. patula* have a preference for NH_4^+ rather than NO_3^- (Marschner, 1995) for this reason nitrate was not examined for the nutrient cycling study.

The N pool in the litter layers is approximately 10 times greater than the N pool in the trees (Fig. 6.1), with 48% of the N in the tree pool being stored in the needle component. Eighty-seven percent of the needle N is returned to the forest floor annually via the litterfall which means that only a small proportion (13%) of the foliar N is retranslocated prior to litterfall. The quantity of N retranslocated would be greater in young stands of *P. patula* as is evident from the litter quality studies which show that litter N concentrations increase with increasing stand age (Fig. 5.1; chapter 5). The quantity of N stored in the litter pool represents an accumulation of 23 years of N input, over half the stand age (42 years). This figure may be an over-estimation as it is based on the assumption that the rate of litterfall remains constant throughout the rotation. However, litter production shows a curvilinear relationship with stand age (Fig. 4.3; chapter 4). The root N pool is greater than the tree pool. In calculating the litter and root pools, some double accounting may have occurred as a result of the intimate association of the roots with the litter which resulted in the roots being difficult to remove from the litter. The annual amount of N mineralised by microbial activity was difficult to determine from this study, as the quantity would be dependent on temperature and moisture conditions throughout the year. Without adequate sampling throughout the year the annual N mineralized cannot be estimated. Results from mineralization studies did, however, show that the INT and soil layers mineralized 4μgN g^{-1} d^{-1} and 5μgN

(1982) reported a DBH of 44.58cm and height of 23.60m for 34 year old *P. patula* in India, values which are similar to those reported in this study. Root mass figures (Table 6.5) calculated using an equation from Morris (1986) may have overestimated the belowground biomass in this study site as the equation was derived from studies of younger trees grown without thinning (1330 sph). Under these circumstances the tree roots may be more compact as they compete for space and nutrients with neighbouring trees. In this study site the trees have been thinned to 274 sph, allowing the roots to spread.

6.4.6 Nutrient reserves

Schutz (1990) calculated the nutrient reserves in the litter and soil of two *P. patula* compartments having litter depths of 10.4cm and 35cm, respectively. These values are presented in Table 6.8 for comparison. Nitrogen reserves in the soil pool were not calculated in Schutz's study. The thickness of the litter in this study site was 21.7cm (217mm), which is intermediate to the two sites shown in Table 6.8. The N reserves in the litter pool in this study (Fig. 6.1) were intermediate to the two values given by Schutz (1990). Phosphorus (Fig. 6.2) and Ca^{2+} (Table 6.6) reserves were lower, while Mg^{2+} and K^{+} reserves were higher in this study than those reported by Schutz (1990) (Table 6.8). In the soil pool the P (Fig. 6.2), Mg^{2+} and K^{+} reserves were higher in this study and Ca^{2+} (Table 6.6) was within the range reported by Schutz (1990) (Table 6.8).

Table 6.8 Nutrient reserves for the litter and soil pools calculated for *P. patula* compartments with accumulated litter depths of 10.4cm (Compartment A) and 35cm (Compartment B), respectively (Schutz, 1990).

Nutrient Pools	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Mg (kg ha^{-1})	Ca (kg ha^{-1})
Litter					
Compartment A	1045	130	82	65	229
Compartment B	2294	381	218	54	272
Soil					
Compartment A		9	54	27	62
Compartment B		2	15	7	2

6.4.4 Root and mycorrhizal distribution

Schutz (1990) showed that there was a correlation between thick litter layers and rooting density in the litter, indicating that thicker litter layers had greater root colonization, occurring in the lower part of the litter layer. Rooting was, however, only assessed visually. In this study 71% of the roots were found in the L2 and F layers. The localization of roots in the litter layers can be attributed to several factors for example, increased rooting volume as litter accumulates; more favourable moisture conditions in the litter layer; nutrient deficiencies in the soil caused by increased nutrient immobilization in the litter; Fe and Al toxicity which inhibits root growth in the soil and reduced soil temperatures (Majdi & Persson, 1995; Schutz, 1990; Vogt *et al.*, 1983).

Approximately half of the roots within the litter layers and soil were ECM (Section 6.3.5); this association was accompanied by the presence of emanating hyphal threads and rhizomorphs which increase the absorption potential of the roots. Because of the proliferation of ECM fine roots, turnover is expected to be slow as mycorrhizal infection prolongs the function of fine roots (Majdi & Persson, 1995). In assessing nutrient pools no attempt was made to quantify the contribution of ECM associations to the nutrient reserves. Inadvertently, nutrient reserves within the hyphal threads and rhizomorphs would have been assessed in the litter, soil and root pools, mainly as a result of these components being difficult to remove from the samples. However, no attempt was made to evaluate the nutrient reserves in the fungal structures such as sporocarps as a result the nutrient budgets presented here would be underestimated.

6.4.5 Biomass

The DBH measurements reported for *P. patula* in the Natal Drakensberg by Bosch (1985) were similar at 40.78cm, while height (22.57m) and crown height (8.77m) measurements were less than those reported in this study (Section 6.3.6). Trees measured by Bosch (1985) were younger than those in the study site and this may account for the height differences. The biomass of the tree components (Table 6.5) were similar with the exception of branchwood mass which was considerably less in the younger trees of the Drakensberg. Singh

6.4.2 Nutrient content of the litter and soil components

Schutz (1990) reported similar litter N and higher litter P concentrations (double) (Table 6.7) than those reported in this study (Table 6.2). In Morris (1986) the N and P concentrations in the the L and F layers were similar to those reported here. The concentrations of the cation K^+, in the L1 and L2 layers were considerably higher (7-8 times higher) than those reported in either Morris's or Schutz's study. The concentrations of Mg^{2+} in the L1 to F layers were also higher (3-7 times higher) than those reported by Morris (1986) and Schutz (1990). Calcium was only detected in the INT layer in this study and was lower than values from Morris's and Schutz's studies (2-4 times higher).

The pH of the litter was within the lower range (2.25 - 5.15) reported by Schutz (1990). The pH of the soil layer was slightly lower than pH values reported for first rotation stands by Morris (1986) which may indicate that as litter accumulates over time pH levels decrease, probably as a result of the accumulation of organic acids. This increase in protons may contribute to soil acidification, although this process is controlled by the buffering capacity of the soil which prevent sharp changes in pH (Sanchez, 1976). A build up of protons may also result in: a decrease in the uptake of nutrients which depends on H^+ extrusion from the roots; a loss of base cations; a reduction in the cation exchange capacity of the soil and an increase of Al^{3+} in the soil solution (Du Toit, 1993). Aluminium displaces cations from adsorption sites such as cell walls and membranes and therefore influences the nutrient supply to the plant (Kuhn, Bauch & Schröder, 1995).

6.4.3 Nutrient content of root component

Morris (1986) reported root N and P concentrations to be 0.15%-0.30% N and 0.01%-0.04% P which were considerably lower than the values reported in this study. Morris did not examine roots within the various litter/soil layers, as was done in this study. The time of root sampling may be relevant, in this study roots were sampled only once, during the active growing season. Ideally roots should be monitored throughout the year to adequately assess biomass, nutrient concentrations and uptake.

Table 6.7 Comparison of nutrient concentrations for various vegetation and litter components reported for *P. patula* plantations. L represents the litter layer; F represents the fermentation layer.

	Morris (1986, 1992)					Schutz (1990)	
Nutrients	Needles	Stemwood	Branchwood	Bark	Litter	Needles	Litter
N (%)	1.55±0.18	0.11±0.03	0.28±0.05	0.52±0.20	L 1.36±0.19 F 1.30±0.19	2.15	1.28±0.16
P (%)	0.13±0.02	0.02±0.01	0.04±0.01	0.05±0.02	L 0.07±0.01 F 0.06±0.01	0.18	0.16±0.01
K^+ (%)	0.83±0.11	0.08±0.05	0.25±0.09	0.53± 7	L 0.11±0.03 F 0.07±0.03	0.87	0.10±0.05
Ca^{2+} (%)	0.30±0.15	0.05±0.02	0.14±0.05	0.23±0.05	L 0.32±0.08 F 0.16±0.06	0.26	0.28±0.21
Mg^{2+} (%)	0.18±0.03	0.01±0.003	0.07±0.03	0.13±0.05	L 0.15±0.02 F 0.08±0.03	0.17	0.08±0.05

6.4 DISCUSSION

6.4.1 Nutrient content of vegetation components

Nutrient concentrations in various vegetation and litter components reported for *P. patula* in studies conducted by Morris (1986; 1992) and Schutz (1990) are presented for comparison in Table 6.7. Morris (1986; 1992) reported higher N concentrations in branchwood (6 times higher), bark (4 times higher) and stemwood (4 times higher), but similar concentrations in the needles. The P concentrations reported by Morris (1986; 1992) in the needles and stemwood were similar to those reported in this study (Table 6.1), while P concentrations in the branchwood (double) and bark (2.5 times higher) were slightly higher. The Ca^{2+} concentrations in needles and branchwood were similar to those reported by Morris (1986; 1992), whereas Ca^{2+} concentrations in the stemwood (5 times higher) and bark (6 times higher) were higher than those reported in this study. However, Mg^{2+} concentrations in all components in this study were higher, and K^{+} concentrations were similar to those reported by Morris (1986; 1992). These differences in nutrient concentrations may be attributed to differences in the tree age as Morris's study included trees up to the age of 21 years, half the age of the compartment used in this study. The decline in some nutrient concentrations with increasing tree age was briefly examined in Morris's (1986) study. He suggested a possible curvilinear relationship, indicating a more rapid decline in concentrations earlier in the rotation. This indicates that as the tree ages nutrients are probably removed from the woody components. Nutrients such as N, P are mobile in the xylem and K^{+} is highly mobile within the xylem and phloem and can be retranslocated within the plant. The mobility of Ca^{2+} from cell to cell and within the phloem is however restricted (Marschner, 1995). Although Mg^{2+} is mobile within the xylem (Marschner, 1995) the element accumulated in the older trees of this study (Table 6.6).

Concentrations of needle P, K^{+} and Ca^{2+} in Schutz's (1990) study were similar to those reported by Morris (1986; 1990) (Table 6.7) and to those in this study (Table 6.1). Needle N concentrations were slightly higher than both Morris's (1986; 1992) study and this study and Mg^{2+} concentrations were lower in Schutz's (1990) study (Table 6.7) than those reported in this study. The differences in nutrient concentrations between these three studies may also be attributed to differences in analytical techniques used in the determinations.

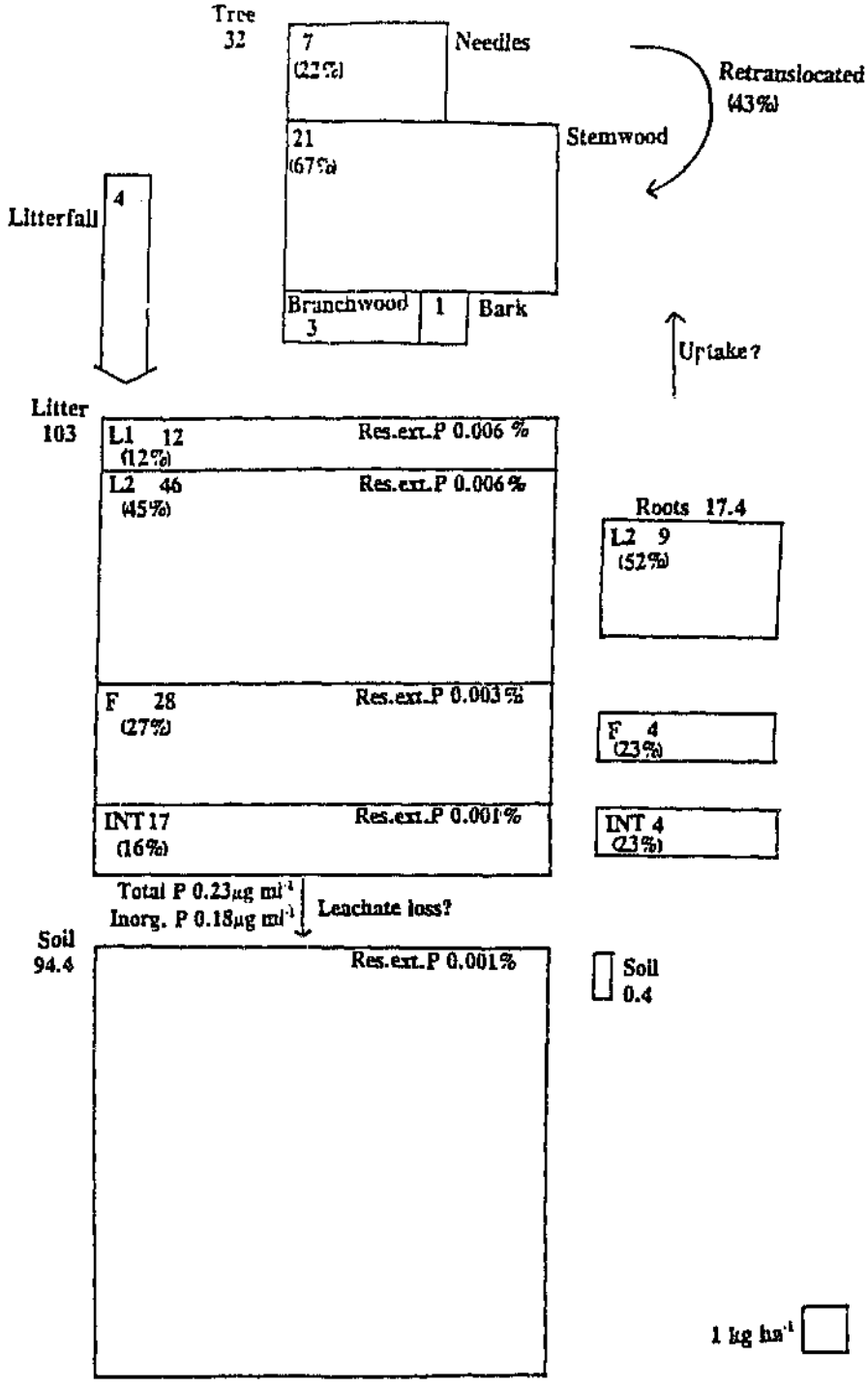


Figure 6.2 The P cycle of a *P. patula* stand in which litter has accumulated. Blocks represent nutrient pools (kg ha^{-1}) and arrows represent nutrient fluxes (kg ha^{-1} yr^{-1}).

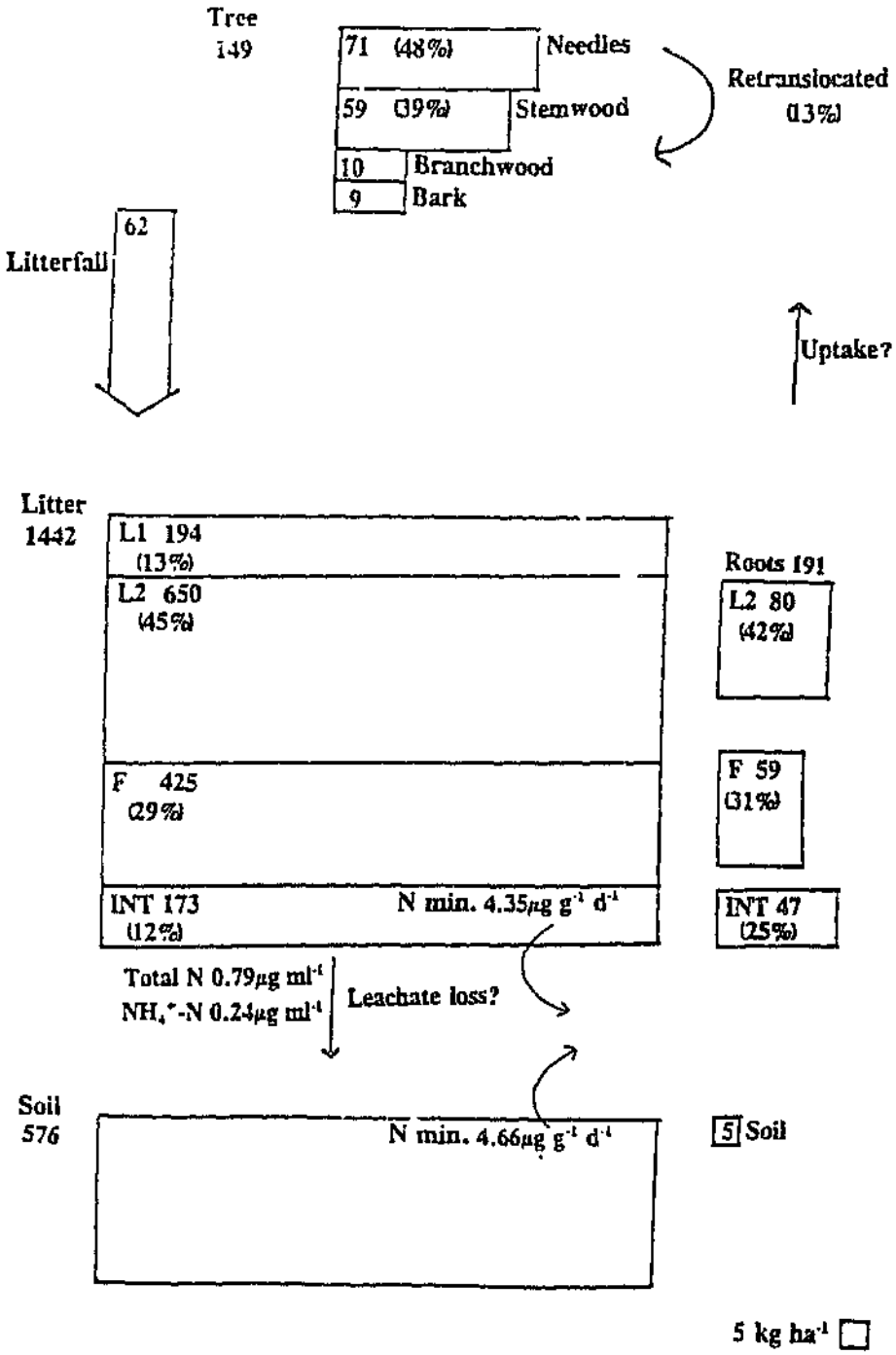


Figure 6.1 The N cycle of a *P. patula* stand in which litter has accumulated. Blocks represent nutrient pools (kg ha^{-1}) and arrows represent nutrient fluxes (kg ha^{-1} yr^{-1}).

accumulation was highest, followed by K^+ and Ca^{2+}. In the litter and soil pools the accumulation of K^+ was the highest followed by Mg^{2+} and Ca^{2+}.

Table 6.6 The distribution of cations in the biomass components.

Components	K^+ (kg ha^{-1})	Mg^{2+} (kg ha^{-1})	Ca^{2+} (kg ha^{-1})
Tree pool			
Needles	120	151	30
Stemwood	50	375	12
Branchwood	59	71	11
Bark	31	14	3
Total tree pool	**260**	**611**	**56**
Litter pool			
L1	121	108	ud*
L2	487	239	ud
F	31	98	ud
INT	53	10	19
Total litter pool	**692**	**455**	**19**
Soil pool			
Soil	226	32	45
Nutrient reserves	**1178**	**1098**	**120**

* ud undetected

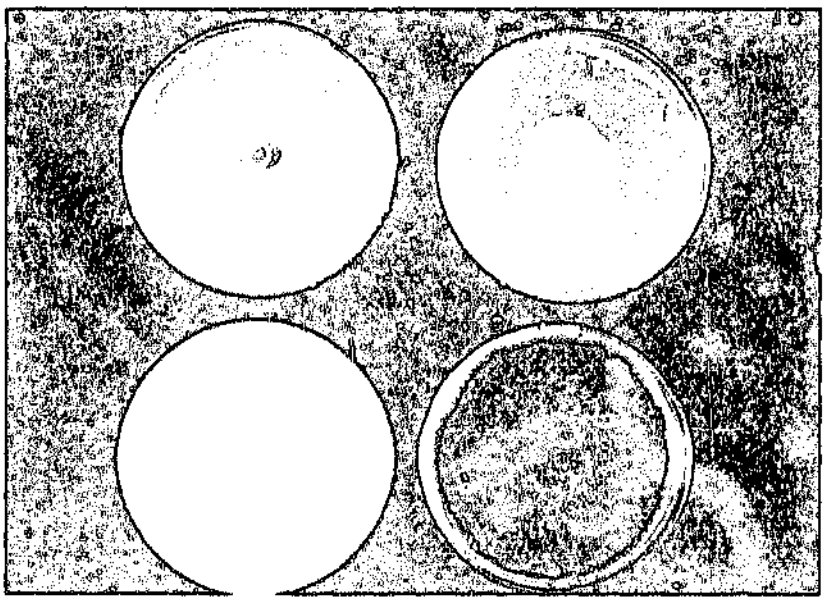


Plate 7 [illegible] WHS 01, WHS 02, WHS 03, WHS 04 and WHS 05

observed. Statistical analyses were conducted using Statgraphics version 5. The temperature, N and P growth curves for each isolate were Log(y + 1) transformed to produce linear growth models. The slopes of these models were tested for homogeneity and any significant differences ($p \leq 0.05$) were subjected to multiple comparisons using the Tukey test ($k \geq 3$). In cases were $k < 3$ comparisons of slopes were made using a t-test (Zar, 1984).

7.3 RESULTS

7.3.1 Isolates

Cultures were obtained from the two types of ECM roots (WITS 01 and WITS 06) and the litter (WITS 02). Cultures of all isolates (including WITS 04) growing on MMN plates are shown in Plate 7.1. Cultural descriptions are presented below:

- WITS 01: Slow growing, pale yellow, velvety culture, margins smooth, hyphae septate with clamp connections
- WITS 02: Fast growing, olive green/brown, flat culture, margins smooth, hyphae septate with clamp connections.
- WITS 04: Fluffy white with aerial growth, margin irregular, hyphae joining to form yellow to brown threads, hyphae septate with clamp connections.
- WITS 06: Growth intermediate, beige, velvety culture, margin smooth, hyphae septate with clamp connections.

