The effect of simulated nitrogen deposition on growth and ecosystem functioning of managed *P. patula* plantation ecosystems in South Africa

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

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Abstract

The managed pine forests of the Drakensberg escarpment area of Mpumalanga Province, South Africa, are characterised by highly leached, acid and occasionally shallow soils in the higher altitude areas. The high altitude areas also receive high nitrogen (N) and sulphur (S) inputs, with the N deposition rate estimated to be ranging from 15 to 24 kg ha^{-1} yr^{-1} at a nitrate:ammonium (NO_3^-:NH_4^+) ratio of approximately 3:1. Most of the N pollution originates from the adjacent, highly industrialized, densely populated Highveld area, where most of South Africa's electricity is generated by coal-driven power stations. Previous studies indicated that shale-derived soil has a moderate level of sensitivity to acidification due to shallow profiles and inherently low base cation status as well as a high N status, suggesting a predisposition to N saturation (Aber *et al.* 1989). Decomposition of *P. patula* litter on shale soil is also inhibited and litter tends to accumulate as the stands mature. The purpose of this study is to investigate the effects of future NO_3^- and NH_4^+ deposition on growth and nutrient cycling in *P. patula* plantations growing on shale-derived soil. This study consists of a field and pot-grown seedling experiments, the former commencing in November 1995 and the latter in August 1996.

The field experiment consisted of three sites (planted in 1973, 1985 and 1991), which represents different age classes of a sawwood *P. patula* rotation (4-, 14- and 22-years-old in 1995). Starting in November 1995, and continuing until 1997, N was sprayed onto the litter layer of each of four plots per site. Four additional plots received no N (the control plots).

NO_3^- leaching. After three years of treatment, the pH of the soil showed no significant differences between the control and the treatment plots. However, changes were detected in the mycorrhizal populations on the roots of *P. patula*, including increases in the mycorrhizal density of one dominant type at the expense of the other. Isotopic analyses of ^{15}N natural abundance in untreated 4-, 14- and 22-year-old *P. patula* ecosystems were also carried out. The L_1 and L_2 layers of all three ecosystems were enriched in ^{15}N and there were no significant differences among sites. The F-layer however, showed a depletion of ^{15}N, which was characteristic of all the sites studied.

In another part of the experiment, N was added to 10-month-old seedlings which were growing in pots containing shale-derived soil taken from a 14-year-old *P. patula* stand. Two different rates of N, equalling 50 and 150 kg N ha^{-1} and three different ratios of NO_3^-:NH_4^+ viz. 1:1, 3:1 and 6:1 were applied to the soil, while the control pots received no N. Results showed that N added to the soil leads to a decrease in the growth rate of *P. patula* seedlings. These trends were well correlated with an increase in the N:P ratio of the foliage of the seedlings, while there was a negative correlation with the root:shoot ratio. These results indicate that the seedlings were experiencing a deficiency in P and reacted by increasing root biomass, while shoot biomass decreased.

The pine forests of the area have high N levels and increasingly low P levels, suggesting that any additional N will be lost from the ecosystem, a situation approximating stage 1 of the theoretical concept put forth by Aber *et al.* (1989). Experimental evidence from both the field and the seedling experiments suggests that the N:P ratio plays a crucial role in the functioning of *P. patula* plantation ecosystems and that increased availability of N through increased N deposition may disrupt this N and P nutritional balance and lead to losses in productivity. The

older stands are especially sensitive, mainly due to immobilization of P in litter and standing biomass, predisposing these stands to N saturation. Nitrogen-induced P deficiencies could be exacerbated by N-induced changes in the mycorrhizal population structure that could lead to dominance of mycorrhizal ecotypes less effective at nutrient uptake. It is expected that increased N deposition will lead to increased NO_3^- leaching from the soil profile, both as a result of an increase in the nitrification rate and as a result of a decrease the the capacity to retain incoming N. This will have adverse consequences for stream water quality in afforested catchments of the Escarpment, and monitoring programmes are needed to track the development of symptoms and to form the basis of a remedial programme.

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My supervisor and mentor Prof. William D. Stock, who has provided guidance, support and inspiration at the right moment. Also to Dr. Peter Dye at the CSIR, who acted as a supervisor and mentor in the CSIR. Dr. Bridget Emmett, Institute of Terrestrial Ecology, Bangor, Wales, UK, provided valuable advice and mentorship, for which she is gratefully acknowledged. The ex-Jonkershoekers who provided logistical and moral support as well as a home base while doing laboratory work at the University of Cape Town. In particular I would like to thank Jan Bosch, Dr. Brian van Wilgen, Adrian Simmers, Harold Julius, Godfrey Moses and Gregory Bailey.

The field experiment was carried out on two Safcol plantations, Brooklands and Ceylon, and personnel stationed there deserve my heartfelt gratitude for technical support and also for providing manual labour during spraying of the experimental plots. Also to Sam Soko and David Maphanga, who provided excellent technical assistance in the field, rain or shine.

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Index

Chapter 1

General introduction

Anthropogenic deposition of N and S compounds in the form of "acid rain" is one of the major consequences of industrialization and fossil fuel burning over the last four decades (Galloway *et al.* 1984; Skeffington and Wilson, 1988; Vitousek *et al.* 1997; Galloway and Mellilo, 1998). Acid rain damage has been linked to the decline of forest trees in Germany and parts of Eastern Europe and this has focussed public and scientific attention on the ecological consequences of N and S deposition (Nihlgård, 1985; Schulze, 1989). Since then many other instances of forest dieback and deterioration of forest health in Europe and North America have been attributed to acid rain (Nihlgård, 1985; Schulze, 1989; Aber *et al.* 1989). A major research effort, focussing on sulphur, culminated in a sharp reduction in emissions of SO_2 in Europe and North America, while emissions of oxidised N gases continued to increase (Aber *et al.* 1989; Galloway and Mellilo, 1998). However, it soon became clear that oxidised and reduced forms of N contribute to some of the reported forest and biodiversity declines that were seen in parts of Europe and North America (Aber *et al.* 1989; Schulze, 1989). Despite numerous attempts to find more environmentally friendly alternatives to fossil fuel burning for energy purposes, no realistic solutions have been found and combustion of fossil fuels is set to continue unabated into the 21st century (Galloway and Mellilo, 1998).

In the atmosphere the dominant N species is the biologically inert gas N_2, which constitutes 78% of the air. It is biologically converted to NH_4^+ and NO_3^- by certain bacteria and fungi, some of which are free living, others forming symbiotic relationships with the roots of

higher plants (Waring and Schlesinger, 1985). The dominant N pollutant species are various oxides of N, nitric oxide (NO), nitrogen dioxide (NO_