All isolates were inoculated individually onto germinated *P. patula* seedlings, however, mycorrhizal infection was poor, although not totally absent. Few roots showed dichotomous branching and no mantles were observed. Roots sectioned with a freeze microtome were examined microscopically and poor 'Hartig' net formation was observed in all seedlings.

7.2.5 Utilization of various P compounds as a source of P

To determine the ability of the four ECM isolates to utilize organic or complexed forms of P, a range of P containing compounds were incorporated into the growth medium. These compounds provided the only source of P in the medium.

The basal medium used was MMN (Table 7.1), excluding the P sources, KH_2PO_4 and $(NH_4)_2PO_4$ and N was supplied as $(NH_4)_2SO_4$ at 60mgN l^{-1}. Phosphorus sources were then added at a concentration of 15mgP l^{-1} and the pH was adjusted to 4.5. Phosphorus sources included:

- an inorganic P source, KH_2PO_4;
- a complexed inorganic P source, $AlPO_4$;
- a complexed P source, phytic acid;
- an organic P source, RNA which would occur naturally in the litter and being of plant, microbial or animal origin;
- an organic P source, glycerophosphate

(Jayachandran, Schwab & Hetrick, 1992; Lapeyrie, Ranger & Vairelles, 1991).

Five replicate plates of each of the amended media were centrally inoculated. An agar disc (7mm in diameter) of each isolate was inoculated onto the amended media. Each isolate was tested individually. Plates were incubated at 25°C. The colony diameter was measured periodically along two transects for a period of 30-35 days. Diameter measurements were averaged to ensure that any irregular colony growth was taken into account. Overall growth rates were calculated by dividing the growth measurement by the number of days growth.

7.2.6 Data analysis

The overall growth rates of different isolates were subjected to one-way ANOVAS to determine the effect of various N and P sources. Where significant ($p \leq 0.05$) differences were evident multiple comparisons using least significant differences, were conducted to establish which nutrient sources contributed to the differences

243mgN l^{-1}. The pH of all the media were adjusted to 4.5. Nitrogen sources included:

- an inorganic NH_4^+-N source, supplied as $(NH_4)_2SO_4$;
- an inorganic NO_3-N source, supplied as KNO_3;
- a protein source, supplied as bovine serum albumin (BSA); This source of protein would not occur naturally in litter but was included as a reference to other studies;
- a tri-peptide source, glutathione;
- various amino acids sources which would occur naturally in the litter and have been reported in forest stands (Abuzinadah & Read, 1988), these included;

    DL-aspartic acid (acidic)

    L-glutamic acid (acidic)

    L-arginine (basic)

    DL-serine (neutral)

    Glycine (neutral)

    L-alanine (neutral)

(Abuzinadah & Read, 1986; Finlay, Frostegård & Sonnerfeldt, 1992; Littke, Bledsoe & Edmonds, 1984).

Five replicate plates of each of the amended media were centrally inoculated. An agar disc (7mm in diameter) of each isolate was inoculated onto the amended media. Each isolate was tested individually. Plates were incubated at 25°C for all isolates except WITS 06 which grew best at 18°C. The colony diameter was measured periodically along two transects for a period of 30-35 days. Diameter measurements were averaged to ensure that any irregular colony growth was taken into account. Overall growth rates were calculated by dividing the growth measurement by the number of days growth. Isolates were qualitatively grouped into 'protein fungi', 'intermediate protein users' and 'non-protein fungi' by comparing the isolates growth curves when growing on the BSA and the $(NH_4)_2SO_4$ amended media. 'Protein fungi' were those isolates whose growth on BSA exceeded their growth on the inorganic N source. 'Intermediate protein users' were those isolates having similar growth on both BSA and the inorganic N source. 'Non-protein isolates' did not grow or only grew poorly on BSA, preferring the inorganic N source (Abuzinadah & Read, 1986).

agar plugs (7mm diameter) onto the amended media (Erland & Söderström, 1990). Each isolate was tested individually. Plates were incubated at 25°C which was the standard incubation temperature used in this laboratory and a temperature at which all isolates grew, with the exception of WITS 06 which grew best at 18°C. The colony diameter was measured periodically along two transects for a period of 30 days. Diameter measurements were averaged to ensure that any irregular colony growth was taken into account. Overall growth rates were calculated by dividing the growth measurement by the number of days growth. From these results the optimum pH for growth for each of the isolates was determined.

Table 7.2 Buffer solution used to adjust the pH of MMN medium (Erland & Soderstrom, 1990; 1991).

Buffer	1000ml
Sodium succinate	1.35g
Glycylglycine	0.66g
Sodium tartrate	1.15g
PIPES buffer	1.51g
HEPES buffer	1.19g

7.2.3 Utilization of various N compounds as a source of N

To determine the ability of the four ECM isolates to utilize both inorganic and organic forms of N, a range of N containing compounds were incorporated into the growth medium. These compounds provided the only source of N in the medium.

The basal medium used was MMN (Table 7.1), excluding malt extract and the ammonium source. Nitrogen sources were then added at a concentration of 60mgN l^{-1}, except BSA which was added at a concentration of

of the isolates, under sterile conditions, in 750ml jars which contained a mixture of perlite and peat moistened with MMN liquid media (Erland & Söderström, 1990). After a period of incubation in a growth chamber, seedlings were examined for ECM roots.

7.2.2 Growth response to temperature

Each of the four isolates were centrally inoculated onto MMN agar plates (Table 7.1), using agar discs (7mm diameter) as inoculum. Each isolate was tested individually. Inoculum was placed at the intersection of two lines drawn across the bottom of the petri dish. Plates were incubated at 7°C, 18°C and 30°C. Five replicate plates of each isolate were incubated at each temperature. The colony diameter was measured periodically along the two transects for a period of 30 days. Diameter measurements were averaged to ensure that any irregular colony growth was taken into account. Overall growth rates were calculated by dividing total growth by the number of days growth. The growth rates for each isolate at 7°C and 30°C were compared with its growth rate at 18°C. These values were used to determine to which growth response category the isolates belonged. Three growth response categories were recognized for each temperature;

- tolerant: where the growth rate is > 50% of the growth rate at 18°C;
- semi-tolerant: where the growth rate is 20-50% of the growth rate at 18°C;
- sensitive: where the growth rate is < 20% of the growth rate at 18°C

(Hutchinson, 1990).

7.2.3 Growth response to pH

The growth response of the four ECM isolates grown on an MMN agar medium (Table 7.1) covering a range of pHs was determined. The pH of a buffer salt solution (Table 7.2) and the MMN medium was adjusted to that required with either NaOH or HCl, and mixed, after autoclaving. The pH values tested were 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00. These pH values were chosen to cover the range of pH in the litter and the soil (Table 6.2; chapter 6) and the higher pH values were included to determine the effect that the pH of lime might have on the growth of ectomycorrhizas. Five replicate plates of each isolate were centrally inoculated, using

Table 7.1 Full strength modified Melin Norkans medium (Molina & Palmer, 1982).

MMN medium	1000ml
Malt extract	3.00g
d-Glucose	10.00g
$(NH_4)_2HPO_4$	0.25g
KH_2PO_4	0.50g
$MgSO_4.7H_20$	0.15g
$CaCl_3$	0.05g
$FeCl_2$	1.2ml (1% solution)
NaCl	0.025g
Thiamine HCl	100μg
pH	5.5

Four isolates which were used in this study (2 of which where isolated from ECM root tips), these isolates were designated;

- WITS 01, isolated from white ECM root tips collected from the *P. patula* compartment, Brooklands, F2 (Table 3.2; chapter 3).
- WITS 02, isolated from litter collected from Brooklands, F2;
- WITS 04, isolated from *P. patula* ECM root tips by Dr. Colin Straker;
- WITS 06, isolated from brown ECM roots collected from Brooklands, F2.

To confirm the mycorrhizal ability of the four isolates used in this study, *P. patula* seeds were surface sterilized by immersion in 30% H_2O_2 for 15min. After germination, *P. patula* seedlings were inoculated with agar plugs

of the *Pinus* litter would contribute to its slow decomposition as decomposition proceeds faster in more neutral environments, the enzymes responsible for decomposition, such as cellulases and amylases, being sensitive to changes in pH (Alexander, 1977). In Mpumalanga, there is concern regarding acid rain and its effect on soil fertility and acidification. The application of dolomitic lime has been suggested as a measure of addressing these concerns (Olbrich, 1995). This measure has also been recommended as a management strategy for sites with accumulated litter layers (De Ronde, 1991). Carlson (1992) has shown that the population structures of ECM associated with *P. patula* roots may change in response to artificially applied acidic rain. Because of these recommendations and the acidic environment in which ECM grow, the growth response of the ECM isolates used in this study, to various pH values was examined.

7.2 METHODS

7.2.1 Ectomycorrhizal isolates

To isolate ectomycorrhizal fungi, blocks of litter were collected (Section 4.2.3; chapter 4) from a site in which litter had accumulated (Brooklands, F2; Table 3.2; chapter 3). Two types of ECM roots were observed in the litter. Because of the intimate association of the ECM roots and the litter, ECM roots were removed by hand. Approximately 30 ECM root tips of each morphological type were removed from the litter and placed in separate nylon mesh bags. The roots were immersed in 0.2% Tween 80 for 5mins and then rinsed in water. This was followed by surface sterilization in 30% H_2O_2 for 1 min. After rinsing, the roots were removed from the bags, placed in a sterile petri dish and macerated (Erland & Söderström, 1990). Pieces of macerated roots were placed on ½ strength modified Melin Norkans (MMN) agar medium (Table 7.1). The medium contained 30mg l^{-1} streptomycin and 1mg l^{-1} Benomyl to reduce bacterial and some fungal contamination. Macerated roots were also plated onto a medium containing only streptomycin, so as not to inhibit any growth by ascomycetes. Plates were incubated at 25°C for 21-30 days and examined periodically for growth and contamination.

have access to unavailable sources of N and P and might, therefore be able to enhance the cycling of nutrients, the following hypotheses were examined;

Hypothesis 8: Ectomycorrhizal fungal isolates of *P. patula* can utilize some organic N, as well as, inorganic N compounds as their sole source of N (Fig. 2.1, chapter 2).

Hypothesis 9: Ectomycorrhizal fungal isolates of *P. patula* can utilize some organically bound or complexed, inorganic P compounds as their sole source of P (Fig. 2.1; chapter 2).

Ectomycorrhizal plants have been successful in a range of habitats. The success of the mycobiont, in colonizing host roots, is dependent on many environmental factors such as temperature, pH, nutrient availability, soil moisture and the influence of other rhizosphere micro-organisms (Marks & Kozlowski, 1973).

The accumulation of litter under *P. patula* has been shown to be correlated with altitude (Schutz, 1990), with thicker litter layers occurring in high altitude sites (Fig. 4.10; chapter 4). This relationship indicates that climatic factors associated with higher altitudes could markedly influence ecosystem processes such as decomposition and nutrient cycling as a result of the generally cooler and wetter conditions experienced (Schutz, 1990). Once the forest canopy has closed, soil and forest floor temperatures can be expected to decrease below the ambient air temperatures because of increased shading (Singh & Gupta, 1977). These cooler temperatures may be responsible for the decrease in decomposition rate observed during the second phase of decomposition (Phase M2; Fig. 5.16; chapter 5).

Because of this interaction with temperature, the growth response of isolated ECM fungi to various temperatures was examined. These tests were conducted as a physiological exercise as well as a taxonomic aid (Hutchison, 1990).

The accumulated litter layer under *P. patula* has been found to be very acidic with pH values decreasing from a pH 3.26 in the upper litter layers, to pH 2.16 in the lower layers (Table 6.2; chapter 6). The acidic nature

lowers the rhizosphere pH. Ectomycorrhizas have also been shown to produce oxalic acid which acts as a chelating agent releasing P from compounds complexed with iron, calcium or aluminium, thereby rendering P more available for uptake (Gianinazzi-Pearson & Gianinazzi, 1989; Nye & Tinker, 1977). In forest soils up to 50% of the phosphorus may be in organic form and a substantial portion of this could be made available by the ECM fungi (Häussling & Marschner, 1989).

Both N and P have been shown to be important in forest ecosystems. Nitrogen is regarded as a limiting factor and there is increasing evidence that, in many forests, P is immobilized in the first stages of decomposition (Attiwill & Adams, 1993). Decomposition studies (Figs. 5.8 & 5.9; chapter 5) in *P. patula* plantations have shown that N is initially mineralized and then rapidly immobilized in the litter layers, while P continues to be mineralized (Figs. 5.12 & 5.13; chapter 5). However, in the nutrient cycling studies (Chapter 6), of an older *P. patula* compartment in which litter has accumulated, it was shown that approximately 50% of the needle P is internally retranslocated prior to litterfall; this indicates that there is a P limitation in this forest ecosystem (Fig. 6.2; chapter 6), which has probably resulted from the complexing of P with aluminium oxides and the P-fixing properties of the soil, thereby reducing availability (Attiwill & Adams, 1993). From the nutrient cycling studies it was evident that the majority of the N and P reserves (1442kgN ha^{-1} and 103kgP ha^{-1}, respectively) were found in the litter layers (Figs. 6.1 & 6.2; chapter 6).

If ECM fungi are capable of decomposing organically bound N compounds and organic or complexed P compounds, they would have the ability to successfully compete with the decomposer micro-organisms for nutrients. It is possible that this would result in decreased microbial activity as suggested by Gadgil & Gadgil (1971; 1975). However, results from the trenching experiment conducted in a *P. patula* compartment, in which litter had accumulated, indicated that the elimination of active ECM roots did not result in increased microbial activity and biomass (Table 5.8; chapter 5). Ectomycorrhizas were, therefore, not responsible for suppressed microbial activity which would contribute to retarded decomposition rates and ultimately accumulation of litter. Conversely, the ability of ECM to utilize organically bound N and organic or complexed inorganic P compounds as sources of N and P would positively contribute to the cycling of nutrients by making unavailable sources of these nutrients more accessible. To determine whether ECM fungi isolated from *P. patula* stands

7 THE ROLE OF ECTOMYCORRHIZAS IN NUTRIENT CYCLING

7.1 INTRODUCTION

It has been well established that ectomycorrhizal (ECM) fungi benefit their hosts by enhancing mineral uptake from the soil and thus contribute to the cycling of nutrients in the ecosystem. The enhanced mineral uptake ability of ECM roots is attributed mainly to the increased surface area, effective in ion absorption (Brundrett, 1991; Harley & Smith, 1983).

This study has shown that ECM roots are a major component of the accumulated litter layers under *P. patula* (Section 6.3.5; chapter 6) and are in intimate contact with the litter. The ECM roots in the litter are well placed to optimise the absorption of nutrients mineralized by the activity of saprotrophic micro-organisms.

Several studies have shown that some ECM fungi are able to utilize complex compounds such as organically bound N and P. Initially, it was thought that ECM fungi lacked the enzymes necessary to exploit complex organic sources of N and that they were dependent on simple organic forms of N such as amino acids, urea and glutamic acid (Marks & Kozlowski, 1973). Experiments have demonstrated that some species of ECM fungi are capable of producing external acid proteases that breakdown protein to amino acids and short chain peptides, and are thus able to utilize proteins as their sole N source (Abuzinadah & Read, 1986; Abuzinadah, Finlay & Read, 1986; Finlay, Frostegård & Sonnerfeldt, 1992; Littke, Bledsoe & Edmonds, 1984). The major sources of N in the litter are likely to be proteinaceous material derived from the litter and from the microbial population itself (Abuzinadah & Read, 1986; Read, Leake & Langdale, 1989).

Ectomycorrhizal fungi have also been shown to produce surface acid phosphatases, of broad specificity, capable of hydrolysing phytates (Antibus, Sinsabaugh & Linkins, 1992; Vogt, Publicover & Vogt, 1991). They not only have the ability to decompose organic or complexed inorganic sources of P, but can also increase the weathering rate of P bound in mineral form as a result of increased CO_2 and organic acid production which

N, P, K^+ and Mg^{2+} was the L2 layer, which was also the layer in which the majority of roots are distributed. This suggests increased microbial activity and nutrient uptake within this layer.

The soil layer holds less nutrients than the litter layers above, with the exception of Ca^{2+}. The distribution of nutrients within the soil was as follows, $N > K^+ > P > Mg^{2+} > Ca^{2+}$. The major nutrient within all the litter/soil layers was N. The accumulation of nutrients within the litter layers and the distribution of roots mainly within the litter layers indicates that nutrients are taken up primarily from these layers. This suggests that nutrients are cycled through a plant-litter-plant in preference to a plant-litter-soil-plant cycle.

6.5 CONCLUSIONS

From the nutrient cycles presented (Figs. 6.1 & 6.2 & Table 6.6) it can be concluded that *P. patula*, growing in a high altitude site in which litter has accumulated, acquires a significant proportion of its nutrients from the litter layers, as is evident from the distribution of roots which occur mainly within the litter layer and the large nutrient reserves within the litter pool, especially with regard to N, K^+ and P. Therefore, **hypothesis 6** and **hypothesis 7** can be accepted. It is evident that litter plays an important role in the cycling of nutrients, with the litter N pool, litter K^+ pool and litter Mg^{2+} pool being 2.5 times, 3 times and 14 times larger than the soil pool, respectively. The litter P pool is similar to that of the soil, however, the P-fixing capacity of the soil may result in a P deficiency which results in the retranslocation of a substantial quantity of needle P prior to needle fall. Nutrients are therefore being cycled through a plant-litter-plant cycle which emphasises the importance of the litter layers. In addition, nutrients are also cycled through a plant-litter-soil-plant cycle as is evident from the leaching of a small quantity of nutrients from the litter into the soil, as well as the presence of roots in the soil layers. However, because of the potential Al^{3+} toxicity of the soil, which decreases the ability of roots to successfully exploit the soils nutrient reserves, it is believed that the plant-litter-plant cycle plays a significant role in the cycling of nutrients in sites which have accumulated litter layers.

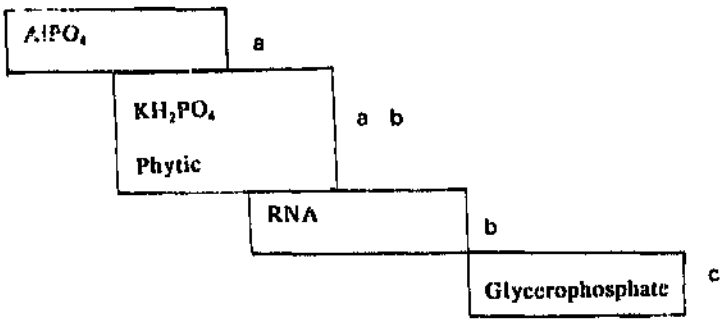


Figure 7.8 Utilization of various P compounds as a P source, isolate WITS 01. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Phosphorus sources listed in descending order according to growth rates.

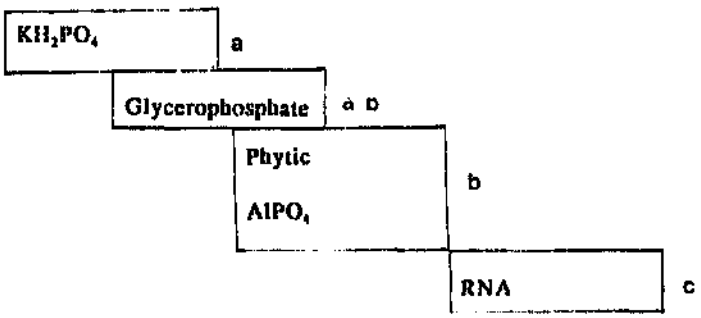


Figure 7.9 Utilization of various P compounds as a P source, isolate WITS 02. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Phosphorus sources listed in descending order according to growth rates.

7.3.5 Utilization of various P compounds as a source of P

The overall growth rates of isolates growing on P amended media are presented in Table 7.6. All isolates grew on all the amended media. However, significant differences in growth rates of isolates on the va us media were evident for all the isolates: WITS 01 ($F_{4,20}$ = 5.86; $p \leq 0.003$); WITS 02 ($F_{4,20}$ = 13.35; $p \leq 0.0001$); WITS 04 ($F_{4,20}$ = 31.75; $p \leq 0.0001$) and WITS 06 ($F_{4,20}$ = 68.69; $p \leq 0.0001$). Using multiple comparisons the differences in overall growth rates on the various P media and are presented graphically (Figs. 7.8, 7.9, 7.10 & 7.11).

Table 7.6 Overall growth rates (mm d^{-1}) of ectomycorrhizal fungal isolates on media amended with various P-sources.

	Isolates			
P source	WITS 01	WITS 02	WITS 04	WITS 06
KH_2PO_4	0.37	1.36	0.44	0.97
$AlPO_4$	0.41	1.11	0.52	0.90
RNA	0.30	0.76	0.11	0.18
Glycerophosphate	0.20	1.20	0.80	0.76
Phytic acid	0.33	1.17	0.38	0.87

Log(y + 1) transformations were conducted on the growth curves to obtain linear growth models which were subjected to statistical analysis to assess differences in the slopes of the models. None of the isolates showed any significant differences between the slopes of all models for growth on all the P amended media

Growth curves for each isolate growing on $(NH_4^+)_2SO_4$ and BSA amended media are presented in figure 7.7. The isolate WITS 04 can be categorized as a 'protein user' as growth on BSA exceeded that on the inorganic source. Isolates WITS 02 and WITS 06 are both 'intermediate protein users' as both isolates showed similar growth on BSA and $(NH_4^+)_2SO_4$. Isolate WITS 01 was a 'non-protein user', being unable to grow on BSA.

Log(y+1) transformations were conducted on the growth curves to obtain linear growth models which were subjected to statistical analysis to assess differences in the slopes of the models. WITS 01, WITS 02 and WITS 06 showed no significant differences between the slopes of all models for growth on all the N amended media. The isolate WITS 04 showed significant differences between the growth model slopes ($F_{0.05,(1),9,23}=7.89$) this was attributable to the slower growth on the $(NH_4^+)_2SO_4$ medium.

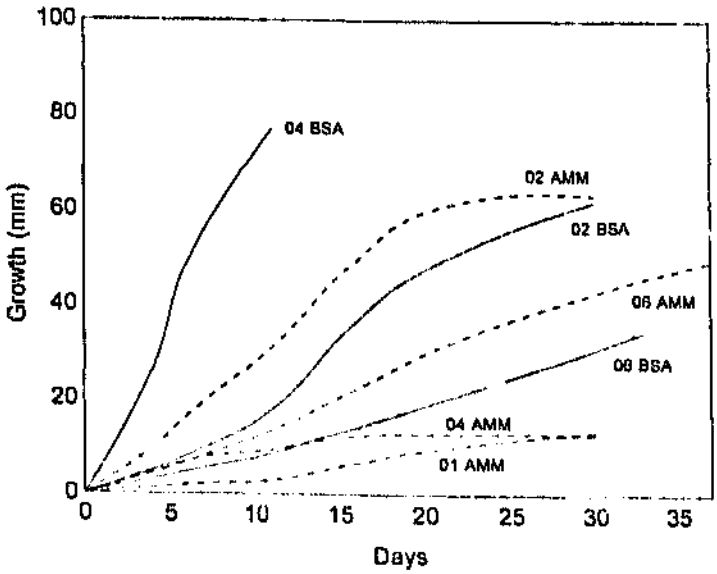


Figure 7.7 Growth of ectomycorrhizal fungal isolates (numbered on graph) on media with $(NH_4^+)SO_4$ (AMM) and the protein bovine serum albumin (BSA) as the sole source of N.

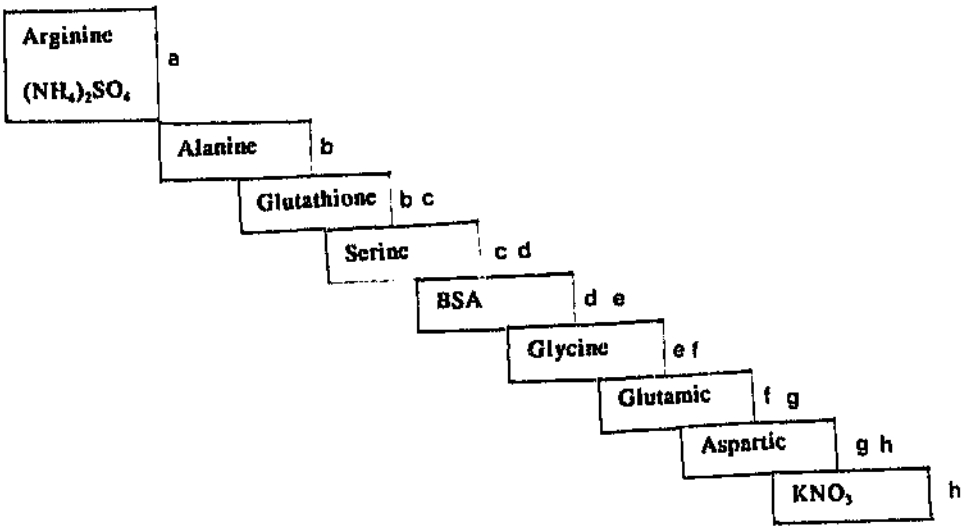


Figure 7.6 Utilization of various N compounds as an N source, isolate WITS 06. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Nitrogen sources listed in descending order according to growth rates.

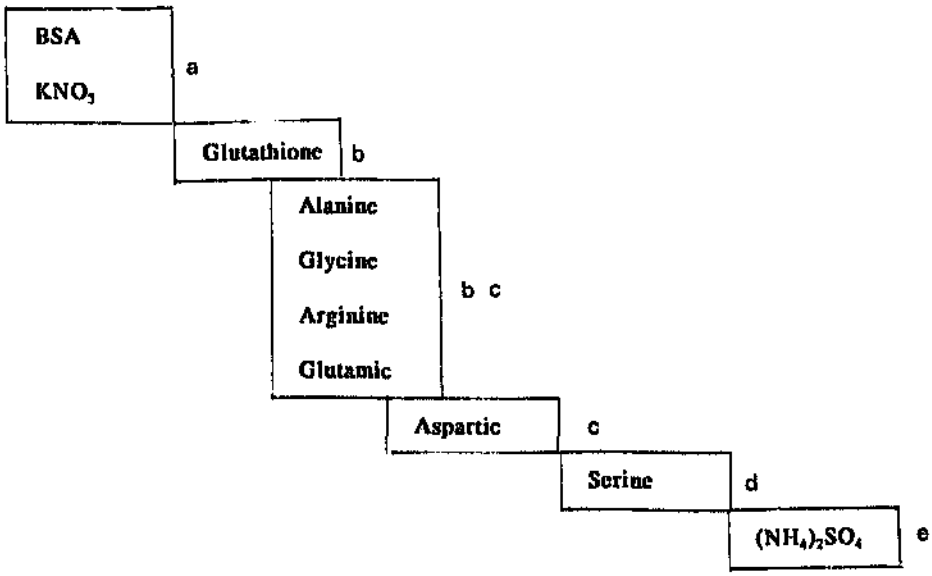


Figure 7.5 Utilization of various N compounds as a N source, isolate WITS 04. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Nitrogen sources are listed in descending order according to growth rates.

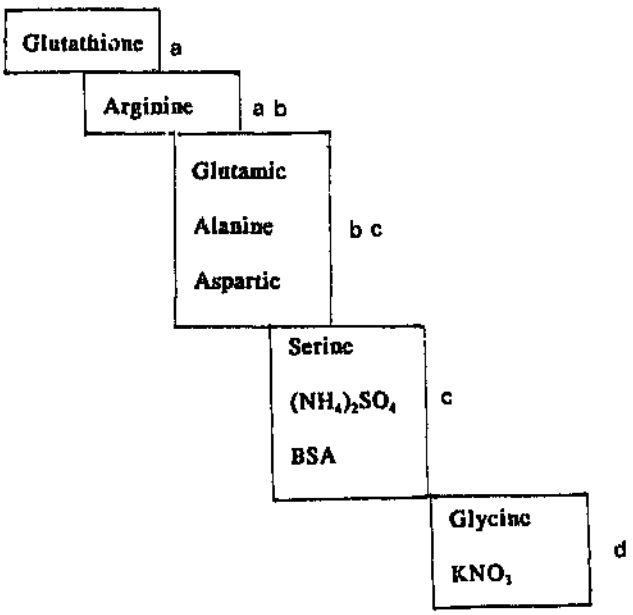


Figure 7.4 Utilization of various N compounds as a N source, isolate WITS 02. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Nitrogen sources are listed in descending order according to growth rates.

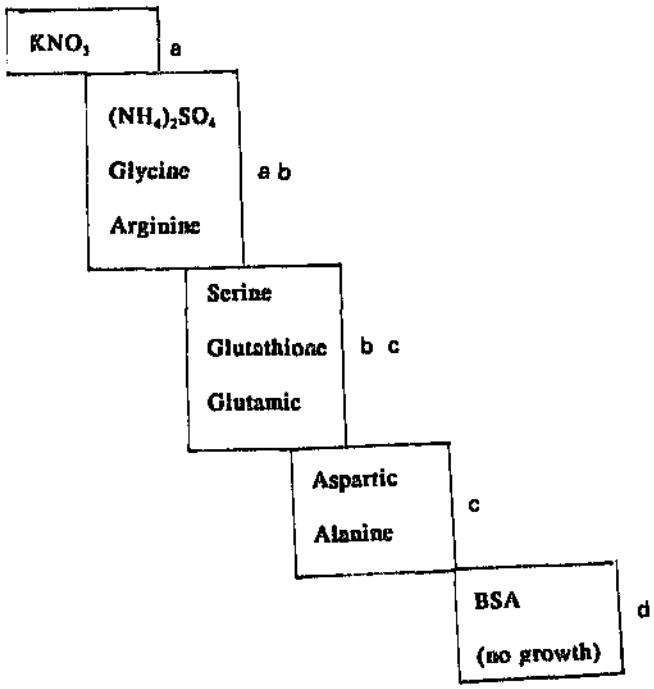


Figure 7.3 Utilization of various N compounds as a N source, isolate WITS 01. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Nitrogen sources are listed in descending order according to growth rates.

7.3.4 Utilization of various N compounds as a source of N

The overall growth rates of isolates growing on N amended media are presented in Table 7.5. All isolates grew on all the amended media, with the exception of WITS 01 which did not grow on BSA and Wits 04 which grew extremely poorly on ammonium. However, significant differences in the growth rates on the various N media were evident for the isolates WITS 01 ($F_{9,40}=21.66$; $p\leq0.0001$), WITS 02 ($F_{9,39}=10.34$; $p\leq0.0001$), WITS 04 ($F_{9,40}=999.99$; $p\leq0.0001$) and WITS 06 ($F_{9,35}=26.99$; $p\leq0.0001$). These differences can attributed to the overall growth on the two inorganic N media as well as on the organic N media and are presented graphically (Figs. 7.3, 7.4, 7.5 & 7.6).

Table 7.5 Overall growth rates (mm d^{-1}) of ECM fungal isolates growing on media amended with various N-sources.

	Isolates			
N source	WITS 01	WITS 02	WITS 04	WITS 06
$(NH_4)_2SO_4$	0.44	2.34	0.42	1.33
KNO_3	0.47	1.81	7.64	0.68
BSA	0.00	2.29	7.64	1.04
Glutathione	.37	2.98	7.54	1.22
DL-aspartic	0.31	2.37	7.38	0.85
L-glutamic	0.36	2.13	7.40	0.89
L-arginine	0.40	2.60	7.45	1.34
DL-serine	0.37	2.36	7.11	1.08
Glycine	0.43	1.91	7.45	1.00
L-alanine	0.29	2.38	7.40	1.17

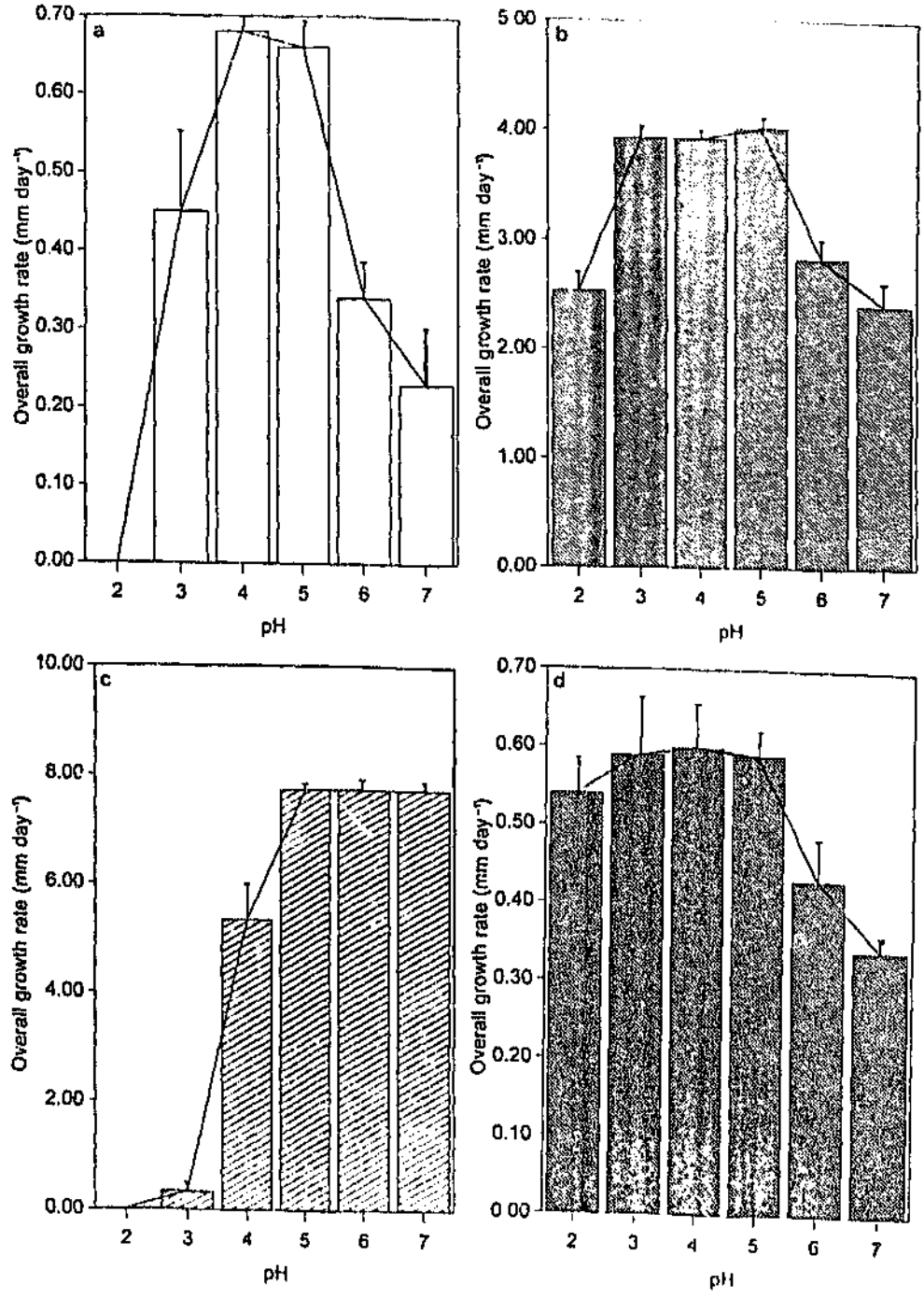


Figure 7.2 Growth response of ECM isolates on media having various pH values; *a* - WITS 01; *b* - WITS 02; *c* -WITS 04 and *d* - WITS 06. Standard deviations (+) are shown on bars. Line graphs represents growth trend.

WITS 06 grew poorly at 7°C (Fig. 7.1d) with significantly faster growth being achieved at 18°C (Table 7.3) and no growth at 30°C. From the overall growth rates obtained for the three temperatures, the isolates can be categorized into their tolerance groupings (Hutchinson, 1990) which are presented in Table 7.4.

Table 7.4 Tolerance groupings of ECM fungal isolates as indicated by their temperature responses.

Isolate	7°C	30°C
WITS 01	sensitive	tolerant
WITS 02	semi-tolerant	tolerant
WITS 04	semi-tolerant	tolerant
WITS 06	sensitive	sensitive

7.3.3 Growth response to pH

The growth rates of isolates WITS 01, WITS 02, WITS 04 and WITS 06, at the various pHs showing optimal pH requirements are presented graphically in figures 7.2 a-d, respectively. Because unrelated isolates are being studied and no growth comparisons are made between isolates the y-axis scaling for each isolate is different. Isolate WITS 01 (Fig. 7.2a) did not grow at pH 2.00 and showed maximum growth at pH 4.00 and 5.00, growth was reduced at pH 3.00, 6.00 and 7.00. Isolate WITS 02 (Fig. 7.2b) showed maximum growth between pH 3.00 and 5.00 with reduced growth at pH 2.00, 6.00 and 7.00. Isolate WITS 04 (Fig. 7.2c) did not grow at pH 2.00 and only grew poorly at pH 3.00. Growth increased at pH 4.00 with maximum growth occurring from pH 5.00 to 7.00. Isolate WITS 06 (Fig. 7.2d) grew well at pH 2.00 with maximum growth being achieved between pH 3.00 and 5.00, growth was reduced at pH 6.00 and 7.00.

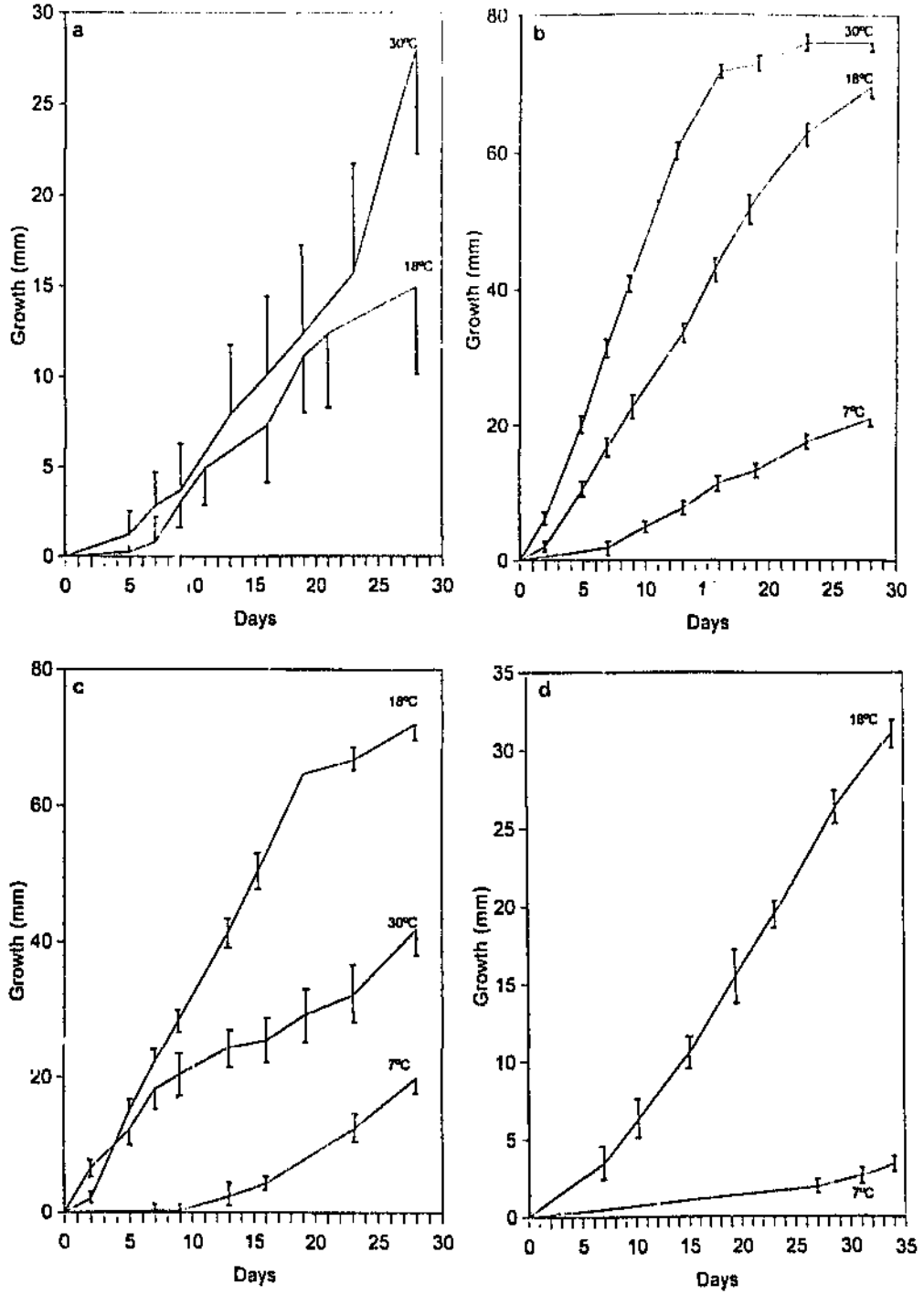


Figure 7.1 Growth curves of ECM isolates at 7°C, 18°C and 30°C; *a* - WITS 01; *b* - WITS 02; *c* - WITS 04 and *d* - WITS 06. Standard deviations (+ and/or -) are shown on the graphs.

7.3.2 Growth response to temperature

Growth curves of isolates WITS 01, WITS 02, WITS 04 and WITS 06, growing at 7°C, 18°C and 30°C, are presented in figures 7.1 a-d, respectively. Because unrelated isolates were used in the study and no comparisons in growth were made between isolates the y-axis scaling on the growth curves are different. Log(y + 1) transformations of the growth curves yielded slightly lower or similar correlation coefficients when compared with those obtained from the linear regression models. This indicated that the cultures were mainly in the linear growth phase. Linear growth models for all isolates growing at all temperatures are presented in Table 7.3.

Table 7.3 Linear growth models obtained from Log(y + 1) transformation of growth curves for ECM isolates growing on MMN medium at various temperatures.

	Temperatures			
Isolates	7°C	18°C	30°C	Significance tests
WITS 01	no growth	$y=0.22+0.04x$	$y=0.13+0.05x$	$t_{0.05,(1),14}=-2.5$
		r 1.00	r 0.94	
WITS 02	$y=0.15+0.05x$	$y=0.57+0.06x$	$y=0.69+0.07x$	$F_{0.05,(1),2,21}=6.5$
	r 0.97	r 0.89	r 0.82	
WITS 04	$y=-0.11+0.05x$	$y=0.61+0.06x$	$y=0.72+0.04x$	$F_{0.05,(1),2,22}=5.89$
	r 1.00	r 0.84	r 0.84	
WITS 06	$y=0.04+0.02x$	$y=0.31+0.04x$	no growth	$t_{0.05,(1),8}=-7.5$
	r 1.00	r 0.95		

Isolate WITS 01 did not grow at 7°C while growth at 18°C was significantly slower than the growth at 30°C (Fig. 7.1a & Table 7.3). Isolate WITS 02 showed significantly slower growth at 7°C when compared with growth at 18°C ($q_{0.05,3,21}=-3.75$) and 30°C ($q_{0.05,3,21}=-5.3$) (Table 7.3). The best growth was at 30°C (Fig. 7.1b) when the stationary phase was reached after 16 days. Isolate WITS 04 grew well at 7°C (Fig. 7.1c) although significantly slower than the growth at 18°C ($q_{0.05,3,22}=-5.87$) and 30°C ($q_{0.05,3,21}=-4.37$) (Table 7.3). Isolate

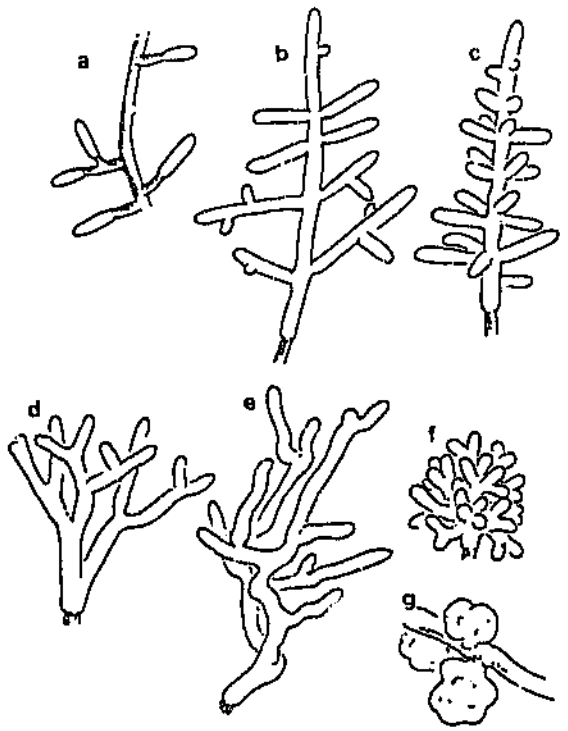


Figure 8.1 Type of ramification; *a* - simple; *b* - monopodial-pinnate; *c* - monopodial-pyramidal; *d* - dichotomous; *e* - irregular pinnate, dichotomous-like; *f* - coralloid and *g* - tubercle-like (Agerer, 1987-1995).

Figure 8.2 Shape of ramification; *a* - straight; *b* - bent; *c* - tortuous and *d* - beaded (Agerer, 1987-1995).

in the litter, WITS 01 and WITS 06 (Section 7.2.1, chapter 7). Approximately 30-50 roots of each morphological type were removed and placed in either sterile, distilled water (fresh samples) or FAA (Formalin : Acetic Acid : 70% Ethanol; 5:5:90; Hawksworth *et al.*, 1995) (Agerer, 1987-1995).

8.2.2 Identification of ectomycorrhizal roots

Morphological characteristics

Mycorrhizal roots were examined with a dissecting microscope and various morphological characteristics were noted such as:

- the colour of fresh mycorrhizas in distilled water and any changes in colour;
- the size of the mycorrhizas, including diameter and length of the ECM tips;
- the type of mycorrhizas, whether simple (unramified), pinnate, pyramidal, dichotomous, coralloid or tubercle (Fig. 8.1);
- the shape of the unramified ends, whether straight, bent, tortuous or beaded (Fig. 8.2);
- features of the mantle surface, whether smooth, reticulate, grainy or woolly (Fig. 8.3);
- the presence and frequency of rhizomorphs and emanating hyphae;
- the attachment of rhizomorphs to the mantle (Fig. 8.4);
- the shape of the rhizomorph (Fig. 8.5) (Agerer, 1987-1995)

Chemical tests

Chemical reactions are generally used as an aid to identify fungal fruiting bodies. This technique has since been applied to fresh whole mycorrhizas (Zak, 1973) and hand sectioned ECM roots (Agerer, 1986). Relatively few chemicals have however been successfully applied to ECM (Agerer, 1994). Chemical reactions were observed on addition of the reagent (reactions occurring within 5 mins) and after rinsing with water. Reagents used included; Cotton-blue-lactophenol, 0.1% Toluidine Blue; 70% Ethanol, 15% KOH, Lactic Acid, Melzer's reagent, IKI and Sulfo-vanillin (Agerer, 1986; Brundrett, Melville & Peterson, 1994; Ingleby *et al.*, 1990; Watling, 1971). The reactions were observed microscopically (Agerer, 1986; 1987-1995). Whole

Ectomycorrhizae' (Agerer, 1987-1995). This atlas is continually updated to include new ECM species and currently contains keys to 218 ECM species occurring on 17 host plants and 90 plates of ectomycorrhizas. An identification manual compiled by Ingleby *et al.* (1990) also employed morphological and anatomical characteristics, concentrating mainly on the mantle.

- Molecular techniques have recently been applied to the identification of ECM. These involve analysing the internal transcribed spacer region in the nuclear ribosomal units using taxon-specific primers (Bruns, White & Taylor, 1991; Bruns & Gardes, 1993; Gardes & Bruns, 1993; Kraigher, Agerer & Javornik, 1995).

Studies on ECM in the forestry regions of South Africa have been sparse. Marais & Kotzé (1977) examined the ECM of *P. patula* at the Belfast plantation in Mpumalanga and identified *Boletus edulis* and *Amanita muscaria* as being ectotrophic, with *Tuber rapaeodorum* and *Lycoperdon umbrinum* as possibly ectotrophic. Van der Westhuizen & Eicker (1987) listed 19 species of basidiomycetes, considered to be ectotrophic, which occur in the major pine growing regions of the country, 10 of which occur in Mpumalanga. Several studies in the Mpumalanga forestry region have classified ectomycorrhizal roots based on morphology alone (Carlson, 1992 & 1994). The aim of this study was to identify the ECM roots collected from a *P. patula*, high altitude site in which litter had accumulated (Section 7.2.1; chapter 7). The identification method used was mainly that of Agerer (1986; 1987-1995). This approach to the identification has not been previously applied to ectotrophic *Pinus* species occurring in South Africa.

8.2 METHODS

8.2.1 Collection of ectomycorrhizal roots

Ten litter samples were collected (Section 4.2.3; chapter 4) from a *P. patula* site in which litter had accumulated (Brooklands, F2; Table 3.2; chapter 3). Ectomycorrhizal roots were interwoven with the litter forming a dense mat. Mycorrhizal roots were, therefore, removed by hand. Two distinct morphological types were observed

8 CHARACTERIZATION OF ECTOMYCORRHIZAS

8.1 INTRODUCTION

Many attempts have been made to identify the fungi responsible for the production of ectomycorrhizas (ECM) in order to correctly interpret and apply various physiological properties that have been demonstrated experimentally (Agerer, 1986). Various methods of identification have been used and include the following:

- Recording the occurrence of sporocarps under host plants has been taken to indicate mycorrhizal ability as the fungi associated with ECM belong mainly to the Basidiomycota and Ascomycota (Marks & Kozlowski, 1973). The disadvantages of this method are (i) the ability of the host plant to form ECM with different mycobionts at the same time (Marks & Kozlowski, 1973) and (ii) an absence of sporocarps which does not necessarily indicate an absence of the fungus (Vogt, Publicover & Vogt, 1991).

- The tracing of rhizomorph connections from a sporocarp to the ECM roots. Although this method provides a link between the fungus and the ECM roots, the tracing of the connections is difficult and some ECM do not produce rhizomorphs (Agerer, 1986; Ingleby *et al.*, 1990).

- The synthesis of mycorrhizas under aseptic conditions, using cultures of known sporocarps. However, difficulties may be experienced in obtaining cultures of some fungal species e.g. some species of *Russula*, *Tuber* and *Lycoperdon* (Agerer, 1986; Marais & Kotzé, 1977).

- The comparison of cultures obtained from ECM roots with cultures of known fungi occurring in the same area. The disadvantages are (i) the difficulty in obtaining some ECM fungi in culture, (ii) the similar growth in culture of many fungi and (iii) strain-specific differences which would not be identifiable in culture (Agerer, 1986).

- The colour of mycorrhizas and several anatomical features observed in cross sections of the ECM roots formed the basis of an ECM key developed by Dominik (1969). The use of these criteria were extended by Zak (1973) to include the reaction of whole mycorrhizas to various chemical reagents and the approach was further extended by Agerer (1986) resulting in the production of a 'Colour Atlas of

The ability of ECM, vesicular-arbuscular and ericoid mycorrhizal seedlings to utilize organic N and P sources has been demonstrated in several studies (Abuzinadah, Finlay & Read, 1986; Jayachandran, Schwab & Hetrick, 1992; Myers & Leake, 1996). However, the extent to which mycorrhizas can utilize these organic sources under field conditions, in competition with the microbial population, is not known. This indicates an area which requires further investigation (Vogt, Publicover & Vogt, 1991).

Differences in the proteolytic and solubilization capabilities between ECM fungi are important in determining their distribution. Mineral soils are progressively transformed through the input of organic matter and in combination with other soil processes, into organic soils (Abuzinadah & Read, 1988). 'Non-protein fungi' may be successful in newly planted first rotation sites, whereas 'protein fungi' would become more important as the stand matures and in successive rotations. This succession of ectomycorrhizal fungi has been reported in a number of ecosystems (Dighton, Poskitt & Howard, 1986; Mason *et al.*, 1983; Natarajan, Mohan & Ingleby, 1992). Succession has not been studied in the Mpumalanga plantations. However, observations made during the study suggests that some succession occurs, with *Amanita* spp. and *Lycoperdon* spp. occurring under younger trees (up to 15 years) and *Boletus* spp. and *Suillus* spp. occurring in older stands. *Scleroderma* spp. occurred mainly from 10 years onward. This is an area requiring further research. Because of the different capabilities of ECM fungi to utilize various organic and inorganic compounds an effort should be made to assess the ECM fungal species occurring in the forestry areas. Combining this information with studies on succession, ECM fungi more suited to specific areas can be identified measures can then be taken to inoculate with the appropriate ECM fungi.

Organic phosphates such as glycerophosphate and RNA are probably enzymatically broken down by fungal phosphatases and the resulting phosphate absorbed by the roots (Häussling & Marschner, 1989; Lapeyrie, Ranger & Vairelles, 1991). Recent studies by Leake & Miles (1996) have shown that the mycorrhizal ericoid fungus *Hymenoscyphus ericae* can hydrolyse DNA and use it as a sole source of P. The activity of the phosphodiesterases was examined and it was concluded that the enzyme was extra-cellular and wall bound and had a pH range of 3.0-6.5 and a pH optimum of 4.5-5.5. Complexed compounds such as phytic acid and $AlPO_4$ are probably solubilized by the action of ECM fungi or a combination of enzyme activity and solubilization. The N source is important in the solubilization process; NH_4^+-N which is found mainly in acidic soils has been shown to stimulate proton extrusion resulting in acidification a process which increases solubilization (Lapeyrie, Ranger & Vairelles, 1991). Phosphatase activity is also affected by the N source, with increased activity occurring when N is supplied as NH_4^+ (Kieliszewska-Rokicka, 1989). Where NO_3^--N is the main source of N, mainly in calcareous soils, oxalic acid excretion is stimulated, the ECM fungi thereby act as a solubilizing agent by acidification and as a chelating agent by chelating iron, calcium and aluminium (Lapeyrie, Ranger & Vairelles, 1991). Rock phosphates have been shown not to be solubilized by ECM fungi, probably as a result of their crystalline structure, although ECM fungi in association with other micro-organisms may be important in this process (Antibus, Sinsabaugh & Linkins, 1992; Lapeyries, Ranger & Vairelles, 1991).

7.5 CONCLUSIONS

From the results presented it is clear that the ECM isolates respond differently to changes in temperature and pH. These are factors which will influence their distribution and ability to colonize host plants. Ectomycorrhizal fungal isolates of *P. patula* were shown to be capable of utilizing organic as well as, inorganic N compounds as their sole source of N therefore, **hypothesis 8** can be accepted. These isolates were also shown to be capable of utilizing organic and complexed inorganic P compounds as their sole source of P therefore, **hypothesis 9** can be accepted.

ECM fungi to breakdown lignin, holocellulose and lignocellulose (Maijala, Fagerstedt & Raudaskoski, 1991; Trojanowski, Haider & Hütterman, 1984) would enable some ECM fungi to be effective competitors with saprotrophs. Although the cellulolytic ability of the isolates used in this study was not examined, ECM roots and rhizomorphs were observed to be growing within decaying wood stumps which may suggest some cellulase activity. Growth of ECM roots within woody debris may be important in maintaining mycorrhizal diversity after disturbances, such as clear felling, and may help to sustain the mycorrhizas during drought conditions. The woody debris retaining moisture longer than the litter layers as well as providing a source of nutrients (Harvey, Larsen & Juergensen, 1976: Parke, Linderman & Trappe, 1983; Vogt *et al.*, 1995).

Abuzinadah & Read (1986) used the pure protein BSA as a model compound and showed that different ECM fungal species had a wide range of degrading capabilities. Using the same model the isolates used in the present study were shown to exhibit different capabilities in their utilization of BSA (Fig. 7.13). This suggests that acid rain (Carlson, 1992) or management strategies such as fertilization (Carlson, 1994) and burning or clearing of the accumulated litter layers (Baar & De Vries, 1995) in the *P. patula* plantations which result in changes to the ECM fungal composition may also result in altered ability to provide access to organically bound nitrogen.

7.4.5 Utilization of various P compounds as a source of P

All isolates were shown to utilize the various P sources, although differences in overall growth rates were apparent (Figs. 7.8, 7.9, 7.10 & 7.11). Statistical analysis of the slopes of linear models indicated that isolates could utilize a variety of P sources with equal efficiency. As a result of the linearization of the growth curves, significant differences indicated when using the overall growth rates were no longer apparent. Differences in the ability of ECM fungi to utilize various P sources, both between and within species, have been observed (Antibus, Sinsabaugh & Linkins, 1992) and these differences may be related to host characteristics, such as nutrient demand. Antibus, Sinsabaugh & Linkins (1992) noted that pH had a significant effect on phosphatase activity with maximum activity occurring at pH 4.5-5.0 for species of *S. cutrinum*.

WITS 04 which showed poor growth on ammonium. These results support studies which have shown that in general ECM fungi can utilize nitrate as well as ammonium although differences between ECM fungal species are apparent which reflects to some extent the distribution of the ECM fungi (Read, Leake & Langdale, 1989). The growth response to the glutathione amended medium was variable but in general good growth was recorded Abuzinadah & Read (1986) found that glutathione was a poor N source for *Laccaria laccata* and *Pisolithus tinctorius* which was attributed to the decline in pH of the growth media as a result of glutathione utilization. This reflects the natural distribution of these fungi which are not well adapted to acidic conditions. For example, *P. tinctorius* is distributed mainly in arid regions where pH is higher and nitrification is promoted, this fungus has also been shown to utilize NO_3^- more readily than NH_4^+ (Read, Leake & Langdale, 1989). All fungi tested by Abuzinadah & Read (1986) were able to utilize alanine peptides, isolates used in this study utilized alanine and a range of other amino acids. Abuzinadah & Read (1986) suggested that a broad spectrum acid protease was involved in the proteolytic process and that the N released was absorbed by the ECM fungi as amino acids or small peptide units rather than as ammonium. Studies have confirmed the presence of a GS-GOGAT pathway in ECM fungi indicating that glutamate can readily enter the cell where it forms the major component of the N assimilation pathway (Rudawska, *et al.*, 1994). This suggests that there is a 'tight cycling' of organic molecules by ECM as the process involves depolymerization rather than mineralization, thus the normal saprotrophic decomposition pathway is short circuited (Read, Leake & Langdale, 1989; Vogt, Publicover & Vogt, 1991).

This study has shown that ECM fungi utilize a range of amino acids and proteins as their sole source of N (Table 7.5). These results support the growing evidence in the literature that ECM and mycorrhizal ericoid fungi are important in providing access to organic sources of N to which their host plants have little or no access (Abuzinadah & Read, 1986; Finlay, Frostegård & Sonnerfeldt, 1992; Read, Leake & Langdale, 1989). The roots of *P. patula* (approximately 50% of which are mycorrhizal) have been shown to be distributed mainly in the litter layers (Section 6.3.5; chapter 6). Results from the litter decomposition (Chapter 5) and nutrient cycling (Chapter 6) studies indicate that N was immobilized within the litter during decomposition. These findings highlight the importance of ECM, which are intimately associated with the litter layers, to provide access to both inorganic and organic N compounds. This proteolytic ability coupled with the ability of some

exhibiting a tolerance to a wider range of pH values, up to pH 5.00. It must be noted that the optimal pH required for fungal growth may differ from that required for actually infection of host roots (Erland & Söderström, 1990). This study only examined fungal growth, as the saprotrophic ability of the isolates was of interest. However, it was noted that ECM roots of the WITS 06 type were distributed mainly in the L2 layer which had a pH of 2.30, while ECM roots of the WITS 01 type were distributed mainly in the F, IN F and Soil layers having pH's of 2.16, 3.29 and 4.08, respectively (Table 6.2; chapter 6).

The decline in pH of forest soils in Mpumalanga, as a result of acid deposition, is a major concern for the industry. Studies by Carlson (1992) have shown that there were no significant differences in the degree of mycorrhizal colonization between root cores acidified with different pH's of artificial rain. However, differences in the mycorrhizal species composition were noted. These changes could have important consequences for the nutrient status of the trees. The widespread use of lime to increase soil pH has been suggested (Olbrich, 1995). Liming would not only reduce acidity, but also reduce the toxic levels of metals, reduce leaching losses of cations and improve nutrient availability (Kreutzer, 1995). All these factors could affect mycorrhizal composition, hyphal growth and colonization.

7.4.4 Utilization of various N compounds as a source of N

All isolates were shown to utilize the various N sources, with the exception of WITS 01 which did not grow on BSA medium, although differences in overall growth rates were apparent (Figs. 7.3, 7.4, 7.5 & 7.6). Statistical analysis of the slopes of linear models indicated that isolates could utilize a variety of N sources with equal efficiency, with the exception of WITS 04 which grew poorly on $(NH_4^+)SO_4^-$. As a result of the linearization of the growth curves, significant differences indicated when using the overall growth rates were no longer apparent. In accordance with other studies all isolates were shown to grow on $(NH_4^+)_2SO_4$ amended media (Abuzinadah & Read, 1986; Finlay, Frostegård & Sonnerfeldt, 1992; Read, Leake & Langdale, 1989). This result was expected as the ECM fungi are growing in an acidic environment where there is an accumulation of organic material, under these conditions the main form of inorganic nitrogen would be NH_4^+ (Marschner, 1995; Read, Leake & Langdale, 1989). Growth on nitrate was slightly lower, with the exception of isolate

This was attributed to the removal of large amounts of N and phenolics with the litter and humus. This would imply that the thick litter layers of the study site suppressed the mycorrhizal diversity. Whether this holds true for *P. patula* and whether low altitude, thin litter layer sites have a greater mycorrhizal diversity is an area that requires further examination.

7.4.2 Growth response to temperature

Variation in the growth rates between the isolates was evident (Table 7.3) and has been observed to occur both between and within species (Marks & Kozlowski, 1973). Hutchison (1990) studied the temperature responses of a large selection of northern hemisphere ECM fungal isolates. *Boletus edulis*, *Amanita muscaria* and *A. rubescens* were shown to be sensitive to both 7°C and 30°C with some *A. muscaria* isolates being semi-tolerant to 30°C. *Suillus granulatus* and *Scleroderma citrinum* were sensitive to 7°C and tolerant to 30°C. The temperature responses of isolates WITS 01, identified as *Scleroderma citrinum* (Chapter 8), and WITS 06, identified as *Boletus pinicola* (Chapter 8), were comparable to similar isolates recorded by Hutchinson (1990). The semi-tolerant response to 7°C of isolates WITS 02 and WITS 04 suggests that these isolates belong to either the *Hebeloma* or *Laccaria* genera. Isolate WITS 04 was initially regarded to be an *Amanita* sp., because of the presence of these fruiting bodies at the time of root collection (Dr. Colin Straker, *pers. comm.*). However, on examination of the temperature response it is evident that the isolates correct identity cannot be conclusively verified. Species of *Boletus*, *Amanita*, *Scleroderma* and *Laccaria* have been recorded and observed to occur in the Mpumalanga plantation (Van Der Westhuizen & Eicker, 1994), although the latter genus was noted only infrequently by the present researcher.

7.4.3 Growth response to pH

The pH optima for most ECM fungi is regarded to be between pH 3.00 and pH 5.00, fungi being generally considered to be acidophilic. Isolates WITS 01 (Fig. 7.2a) and WITS 02 (Fig. 7.2b) were shown to have pH optima within this range. Isolate WITS 04 (Fig. 7.2c) exhibited optimal growth above pH 5.00, being less adapted to acidic conditions. Isolate WITS 06 (Fig. 7.2d) grew well under very acidic conditions (pH 2.00),

7.4 DISCUSSION

7.4.1 Isolates

The isolation method described by Erland & Söderström (1990) was effective in isolating ECM fungi from infected root tips. Contamination was minimal, with some *Penicillium* and *Trichoderma* species being recorded. Reinoculation of *P. patula* seedlings with the isolates was not totally successful, with only a few dichotomous roots, poor mantle and 'Hartig' net formation. Ectomycorrhizal fungi have been described as either 'early-' or 'late-stage' fungi, which refers to the succession of mycorrhizas as the host plant ages (Dighton, Poskitt & Howard, 1986; Mason *et al.*, 1993; Natarajan, Mohan & Ingleby, 1992). There have been several reports regarding the difficulties experienced when inoculating seedlings with 'late-stage' ECM fungal isolates, which have been related to the 'late-stage' isolates high demand for glucose (Gibson & Deacon, 1990). These isolates are probably 'late-stage' isolates with WITS 01, WITS 02 and WITS 06 being isolated from a 42 year-old *P. patula* compartment and WITS 04 coming from a 15-20 year-old compartment (Dr. Colin Straker, *per comm*). Alternatively, the formation of only a few mycorrhizal roots may have indicated that under the experimental conditions used, the fungal isolates were less vigorous in the colonization of roots (Pera & Alvarez, 1995). Marais & Kotzé (1979) suggested that the source of *P. patula* seed may affect ECM colonization, and reported that the incidence of ECM infection improved when seeds from higher altitude sites were used. The seeds used in this study originated from Witklip and Spitskop plantations, both low altitude areas. This is a factor which would have to be considered in any future ECM studies.

Only two morphological types of ECM roots were observed in the litter and soil at the study site (Brooklands, F2; Table 3.2; chapter 3) not only at the time of sampling but throughout the study period (1992-1994). This suggests poor mycorrhizal diversity which may be attributed to the lack of inoculum of other mycorrhizal types. Most mycorrhizal species associated with *Pinus* spp. in South Africa were introduced in litter and humus after the repeated failure of *Pinus* spp. planted in plantations (Poynton, 1980). A study conducted by Baar & De Vries (1995) in The Netherlands concluded that the removal of litter and humus from stands of *P. sylvestris* resulted in increased number of ECM types observed inoculum presumably being supplied from adjacent areas.

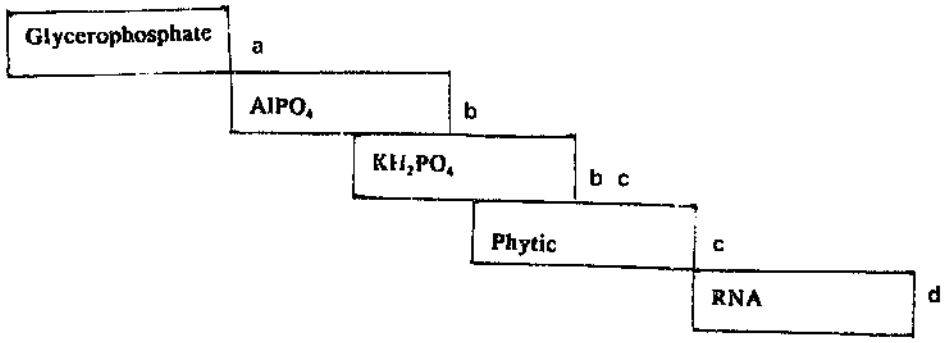


Figure 7.10 Utilization of various P compounds as a P source, isolate WITS 04. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Phosphorus sources listed in descending order according to growth rates.

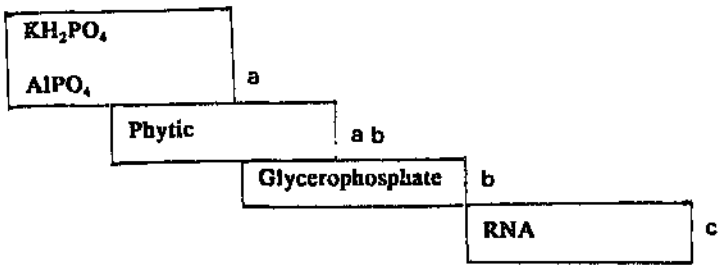


Figure 7.11 Utilization of various P compounds as a P source, isolate WITS 06. Overlapping blocks (similar letters) and compounds within the same block are not significantly different with regard to their overall growth rates. Compounds shown in blocks that do not overlap (different letters) are significantly different ($p \leq 0.05$) from each other. Phosphorus sources listed in descending order according to growth rates.

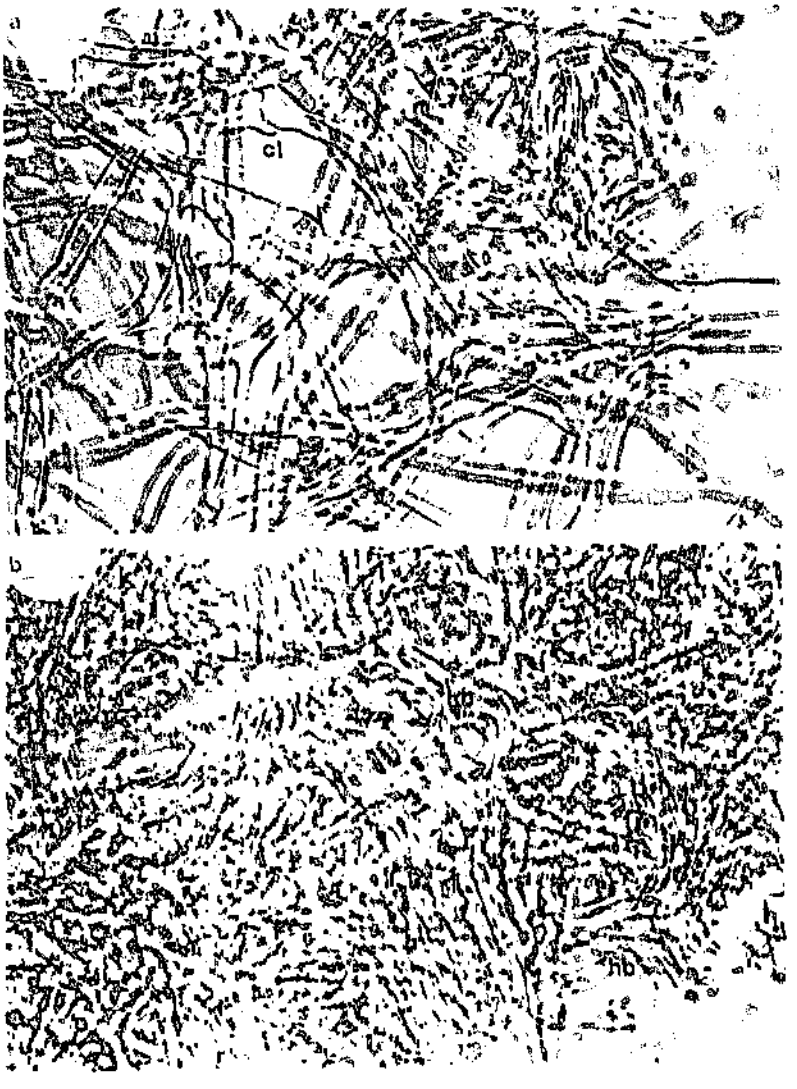


Plate 8

Anatomical characteristics

Dolipore/Woronin bodies: none observed

Lactifers: none

Outer mantle: (Plate 8.3a)

Hyphal arrangement : Plectenchymatous, loose, no distinct pattern

Shape of hyphal cells: Elongated, rectangular with clamp connections

Thickness of walls: 0.3-0.4μm

Length and diameter of hyphal cells: 8-10μm x 3-4μm

Inner mantle: (Plate 8.3b)

Hyphal arrangement: Plectenchymatous, hyphae more compact, showing evidence of ring-like arranged hyphal bundles

Shape of hyphal cells: elongated, rectangular

Thickness of walls: 0.3-0.4μm

Length and diameter of hyphal cells: 4-5μm x 3-4μm

Rhizomorphs: (Plate 8.4a, b & c)

Colour: pale yellow

Connection with mantle: restricted

Margin: smooth

Mode of ramification: undifferentiated, hyphae compactly woven.

Mode of hyphal connections: anastomoses between middle part of hyphal cell and a clamp and between inverted clamps

Arrangement of hyphae: Parallel, interwoven, branching

Diameter: 22.0 ± 8.0μm

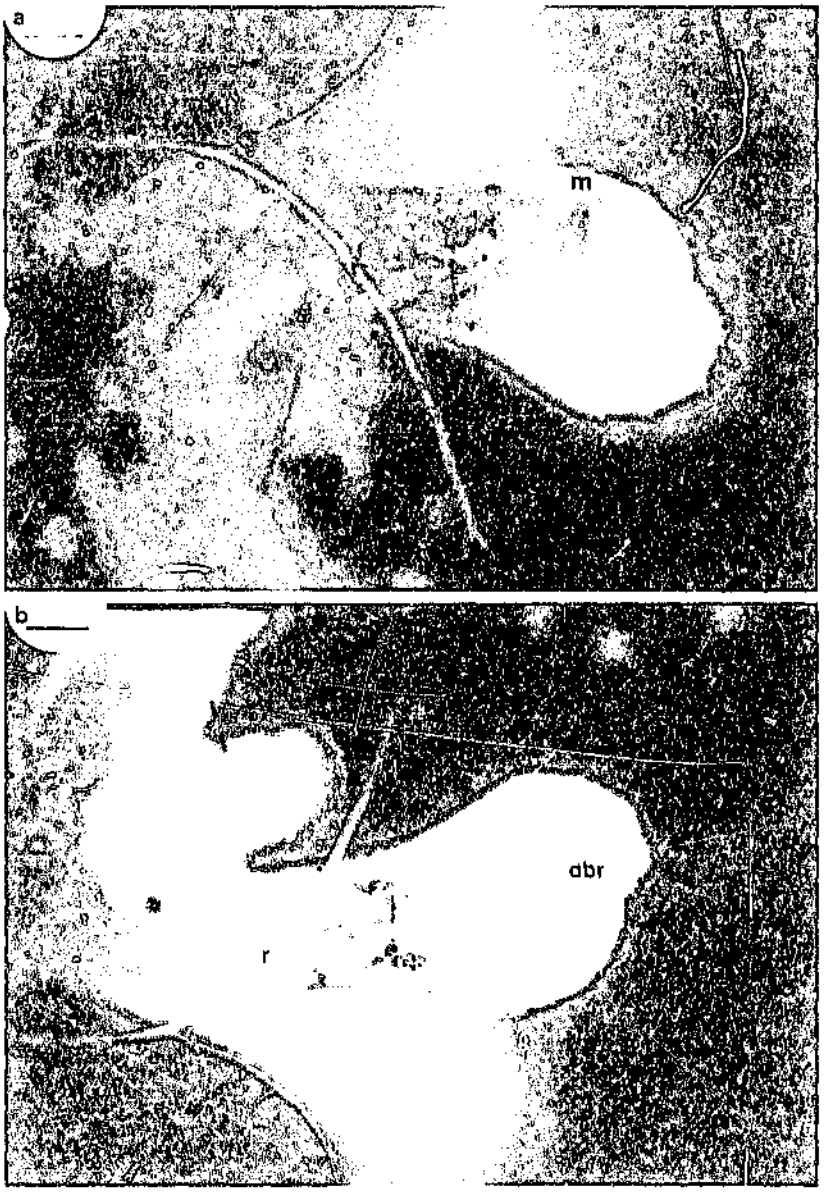


Plate 8.2 [illegible]

Chemical tests

Cotton blue-lactophenol - whole: blue

cross section: cortical cells, blue; hyphae, blue

Toluidine Blue 0.1% - whole: mantle no change; roots, blue

cross section: cortical cells, blue

Ethanol 70% - whole: no change

cross section: no change

KOH 15% - whole: no change

cross section: no change

Lactic acid - whole: no change

cross section: no change

Melzer's reagent - whole: orange

cross section: hyphae, pale yellow

IKI - whole: no chnge

cross section: no change

Sulfo-vanillin - whole: red

cross section: cortical cells, red

(no change after rinsing unless stated)

Autofluorescence

390-420nm: entire mycorrhiza fluoresces green (Plate 8.2a)

450-490nm: entire mycorrhiza fluoresces yellow (Plate 8.2b)

Siderophilous granulations: none

Plate 8.1 WITS-3: [illegible]

8.2.4 Photography

Photographic records of the whole mycorrhizas were taken using a Wild 7A dissecting photomicroscope. Ectomycorrhizal roots were placed in a petri dish and submerged in water. To obtain good quality photographs ECM roots were photographed on a black background (Agerer, 1987-1995). Sectioned ECM roots were examined and photographed with a Zeiss photomicroscope III, using Normarski's Differential Interference Contrast (NIC). This microscope was also used to examine autofluorescence.

8.3 RESULTS

8.3.1 Identification of ectomycorrhizal roots

ISOLATE WITS 01

Morphological characteristics

<u>Whole mycorrhiza:</u> (Plate. 8.1a & b)

Type of ramification: dichotomous

Length of ramified system: 2294.8 ± 98.8µm

Length of unramified ends: 841.3 ± 218.2µm

Diameter of unramified ends: 385.6 ± 13.0µm

Shape of unramified ends: straight

<u>Mantle:</u>

Mantle description: shiny; smooth

Mantle colour: white

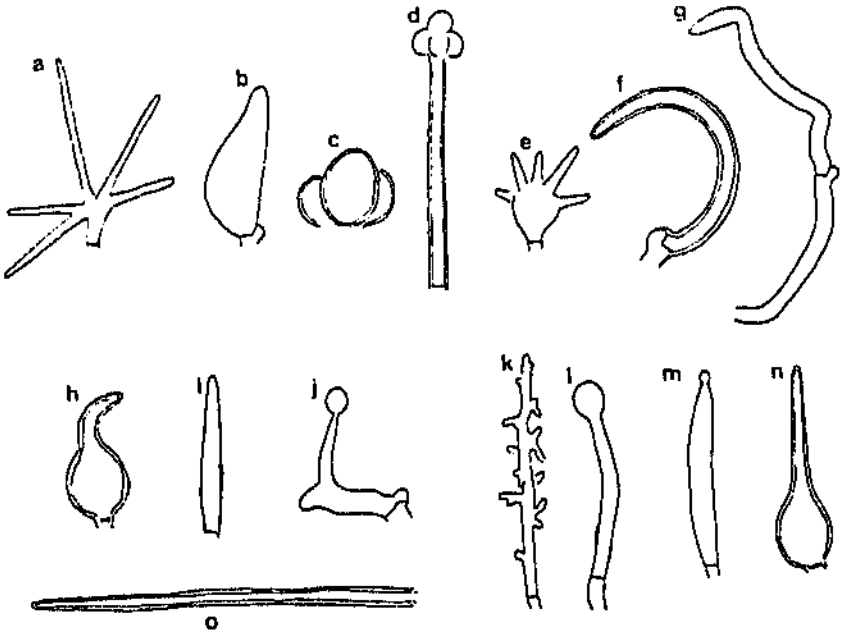


Figure 8.9 Shape of cystidia; *a* - ramified; *b* - fusiform; *c* - globular; *d* - bristle-like with apical lobes; *e* - bottle-shaped with finger-like outgrowths; *f* - sickle-shaped; *g* - normal hyphaa, often twisted like a cork-screw; *h* - bottle-shaped with a bent neck; *i* - thin-walled, slightly acuminated; *j* - lateral, eith tapering, knob-bearing outgrowth; *k* - acanthocystidia; *l* - capitate; *m* - flask-shaped with apical knob; *n* - bottle-shaped with straight neck and *o* - awl-shaped, bristle-like (Agerer, 1987-1995).

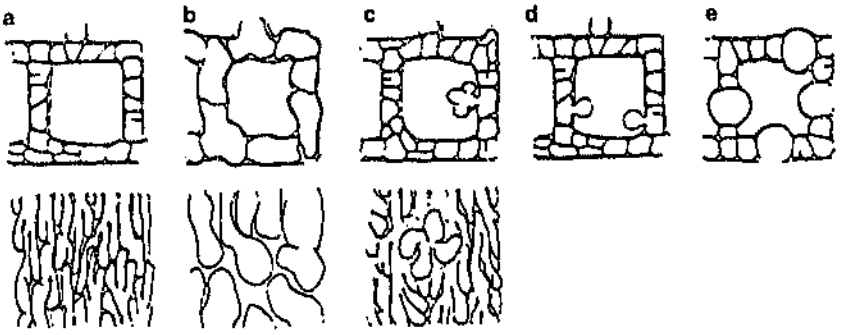


Figure 8.10 Shape of 'Hartig' net; *a* - common type with typical palmetti and single hyphal rows; *b* - coarse 'Hartig' net with broad infrequently ramified lobes; *c* - like type *a*, but lobed or ramified haustoria originate from the 'Hartig' net; *d* - like type *a*, but small globular haustoria-like structures occur and *e* - beaded 'Hartig' net with globular thickenings, often combined with haustoria (Agerer, 1987-1995).

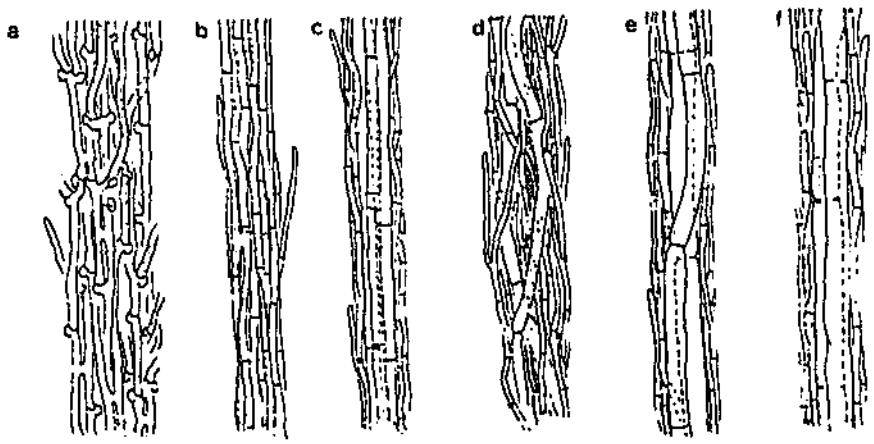


Figure 8.7 Arrangement of hyphae within rhizomorph; *a* - undiffrerentiated, hyphae loosely woven; *b* - undifferentiated, hyphae compactly arranged; *c* - slightly differentiated, central hyphae somewhat enlarged; *d* - differentiated, randomly distributed very thick hyphae; *e* - differentiated, thick hyphae forming a central core and *f* - highly differentiated, thick hyphae forming a central core, septa partially or completely dissolved (Agerer, 1987-1995).

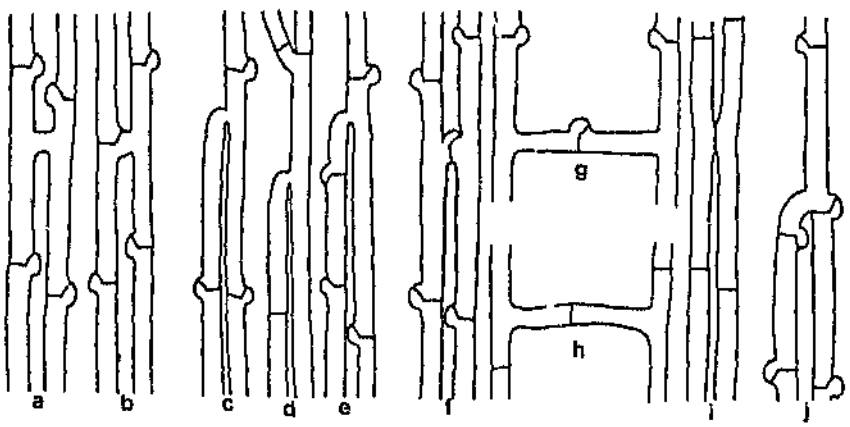


Figure 8.8 Hyphal anastomoses; *a* - between middle parts of hyphal cells; *b* - between middle part of hyphal cell and a clamp; *c* - hyphal tip with a hypha (each with clamps); *d* - the same without clamp; *e* - reversely oriented clamp, after anastomosis of a hyphal tip with a hypha; *f* - contact-clamp; *g* - anastomosis with a clamp; *h* - anatomosis with a septum; *i* - contact-septum and *j* - reversely orientated ramification (Agerer, 1987-1995).

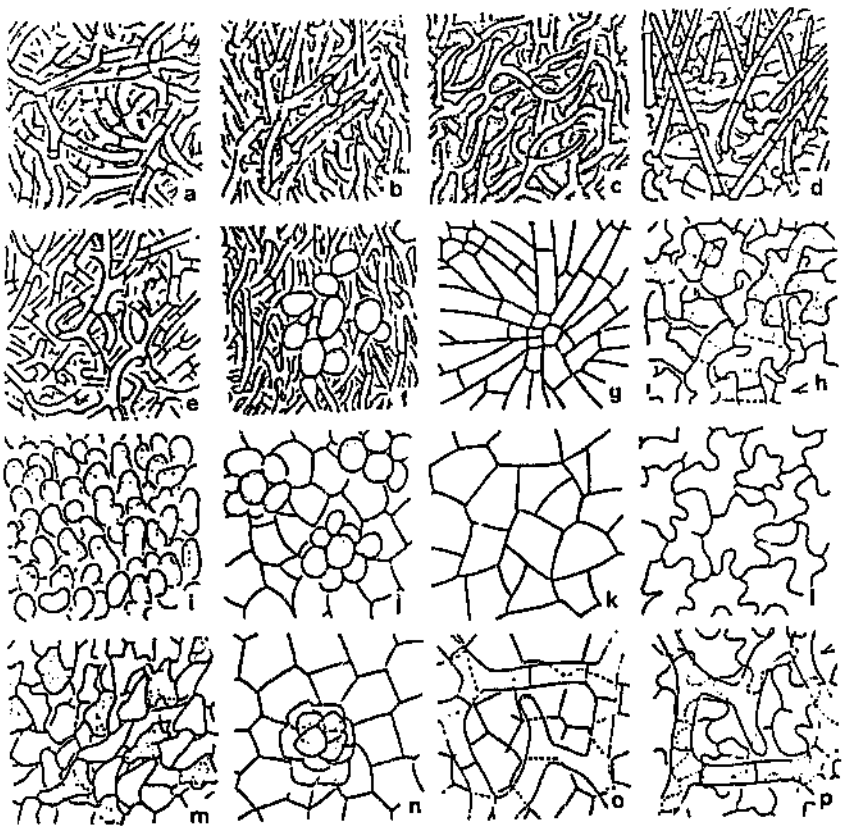


Figure 8.6 Mantle types; *a* - plectenchymatous with ring-like arrangement of hyphal bundles; *b* - plectenchymatous, no pattern discernable; *c* - plectenchymatous with gelatinous matrix between the hyphae; *d* - plectenchymatous, hyphae arranged net-like with prominent cystidia; *e* - plectenchymatous, hyphae arranged net-like, repeatedly branched; *f* - plectenchymatous with occasional roundish cells lying on the mantle; *g* - plectenchymatous, hyphae in star-like arrangement; *h* - transitional form between plectenchymatous and pseudoparenchymatous, irregularly shaped hyphae form a coarse net; *i* - plectenchymatous, mantles with hymeniform; *j* - pseudoparenchymatous, composed of angular cells, bearing mounds of roundish cells; *k* - pseudoparenchymatous, mantles with angular cells; *l* - pseudoparenchymatous, mantles with epidermoid cells; *m* - pseudoparenchymatous, mantles with some cells containing droplets, staining in sulfo-vanillin; *n* - pseudoparenchymatous, mantles with angular cells and mounds of flattened cells; *o* - pseudoparenchymatous, mantles with angular cells bearing a delicate hyphal net and *p* - pseudoparenchymatous, with epidermoid cells bearing a delicate hyphal net (Agerer, 1987-1995).

- Small sections of the mantle were removed with fine forceps and examined microscopically with Normarski's interference contrast (NIC). The arrangement of the mantle hyphae from the outer to the inner surface (Fig. 8.6) and the presence of a dolipore septum, woronin bodies and lactifers were recorded (Agerer, 1987-1995).

- Rhizomorphs were examined microscopically with NIC noting the arrangement of hyphae (Fig. 8.7) and mode of hyphal connections (Fig. 8.8) (Agerer, 1987-1995).

- Emanating hyphae were examined for septa, thickness of walls and diameter. The presence and shape of cystidia was also noted (Fig. 8.9).

- Cross and longitudinal sections (± 50μm thick) of ECM roots were made using a cryomicrotome (Brundrett, Melville & Petersen, 1994). Sections were mounted in polyvinyl lactic acid (Omar, Bollard & Heather, 1979) and examined microscopically using NIC for mantle thickness, shape of tannin and cortical cells, thickness of cell walls and arrangement of 'Hartig' net (Fig. 8.10) (Agerer, 1987-1995).

Many characteristics are used in Agerers' (1987-1995) atlas to describe ECM and a few of the basic characteristics have been mentioned above. In the following results section a more complete list of characteristics, modified from Check-List A, B, C1, C2, D1 and D2 (Agerer, 1987-1995) will be provided for each of the mycorrhizal types examined.

8.2.3 Tracing mycorrhizal links to sporocarps

Sporocarps occurring in the study area were recorded and identified using various identification manuals (Levin *et al.*, 1987; Singer, 1962; Van Der Westhuizen & Eicker, 1994). Sporocarps and the litter in which they were growing were removed and any connections with rhizomorphs and mycorrhizal roots were examined using a hand-held magnifying glass.

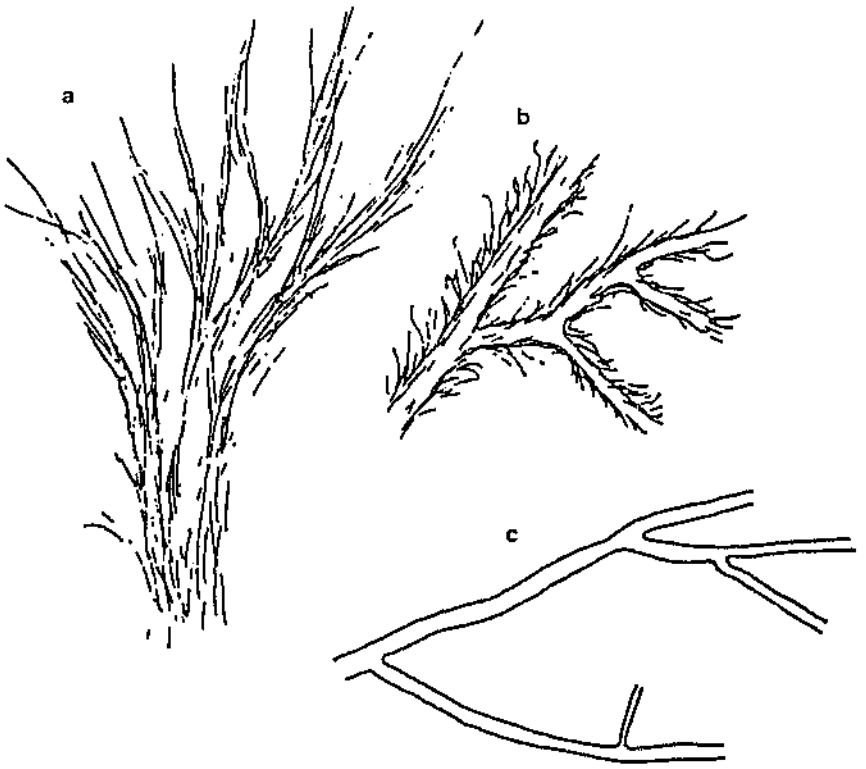


Figure 8.5 Shape of rhizomorphs; *a* - interconnected filaments; *b* - smooth margins and *c* - ensheathed or hairy (Agerer, 1987-1995).

mycorrhizas, mounted in lactic acid, were examined with a fluorescent microscope using a UV (390-420nm) and a Blue (450-490nm) filter, to assess autofluorescence. The occurrence of siderophilous granulations in either the mantle or emanating hyphae was also recorded (Agerer, 1986; 1987-1995).

Anatomical characteristics

Recent studies have shown that ectomycorrhizas formed by different fungi are structurally distinct, therefore anatomical studies have become increasingly important in the characterization of ECM (Agerer, 1994). Anatomical details were recorded for the mantle, rhizomorphs, emanating hyphae and cross and longitudinal sections of the ECM roots. The procedures and characteristics examined were as follows:

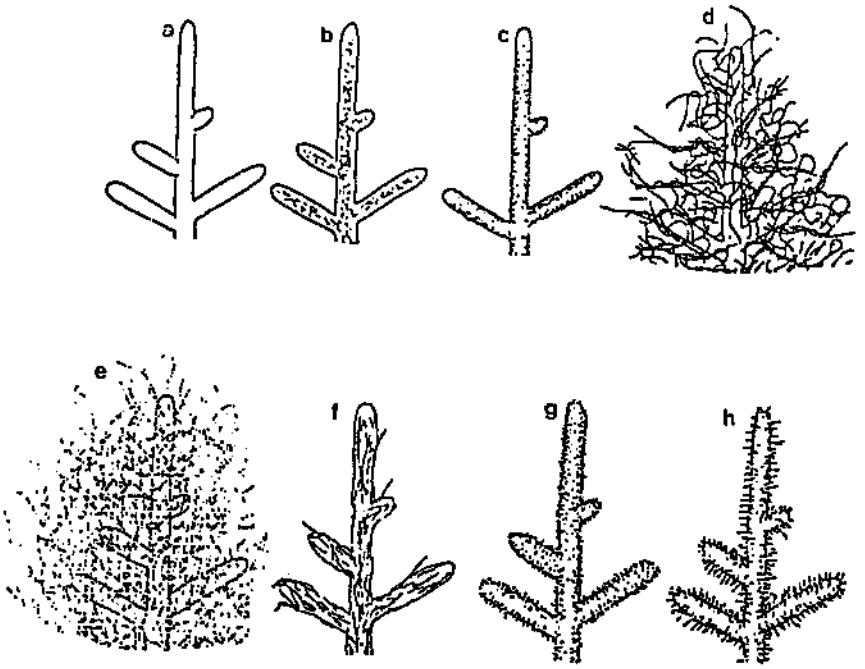


Figure 8.3 Features of the mantle surface; *a* - smooth; *b* - reticulate; *c* - grainy (warty); *d* - woolly; *e* - cottony; *f* - stringy; *g* - short-spiny and *h* - long-spiny (Agerer, 1987-1995).

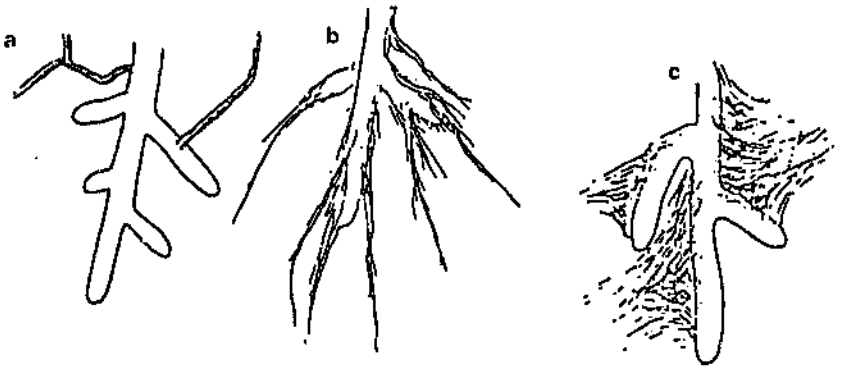


Figure 8.4 Rhizomorphal connection with mantle; *a* - restricted; *b* - rhizomorphs growing off in flat angles and *c* - hyphal fans (Agerer, 1987-1995).

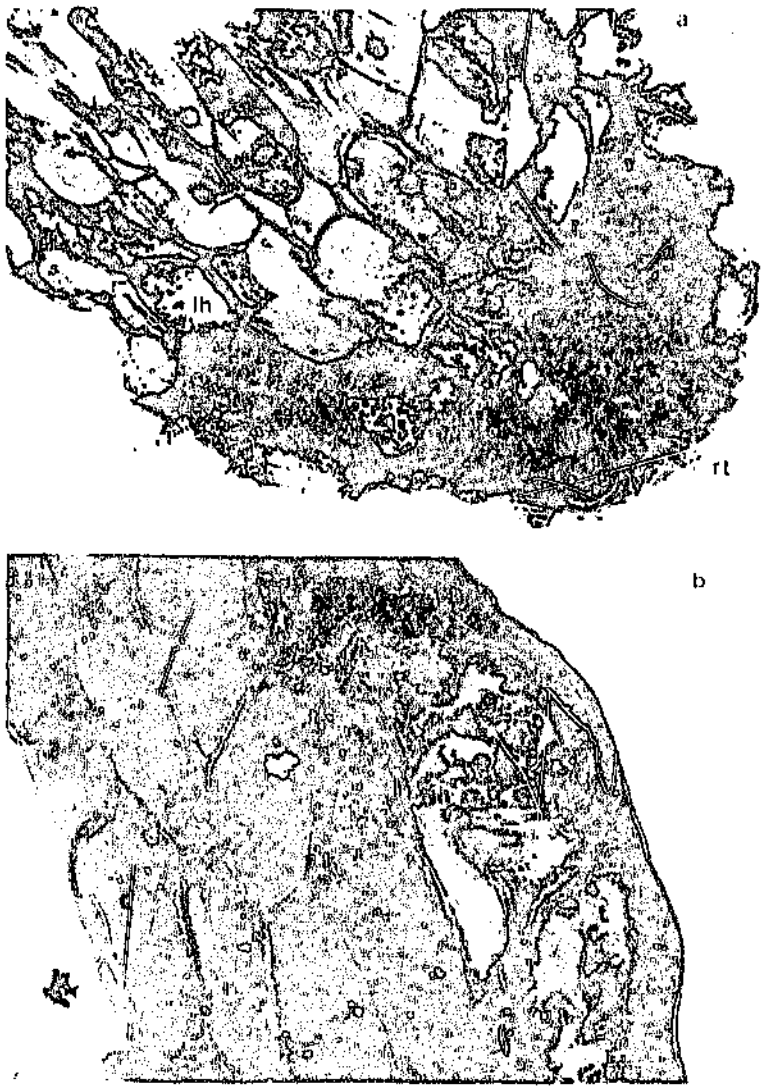
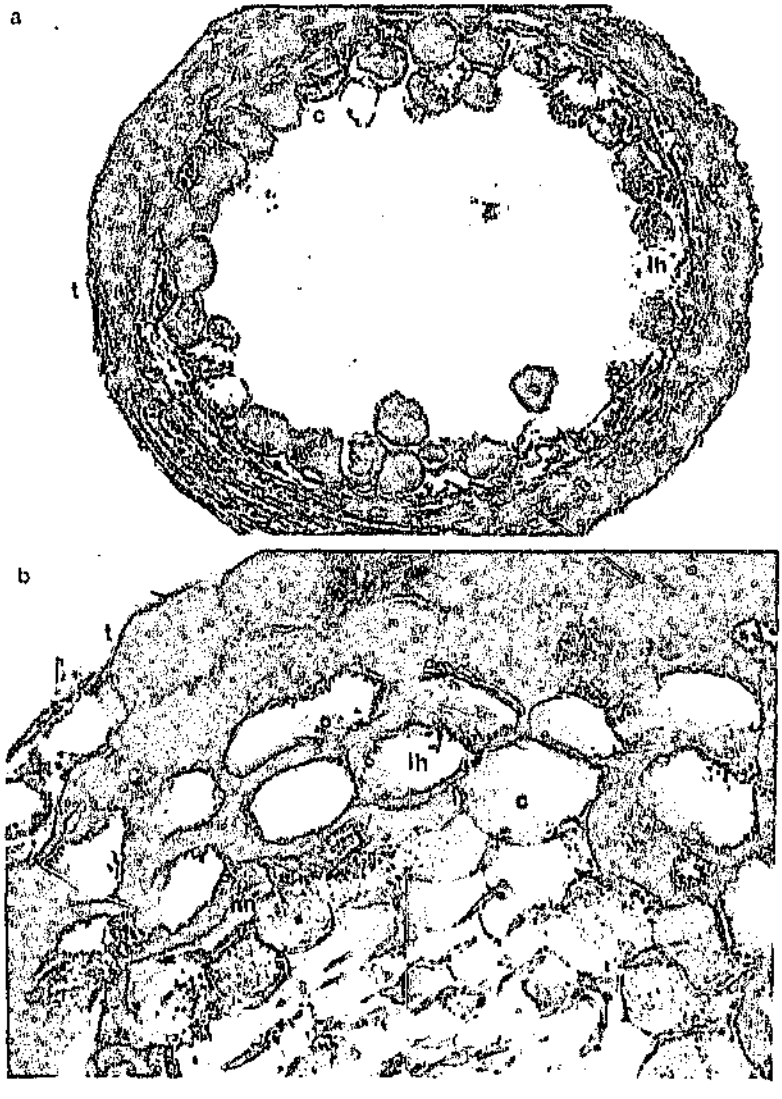


Plate 8.10 [illegible]


a
c
lh
t
b
t
lh
c
m

Cystidia:

Occurrence: none

Cross and longitudinal sections:

Cross sections: (Plate 8.9)

Root cells with 'Hartig' net:

Tannin cells - number of rows: 1-5

- shape: rectangular

- measurements: $8.7 \pm 1.2\mu m \times 1.56 \pm 0.2\mu m$

Cortical cells - number of rows with 'Hartig 'net: 1-3

- shape: oval to circular

- measurements: $6.5 \pm 0.7\mu m \times 5.4 \pm 0.9\mu m$

'Hartig' net:

Thickness of 'Hartig' net around tannin cells:

cortical cells: $0.5 \pm 0.1\mu m$

Number of hyphal rows around tannin cells: 1

cortical cells: 1

Shape of 'Hartig' net around tannin cells: common type A, palmetti

cortical cells: type A, palmetti with lobed haustoria originating from 'Hartig' net

Longitudinal sections: (Plate 8.10)

Root cells with 'Hartig' net:

Tannin cells - number of rows: 1-5 rows

- shape: oval to rectangular

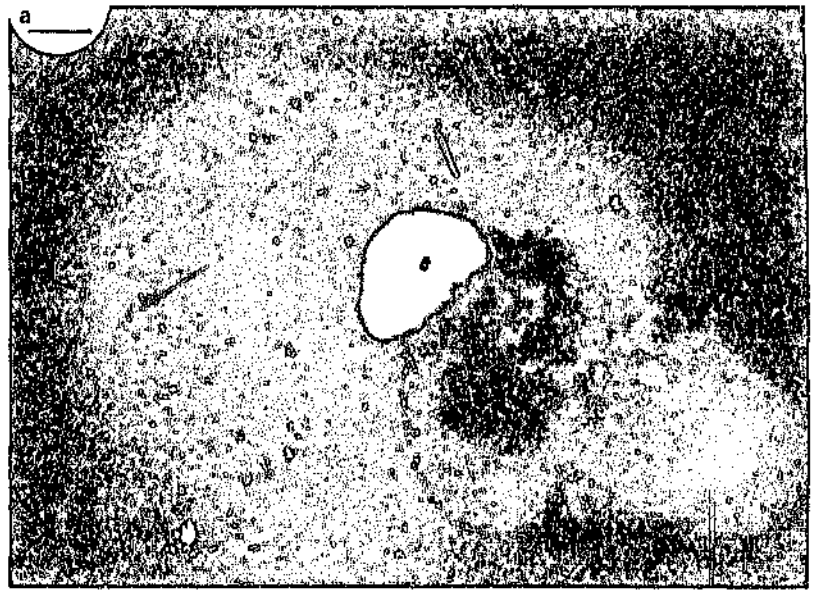


Plate 8.8 WUS 16. Autofluorescence of ectomycorrhizal root tip. *a* = 390–420nm fluoresces green at tip (Scale bar = 70μm)

Lactic acid - whole: no change

cross section: no change

Melzer's reagent - whole: no change

cross section: no change

IKI - whole: no change

cross section: no change

Sulfo-vanillin - whole: red

cross section: tannin and cortical cells red

(no change after rinsing unless stated)

Autofluorescence

390-420nm: fluoresces green at tip (Plate 8.8)

450-490nm: fluoresces green at tip

Siderophilous granulations: None

Anatomical characteristics

Dolipore/Woronin bodies: none observed

Lactifers: none

Mantle: none

Rhizomorphs: none

Emanating hyphae:

Occurrence: rarely

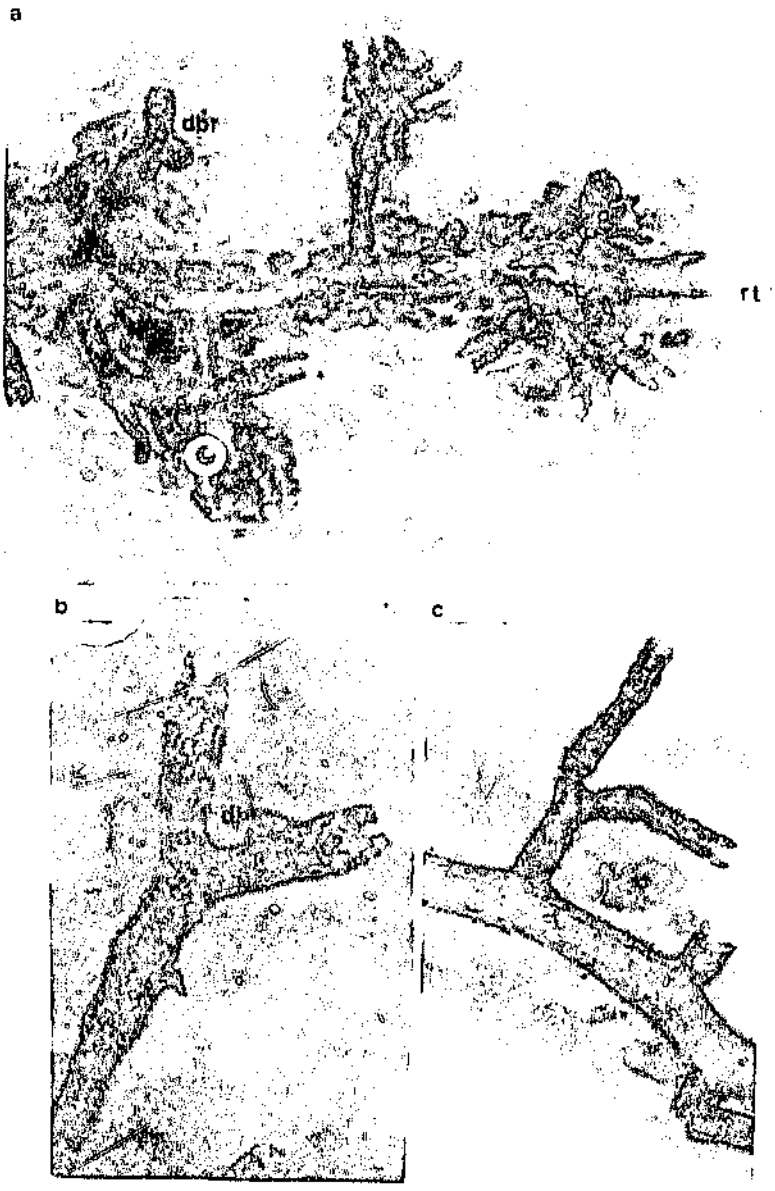


Plate 8.7 WILSON. [illegible]

ISOLATE WITS 06

Morphological characteristics

Whole mycorrhiza: (Plate 8.7)

Type of ramification: simple to dichotomous

Length of ramified system: simple - 866.5 ± 305.9μm,

dichotomous - 1660.3 ± 622.2μm

Length of unramified ends: 614.0 ± 188.3μm

Diameter of unramified ends: 348.6 ± 36.2μm

Shape of unramified ends: straight, older mycorrhizas occasionally beaded

Mantle:

Mantle description: No distinct mantle, mycorrhiza smooth, sparse hyphae occurring on root surface

Chemical tests

Cotton blue-lactophenol - whole: blue

cross section: cortical cells, blue; hyphae, blue

Toluidine Blue 0.1% - whole: blue

cross section: cortical cells, blue; hyphae, blue

Ethanol 70% - whole: no change

cross section: no change

KOH 15% - whole: no change

cross section: no change

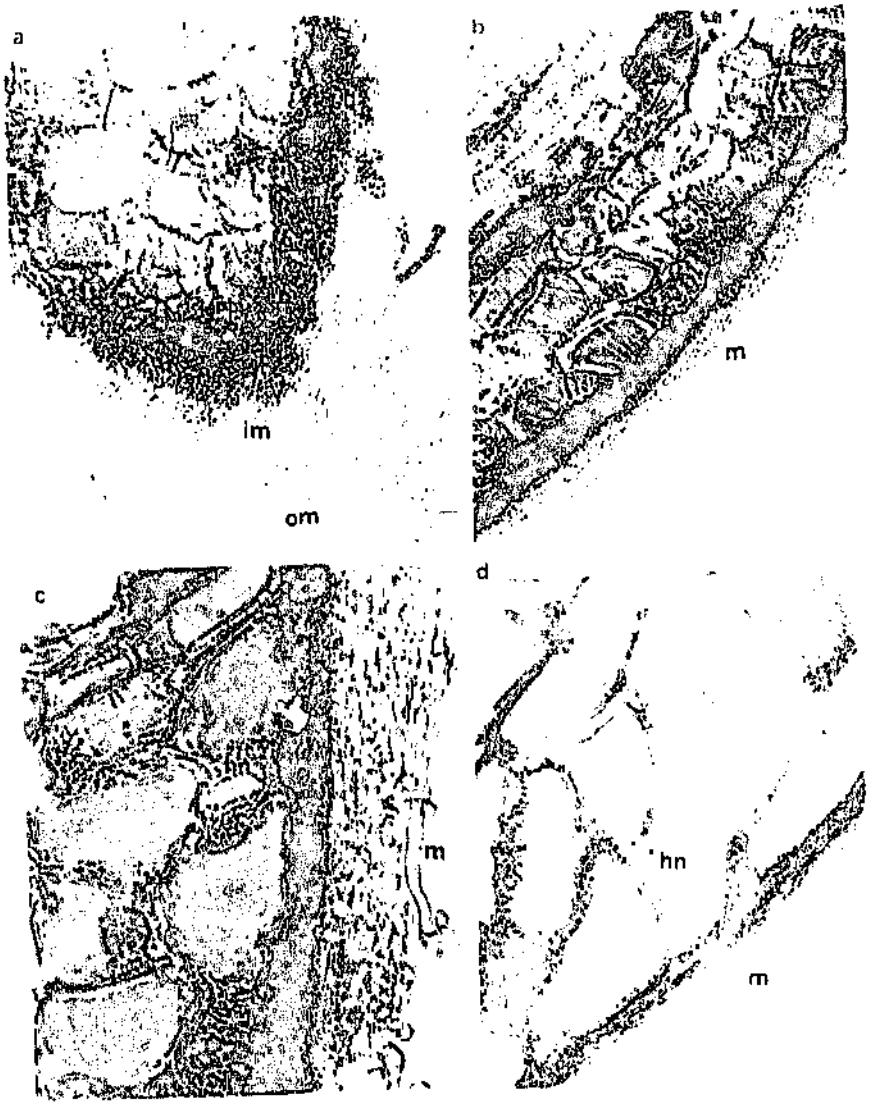


Plate 8.6

'Hartig' net:

Thickness of 'Hartig' net around tannin cells: 0.5-1.0μm

cortical cells: 2.7-5.4μm

Number of hyphal rows around tannin cells: 1

cortical cells: 1-2

Shape of 'Hartig' net around tannin cells: common type A, palmetti

cortical cells: common type A, palmetti

Longitudinal sections: (Plate 8.6)

Measurements of mantle hyphae - tangential: 5.6μm

- radial: 0.56μm

- thickness of wall: 0.3-0.5μm

Root cells with 'Hartig' net:

Tannin cells - number of rows: 1

- shape: rectangular

- measurements: $3.5 \pm 1.2\mu m \times 2.4 \pm 0.5\mu m$

Cortical cells - number of rows with 'Hartig' net: 1-2

- shape: oval to rectangular

- measurements: $4.9 \pm 1.2\mu m \times 6.8 \pm 1.0\mu m$

'Hartig' net:

Thickness of 'Hartig' net around tannin cells: 1-2μm

cortical cells: 2-4μm

Number of hyphal rows around tannin cells: 1

cortical cells: 1

Shape of 'Hartig' net around tannin cells: common type A, palmetti

cortical cells: common type A, palmetti

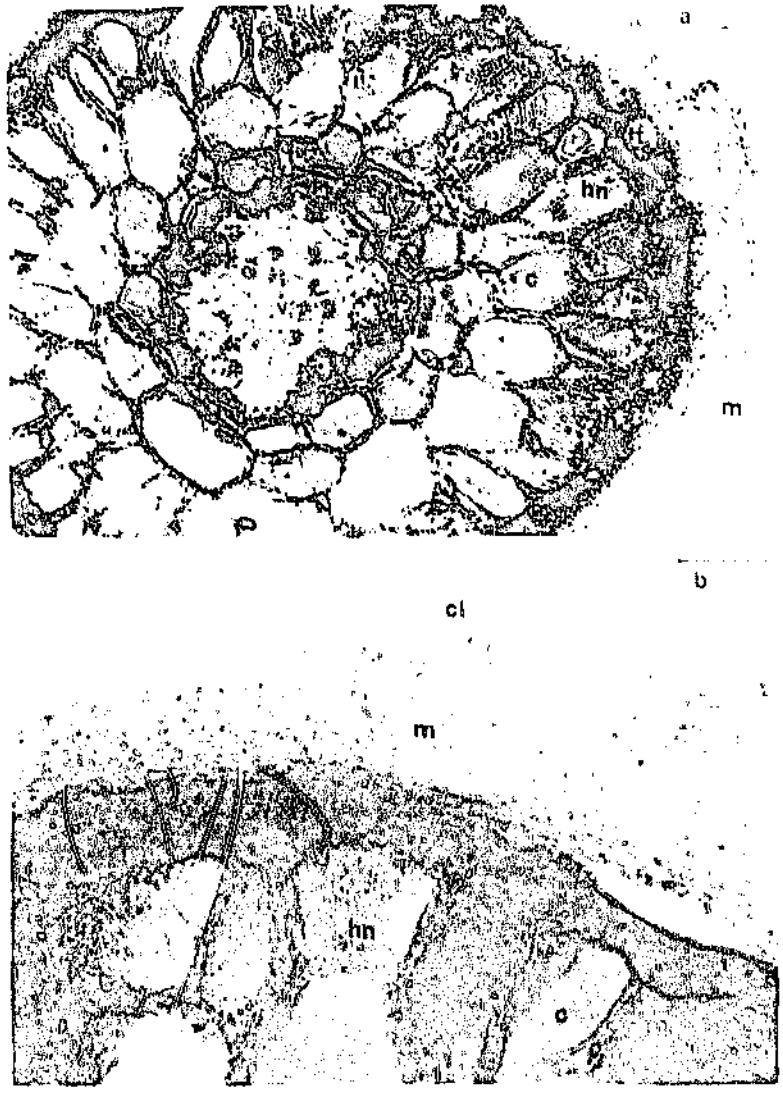


Plate 8

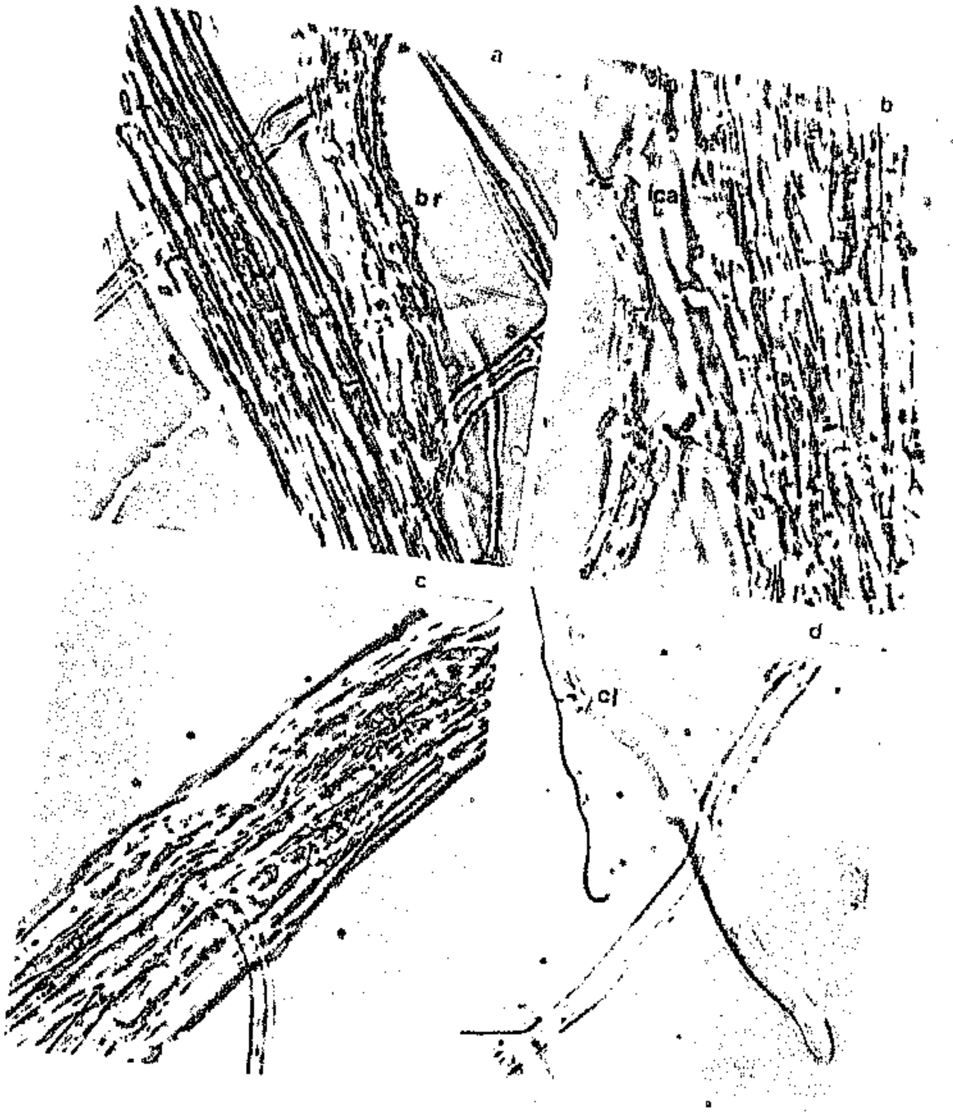


Plate 8.4 [illegible]

hyphae [illegible]

Emanating hyphae: (Plate 8.4d)

Occurrence: occasional, hyaline

Septa: present with clamp connections

Diameter: 5μm

Thickness of wall: 0.3-0.5μm

Cystidia:

Occurrence: None

Cross and longitudinal sections:

Mantle thickness: 22.7±7.8μm

Cross sections: (Plate 8.5)

Measurements of mantle hyphae - tangential: 2.8μm

- radial: 0.28-0.56μm

- thickness of wall: 0.2-0.5μm

Root cells with 'Hartig' net:

Tannin cells - number of rows: 1

- shape: oval to rectangular

- measurements: 10.8 x 11.5μm

Cortical cells - number of rows with 'Hartig' net: 1-2

- shape: oval

- measurements: 58.7±14.2μm x 35.1±8.1μm

application may also be of importance *i.e.* broadcasting the lime onto the litter or rotovating into the litter. Field trials of at least 3 years duration (Morris, 1986) would be required to determine the effectiveness of lime applications and these studies should also monitor decomposition, microbial activity and biomass and changes in the ECM fungal population composition.

Little can be done to increase ambient temperatures, in the short term (global warming excluded) and increasing the temperature of the forest floor is a difficult task. Measures can be taken to increase the planting distance of trees (currently 2.7m) to allow for a more open canopy structure (Singh & Gupta, 1977). This would allow radiation to reach the forest floor, thus increasing surface temperatures. However, the cost of reducing the number of stems per hectare would have to be carefully analyzed. Reducing the stems per hectare (sph) would reduce or eliminate the need for thinning, an operation which supplies timber for pol- he loss of income from thinning operations would have to be taken into account. However, forestry ma-gement strategies indicate a move to increase the number of sph with a reduced rotation. The more open canopy structure resulting from reduced sph plantings would allow increased understorey growth of weeds and other vegetation, which increases the competition for nutrients and moisture reducing tree growth and hence production. However, understorey vegetation even if not of commercial value could promote N cycling and help mitigate potential soil degradation as the litterfall is more easily decomposed by soil micro-organisms. A strategy of mixed plantations could act as a N pump and also help to maintain higher levels of available N in the litterfall which is readily mineralized without accumulating on the forest floor (Liu, 1995). Several mixed plantation projects have been conducted in South Africa involving interplanting *Pinus* with groundnuts and *Eucalyptus* with cowpea, however, no decomposition or nutrient dynamic studies have been conducted on these sites. Alternatively, measures could be taken to install water pipes beneath the litter. Water could be heated, using solar energy and recycled through the pipes in order to achieve increased forest floor temperatures. The cost of this measure and the feasibility in terms of security of equipment would need to be assessed.

ensure that *P. patula* planted on high altitude, shale sites would invariably accumulate litter. This emphasises h importance of site/species matching and indicates that shale sites, in the Mpumalanga escarpment area, above an altitude of 1200m should not be planted to *P. patula*. Furthermore any study which examines the factors that influence microbial activity should not ignore the micro-organisms or the processes in which they are involved.

9.1.3 Measures to be taken to improve decomposition

It is important to stress the role of the microbial population in the decomposition process. Efforts should be focused on making conditions in the litter more favourable for microbial activity. It must be remembered that microbial activity does not result from the addition of simple effects, but rather depends on interactions between individual environmental factors (Nicolardt, Fauvet & Cheneby, 1994). The factors which are most important with regard to litter accumulation, are the acidity of the litter and temperature, factors which should be given consideration.

It is di cult to control environmental factors under field conditions. Some management steps could be taken to make conditions more favourable for microbial activity. Alleviating the acidic conditions in the litter could be achieved by the application of dolomitic lime, which provides a source of both the cations Ca^{2+} and Mg^{2+}. The application of dolomitic lime has also been suggested as a measure of addressing soil fertility declines as a result of acid rain. It has been suggested that the application of lime could be carried out prior to planting at the start of each rotation (Olbrich, 1995). However, acid rain also increases needle loss and liming has been shown not to improve needle retention therefore, high litter production would prevail. Liming has been shown though to improve decomposition and reduce litter layers (Huettl & Zoettl, 1993). Litter accumulation was shown to increase with stand age and in high altitude sites maximum litter depths were recorded in 25 year old stands. The application of dolomitic lime prior to planting may not be sufficient to maintain increased pH throughout the rotation; therefore, mid rotation applications may have to be considered. The method of

influenced by rainfall; however, as no evidence was found to suggest that anaerobic conditions developed in the litter (reinforced by the presence of numerous ECM roots and hyphal threads) increased rainfall was probably not responsible for the litter accumulation observed.

Temperature has also been shown to be positively correlated with decomposition with increased temperatures stimulating microbial activity (Kirschbaum, 1995). The cooler temperatures of the higher altitude sites are considered to be the main cause of litter accumulation. The mean monthly minimum temperature during June, at the Mokobulaan station (high altitude) during 1993 was 7.55°C (lowest temperature 4.50°C) while at the D.R. De Wet station (low altitude), 10.95°C was recorded (lowest temperature 8.58°C).

It is important to stress that in Schutz's (1990) study several site factors were found to be correlated with litter accumulation. Lower temperatures in high altitude sites which results in retarded decomposition rates may be critical in combination with other site factors. These factors included;

- geology: thin litter layers occurring on soils derived from dolomite with thick litter layers occurring on soils derived from Timeball (shale);
- subsoil colour: litter thickness increases as the hue of the B horizon changes from red to yellow, a yellowing subsoil indicating increasing wetness. This can be linked to the higher rainfall at high altitudes;
- soil texture: litter thickness decreased with increasing coarse sand in the A horizon, indicating better drainage;
- site index: Schutz (1990) suggested that this correlation implied that litter decomposition was strongly dependent on favourable environmental conditions. This variable encompasses several site and environmental factors (Schutz, 1990).

However, none of the above factors could be solely responsible for influencing the breakdown of litter. Litter decomposition, being a biological process, is ultimately controlled by the micro-organisms. The unfavourable conditions in the micro-environment caused as a result of the cooler temperatures and acidic conditions would

not all micro-organisms are easily cultured and therefore not included in standard enumeration techniques (Wollum II, 1982), enzyme activity should also be monitored (Kshattriya, Sharma & Mishra, 1992). Differences in the microbial populations can therefore not be excluded as a possible explanation for reduced decomposition. This study showed that soil fauna were not an important influence on decomposition at the time of sampling. However, this area would also require further investigation.

9.1.2 Abiotic factors

Regardless of any differences there may be between the microbial population numbers at low and high altitude sites, microbial activity and biomass itself may be altered by a number of abiotic factors. The pH of the soil and litter is one of these factors. Thick litter layers have been shown to be correlated with soil acidity (Schutz, 1990). However, it is difficult to determine whether soil acidity is the result of or the cause of litter accumulation. Afforestation has been shown to acidify the soil (Du Toit, 1993). The accumulated litter layers are very acidic, decreasing to a pH of 2.16 in the F layer (Table 6.2; chapter 6), although the litter leachate was also shown to be acidic (pH 3.88) (Table 6.4; chapter 6). The soil beneath the litter was less acidic (Table 6.2; chapter 6) probably a result of the buffering capacity of the soil (Sanchez, 1976). This indicates that conditions unfavourable for microbial activity exist in the litter and this would affect decomposition and possibly nutrient uptake by the ECM. The continued accumulation of litter, in combination with acid rain, would exacerbate these unfavourable conditions. Microbial activity and biomass may be enhanced under higher pH conditions which allows the enzymes involved in the degradation of the organic matter to function under optimal conditions (Alexander, 1977).

Rainfall influences the decomposition process, during both the initial leaching phase and the biological phase, by maintaining the moisture content of the litter at a level which sustains microbial activity (Vanlauwe *et al.*, 1995). High altitude stands receive more precipitation, both as a result of higher rainfall and the frequent occurrence of mists on the escarpment. Depending on the drainage of the site, this may result in anaerobic conditions in the forming within the litter. The initial phase of decomposition (Phase M1) was shown not to be influenced by altitude (Section 5.3.3; chapter 5). The leaching of soluble compounds from litter is

9 CONCLUSIONS AND RECOMMENDATIONS

9.1 WHY DOES LITTER ACCUMULATE UNDER *P. patula* IN HIGH ALTITUDE SITES?

Litter accumulation has been shown both by Schutz (1990) and this study to increase with increasing altitude. Altitude, however, has been shown not to influence litter production (Fig. 4.4; chapter 4). Therefore, increased litter production, in high altitude sites, is not responsible for the litter accumulation observed under *P. patula*. Litter production is a process by which organic matter enters the ecosystem and this input process would ideally be balanced by the output or release of nutrients through the decomposition of litter. As the input of litter does not increase with increasing altitude, the imbalance between the input and output process must be a result of the decomposition process.

The rate of decomposition was shown to decrease during the second phase of decomposition (Phase M2, 6-52 weeks), with increasing altitude (Fig. 5.16; chapter 5). This was further emphasized on examination of the decomposition constants (Table 5.6; chapter 5). Many factors, both abiotic and biotic, regulate the decomposition process and a combination of these may be responsible for the reduced decomposition rates observed in high altitude sites (Dickinson & Pugh, 1974).

9.1.1 Biotic factors

An examination of biotic factors which influence decomposition revealed that litter quality did not decrease with increasing altitude (Chapter 5). *P. patula* growing in high altitude sites does not have a poorer litter quality than trees growing at lower altitudes. Enumeration of the microbial populations at both a low and a high altitude site showed no significant differences at the time of sampling, although there were indications that fungal counts were higher in high altitude sites and both bacterial and fungal counts generally decreased down through the litter/soil profile (Figs. 5.25 & 5.26; chapter 5). This area would, however, require further study. Such a study should examine seasonal changes in the composition of the bacterial and fungal populations. As

descriptions provided are the most comprehensive for mycorrhizas of any *Pinus* spp. in South Africa. The understanding of forest ecosystems has become crucial in determining how to sustain long-term timber production. These ecosystems cannot be fully understood if the role of ECM is ignored. Ectomycorrhizas formed by different fungi have been shown to be structurally distinct and studies have indicated that different species behave different in several respects such as nutrition and growth-promoting efficiences (Agerer, 1994). It is therefore essential that ECM are correctly identified so that interactions observed and studied can be correctly interpreted. The lack of adequate ECM descriptions has hampered mycorrhizal research in this country. The author suggests that a project be initiated to document and describe ECM associated with *Pinus* spp. growing in the country. As an exotic host with a limited range of potential mycorrhizal fungi this would be not only a possible but a worthwhile task. On the basis of this, decisions regarding the introduction of other ECM species more suited to our climatic and growing conditions could be taken.

Table 8.1 Ectomycorrhizal fungi associated with *P. patula* growing in South Africa.

Species	Reference / Comment
Scleroderma citrinum	This study
Boletus pinicola	This study
Boletus edulis	Marias & Kotzé (1977)
Amanita muscaria	Marias & Kotzé (1977)
Tuber rapaeodorum	Marias & Kotzé (1977) / possible ECM, Ascomycetes
Lycoperdon umbrinum	Marias & Kotzé (1977) / possible ECM
Thelephora terrestris	Van Greuning & Van Der Westhuizen (1990) / on seedlings in nurseries

Carlson (1992; 1994) examined ECM roots of *P. patula* in Mpumalanga. However, characterizations were based on colour and morphology. From the descriptions provided it was difficult to assess whether ECM of *S. citrinum* and *B. pinicola* were represented. Species 4 (Carlson, 1992) and Species 3 (Carlson, 1994) may be *S. citrinum* and Species 1 & 2 (Carlson, 1992) and Species 2 (Carlson, 1994) may be *B. pinicola*; however, these relationships cannot be positively confirmed.

8.3 CONCLUSIONS

The mycorrhizal roots of WITS 01 and WITS 06 were characterized as being formed by *S. citrinum* and by *B. pinicola*, respectively. This is the first record of *B. pinicola* occurring in Mpumalanga. The mycorrhizal

The isolate WITS 06 was identified as *B. pinicola*, a species which has not been previously recorded in Mpumalanga (Levin *et al.*, 1987; Van Der Westhuizen & Eicker, 1994). Pearson (1950) recorded the occurrence of *B. pinicola* under *Pinus* in the Western Cape, this was the only known record of the species. The sporocarps occurring in the study site were similar in appearance to *Suillus granulatus* (Van Der Westhuizen & Eicker, 1994), however, no latex exudates or granulations were observed on the stipe. On collection of sporocarps, the yellow pore layer and inner cap tissue showed a blue bruising where touched. The species was also similar to *Xerocomus* (*Suillus*) *badius* as recorded in Levin *et al.*, (1987); however the deep chestnut colour of the cap, characteristic of this species, was not evident (Dickinson & Lucas, 1979). Ectomycorrhizal roots of *X. badius* as shown to occur on *Picea*, in Agerer's (1987-1995) atlas, were not similar to the ECM roots described in this study. Agerer (1994) stated that from the descriptions in the atlas it was possible to make some general comments about the ECM fungi, one being that smooth ECM with scant or no rhizomorphs were chartacteristic of some *Boletus* spp. which supports this studies findings. Species similar to *B. pinicola* that have been recorded as occurring in Mpumalanga include *S. granulatus*, *S. bovinus* and *Boletus edulis* (Lundquist, 1986; Van der Westhuizen & Eicker, 1987 & 1994). These species were observed to occur in various plantations however, none showed the characteristic bruising. *B. pinicola* has been recorded under conifers and some deciduous species (Dickinson & Lucas, 1979).

An updated list of ectomycorrhizal fungi known to be associated with *P. patula* growing in South Africa is presented in Table 8.1.

The majority of the species listed in Table 8.1 were observed to occur under *P. patula* with the exception of *Tuber rapaeodorum* and *Thelephora terrestris*. Other fungal species reported as occurring in *P. patula* plantations and which are known to be ECM are *Tricholoma* sp., *Russula* sp., *Laccaria laccata*, *Rhizopogon luteolus*, *Suillus brevipes*, *S. subluteus* and *Lycoperdon perlatum* (Lundquist, 1986; Miller Jr, 1982; Natarajan, Mohan & Ingleby, 1992; Trappe, 1962). Of these species only *L. laccata* were observed in the Mpumalanga plantations.

8.4 DISCUSSION

8.4.1 Identification of ectomycorrhizal roots

On examination of the morphological ECM types and sporocarps observed occurring at the study site it was concluded that WITS 01 was formed by *S. citrinum* and WITS 06 was formed by *B. pinicola*. The remaining two mycorrhizal isolates used in this project, WITS 02 and WITS 04 (Section 7.2.1; chapter 7) were not positively identified as difficulties were experienced in achieving good mycorrhizal infection under aseptic conditions, this was discussed in the previous chapter (Section 7.4.1; chapter 7).

In Agerer's (1987-1993) atlas an ECM similar to WITS 01 was described for *S. citrinum* forming a mycorrhizal association with *Betula pendula*. These mycorrhizas were similar morphologically and anatomically with regard to mantle and Hartig net type (Agerer 1987-1995). Several differences were however noted and included:

- The ECM roots of *Betula* showed monopodial-pyramidial branching opposed to the dichotomous branching observed on *P. patula*. However, dichotomous branching of ECM roots has only been observed in *Pinus* spp. (Marks & Kozlowski, 1973).
- The rhizomorphs from the *Betula* ECM roots are white as opposed to pale yellow and contain thick central hypha, which was not observed in the rhizomorphs from the *Pinus* ECM roots. No evidence in the literature could be found pertaining to the stability of the rhizomorph anatomy or whether variability in these characteristics is dependant on host species or strains of the fungus. Colour however, is variable, especially with age and photographically may be dependant on the light source (Agerer, 1986).

Several *Scleroderma* species are recognized as being mycorrhizal with a broad host range (Miller Jr., 1982; Trappe, 1962). *S. citrinum* is acidophilous and is mainly distributed on raw humus and peat (Agerer, 1987-1995). In South Africa the species is widely distributed throughout the wetter parts of the country, occurring under *Pinus* spp. (Van Der Westhuizen & Elcker, 1994).

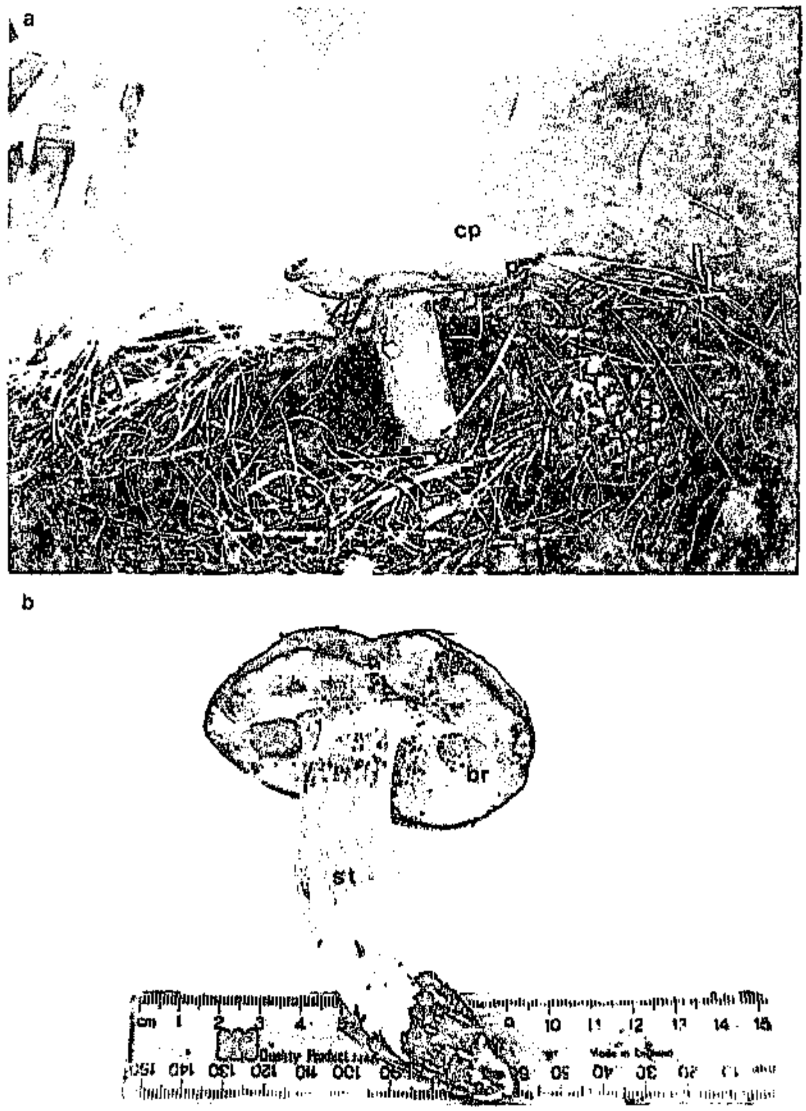


Plate 8.12 [illegible]

characteristic [illegible]

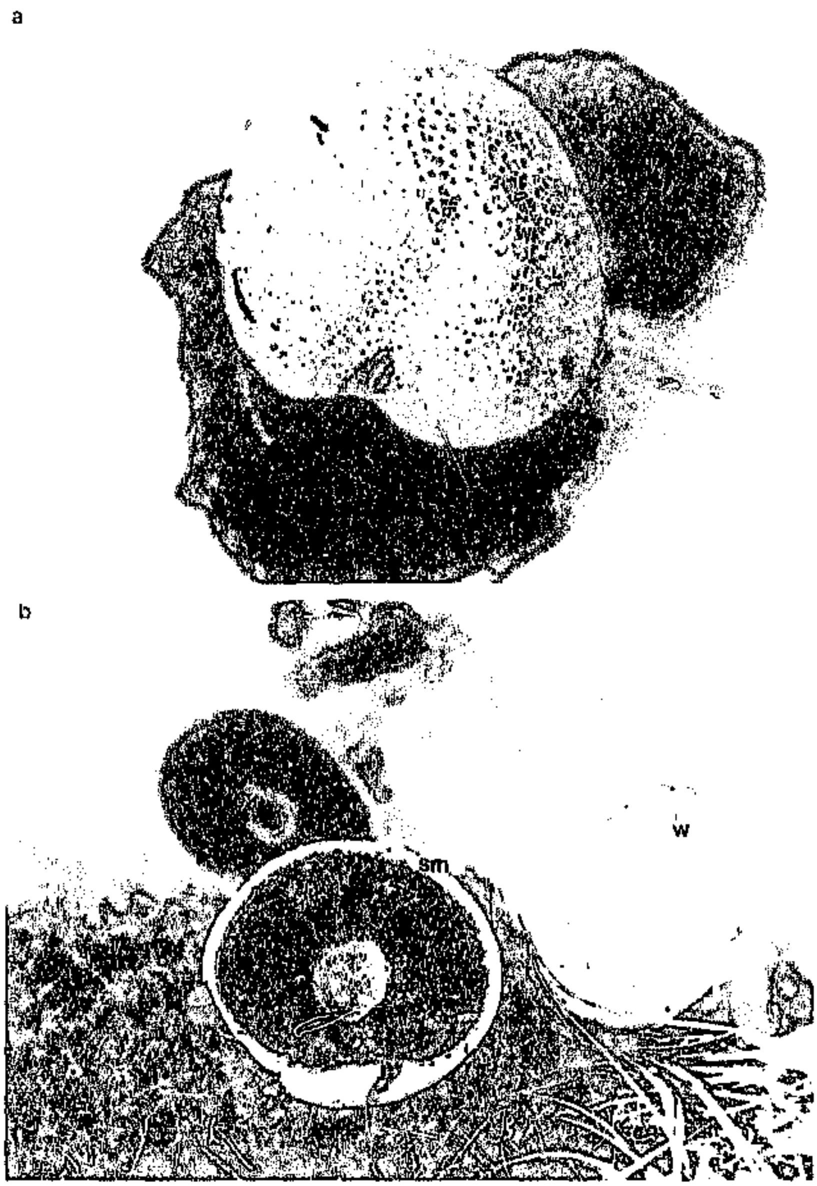


Plate 8.11 WHS01 *Scleroderma citrinum* Pers. sporocarps (a & b) sm - dark coloured internal spore mass w - wart like protuberances

- measurements: 0.8μm

Cortical cells - number of rows with 'Hartig' net: 1-3

- shape: rectangular

- measurements: 15.4$\pm$4.2μm x 4.0$\pm$0.9μm

'Hartig' net:

Thickness of 'Hartig' net around tannin cells: 0.1-0.5μm

cortical cells: 0.28-0.56μm

Number of hyphal rows around tannin cells: 1

cortical cells: 1

Shape of 'Hartig' net around tannin cells: common type A, palmetti

cortical cells: type A, palmetti with lobed haustoria originating from 'Hartig' net

8.3.2 Tracing mycorrhizal links to sporocarps

Two main types of sporocarps were collected from the study site and identified as *Scleroderma citrinum* Pers. (previously *S. aurantium*) (Plate 8.11) and *Boletus pinicola* Vitt. (Plate 8.12a & b) (Buczacki, 1989; Pearson, 1950; Bottomley, 1948; Van Der Westhuizen & Eicker, 1994). Identifications were confirmed with Dr. Van Der Westhuizen (*pers. comm.*). Rhizomorphs of *S. citrinum* were similar to the rhizomorphs observed on the WITS 01 ectomycorrhizal roots and connections between roots and sporocarps were confirmed. *B. pinicola* sporocarps did not have any rhizomorphs and hyphal connections with roots were difficult to trace. However, ECM roots of the WITS 06 type were frequently found associated near or on the base of the *B. pinicola* stipe.

FINLAY, R.D., FROSTEGÅRD, Å. & SONNERFELDT, A.-M. (1992). Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex Loud.

FOGEL, R. & CROMACK JR., K. (1977). Effect of habitat and substrate quali on Douglas-fir litter decomposition in western Oregon. *Canadian Journal of Botany* **55**, 1632-1640.

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9.3.3 Global changes

Global warming is a reality and it is predicted that CO_2 levels (current levels 360ppm) will have doubled within the next fifty years i.e one to two rotations (Scholes & Bailey, 1996). Increased concentrations of CO_2 would increase the rate of photosynthesis and hence production. This fertilization effect may result in trees growing faster and larger, increasing the storage of C in the ecosystem (Scholes & Bailey, 1996). Research into the effects of these increased CO_2 levels on commercial plantations should be initiated. Increased C allocation to the roots may alter the structure of both microbial and mycorrhizal populations, resulting in the potential to change mineralization patterns and nutrient pool sizes (Curtis *et al.*, 1994). Increased temperatures are also expected to occur as a result of global warming (Scholes & Bailey, 1996); this would be beneficial to decomposition in high altitude sites. However, drier conditions are also expected (Scholes & Bailey, 1996) and this could have a negative effect on decomposition. Because of these expected changes every effort should be made to make conditions more favourable for microbial activity in high altitude sites in which litter accumulation is a problem. Furthermore, any studies examining tree growth under elevated CO_2 conditions should also examine decomposition and nutrient dynamics. Addressing these problems now would ensure that the ecosystem would be better able to withstand future stress conditions.

9.3.2 Fertilization

In order to meet the increasing demand for timber steps need to be taken to increase and maintain productivity on existing plantations. Declining soil fertility has been identified as a factor contributing to reduced productivity and fertilization has been recommended (Olbrich, 1995). The effective and efficient use of fertilizers has been an area of intensive research. It is necessary to have quantitative information on how fertilization of stands, particularly in high altitude sites, affects litter production, decomposition of litter and the release of nutrients in the litter layers. During fertilization trails it is essential to measure rates of biological processes in addition to timber yields, all studies should be long-term. Several studies have shown that fertilization increases litter production and improves litter quality (Theodorou & Bowen, 1990; Wienand, 1992; Wienand & Stock, 1995). However the effect of fertilization on litter decomposition rates have been contradictory, with reports of the rates increasing, decreasing or remaining the same (Prescott, 1995). Ammonium fertilization has been shown to reduce ECM colonization and the production of ECM fruiting bodies. High P fertilization levels have been shown to reduce the development of extramatrical mycelium and ECM colonization (Arnebrant, 1994; Clemensson, Lindell & Persson, 1995; Wallander & Nylund, 1992; Wallander, 1995). Fertilization trials in South Africa have mainly examined the application of NPK, both singly and in combination. These trials have resulted in a variety of responses. Although, recommendations for fertilizer application have been made, these are generally site specific (Freimond, 1994; Schutz, 1976). Du Toit (1994, cited in Freimond, 1994) obtained good growth responses of pines growing on shales, using N, P, K^+ and Ca^{++} fertilizers. Considering the acidic nature of the soil, the low cation concentrations and the potential for reduced cation availability as a result of acid rain, fertilizations trials should include the cations, Mg^{2+} and Ca^{2+}. More attention should also be placed on the effects of fertilization on fine root production which is essential for nutrient and water uptake (Clemensson, Lindell & Persson, 1995) and on soil microbial activity.

chopper-rolling does not address the acidity problem and does not significantly alter the microbial activity. This method in combination with the application of dolomitic lime may be more successful, and is an area that requires further investigation.

9.3 AREAS REQUIRING FURTHER RESEARCH

9.3.1 Acid rain

In Sweden atmospheric deposition has resulted in soil acidification, cation losses and N saturation. Forest tree productivity has not been affected and many stands are showing growth responses to the increased N input. However, in Germany soil acidification has been more extensive and declining tree vitality has been noted (Clemensson, Lindell & Persson, 1995; Likens, Driscoll & Buso, 1996). Research has been conducted on the impact of air pollution on commercial forests in South Africa. These studies have examined the effect of acid rain on soil fertility and acidification as well as nutrition and nutrient cycling (Olbrich, 1995). Studies by Carlson (1992) have indicated that acid rain could alter the uptake of Ca^{2+} and Mg^{2+} by plant roots, by decreasing the availability of these cations in the soil. The present study has highlighted the role of ECM fungi in a forest ecosystem, particularly in connection with nutrient dynamics. Studies by Carlson (1992) have shown that there was no significant difference in the degree of mycorrhizal colonization between root cores acidified with different pHs of artificial rain. However, there was a difference in the mycorrhizal species composition. This change in ECM species could have important consequences to the nutrient status of trees as different mycorrhizal species not only differ in their ability to supply nutrients to the trees but also differ in their ability to utilize various sources of N and P (Chapter 7). Further studies are required to characterize ECM species and to assess their ability to utilize various nutrient sources. A survey of ECM types should also be conducted so that any changes in mycorrhizal composition and subsequent functioning can be monitored.

9.2.3 Enhancing the release of nutrients from the litter

Litter accumulation has been shown to increase as much as 37% from the first to second rotation (Morris, 1986). At present the action of ectomycorrhizas and their ability to utilize organic source of N and organic and complexed sources of P are assisting in providing the nutrients required for tree growth. To ensure the continued supply of nutrients from the organic litter layers ectomycorrhizal fungal isolates should be screened to determine which species are efficient organic N and complexed P users. Selected species could be used to inoculate *P. patula* seedlings destined to be planted into litter accumulation sites. This would ensure that seedlings have access to the nutrients locked up in the litter, thereby alleviating any deficiencies in younger stands.

Steps can also be taken to reduce the organic matter on the forest floor after clearfelling. The most obvious method would be to burn the litter. The burning of litter is known to release nutrients, however benefits obtained from burning are short term. Substantial quantities of N and P are lost during burning and added to nutrient losses as a result of harvesting, these losses could account for as much as 66% of the N and 35% of the P (Goh & Phillips, 1991). This amounts to a substantial loss of the nutrient reserves. Because of the large quantities of both N and P stored in the litter layers, the loss of nutrients from the stand should be minimized, therefore burning of the litter after clearfelling is not recommended. Alternatively, if favourable burn conditions are maintained, *i.e.* a slow, cool burn at low temperatures and low wind speeds and high relative humidity, the loss of nutrients can be minimised. Serious consideration to the release of nutrients from the litter layers may have to be considered in the light of proposed reduced rotations because shorter rotation times may result in nutrient losses from sites exceeding nutrient inputs which would translate into imbalances in nutrient cycling.

Increasing the surface area of litter available for decomposition by chopping the litter (i.e. chopper-rolling) has also been suggested as a means of treating the litter after clearfelling. This method was suggested as a means by which more nutrients could be released from the litter. However, trials conducted by the Institute for Commercial Forestry Research have not been promising (Freimond, *pers. comm.*). This is probably because

younger stands, suggesting N limitations. Host trees, including *Pinus*, are associated with different mycobionts forming ECM at different times during their development. The mycobionts occurring on seedlings become replaced as the host plant matures. This results in a succession of ECM types (Dighton, Poskitt & Howard, 1983; Malloch, Pirozynski & Raven, 1980). Younger *P. patula* trees are probably associated with non-protein ECM fungi while older trees are associated with protein fungi. Studies on ectomycorrhizal succession have not been undertaken in Mpumalanga and indicates an area for further research. From observations made during the study some succession was evident with *Amanita* spp. and *Lycoperdon* spp. occurring mainly in younger stands (up to 15 years) and *Boletus* spp. and *Suillus* spp. occurring in older stands. *Scleroderma* spp. occurred mainly from 10 years onwards.

9.2.2 The P cycle

A large proportion of P was retranslocated prior to needle fall, indicating a P deficiency in the system (Fig. 6.2; chapter 6). The results from the decomposition study revealed that P was mineralised and was available for plant uptake (Figs. 5.12 & 5.13; chapter 5). However, the rate of P release decreased with increasing altitude during the second phase of decomposition (Fig. 5.24; chapter 5). The soils of these sites are known to be P-fixing soils which alter the availability of P, resulting in the transformation of soluble phosphates into less soluble Al or Fe phosphates (Sanchez, 1976). This occurs especially in soils with high exchangeable acidity, as shown in this study (Table 6.2; chapter 6). This would account for the P limitations observed. Ectomycorrhizal fungal isolates were shown to grow on inorganic P sources as well as complexed inorganic and organic sources of P. Some isolates grew particularly well on complexed P sources such as $AlPO_4$, the form in which Al is precipitated from the exchange bases (Section 7.3.5; chapter 7). This indicates that ECM fungi play an important role in the P cycle, providing some access to otherwise unavailable or poorly available sources of P. Litter quality studies revealed that litter P in younger stands was lower than in older stands (Fig. 5.3; chapter 5). This indicates that a greater proportion of P may be retranslocated from the needles, suggesting increased P limitations in younger stands. This again may be attributed to ECM succession.

9.2 WHAT ARE THE NUTRIENT DYNAMICS OF A *P. patula* SITE IN WHICH LITTER HAS ACCUMULATED?

The N and P nutrient cycles examined in the high altitude, litter accumulation site revealed that the majority of these nutrients are stored in the forest floor component, followed by the soil and tree pools (Figs. 6.1 & 6.2; chapter 6). Because of the large reserves of N and P in the litter layers and the distribution of the roots within these layers (Section 6.3.5; chapter 6), it can be assumed that litter is critical to nutrient uptake, indicating that these nutrients are cycled through a plant-litter-plant cycle. Nutrients are also cycled through a plant-litter-soil-plant cycle, as was evident from the distribution of some roots within the soil (Section 6.3.5; chapter 6) and the leaching of nutrients from the litter into the soil (Table, 6.4; chapter 6). However, the potential Al^{3+} toxicity of the soil, limits the successful exploitation of the soil nutrient reserves. Of the cations, Ca^{2+} was particularly low in the litter and soil layers. These low cation concentrations were expected under the acidic conditions within these layers. It has been proposed that cation requirements are met through weathering of the parent rock material, cations being taken up by deep roots (Morris, 1995). This area requires further investigation.

9.2.1 The N cycle

Only 13% of needle N was retranslocated prior to needle fall (Fig. 6.1; chapter 6). This indicates that the N requirements of the tree are probably being met even though N was shown to become immobilized in the litter during decomposition (Figs. 5.8 & 5.9; chapter 5). It is evident that the ECM roots, which are in intimate contact with the litter layers, not only increase the uptake of inorganic N, but also aid in the breakdown of organic N compounds thus supplying an additional quantity of N to the tree (Section 7.3.4; chapter 7). Litter quality studies revealed that litter N in younger stands was lower than in older stands (Fig. 5.2; Chapter 5). This may indicate that a greater proportion of N is retranslocated from the needles prior to needle fall in the

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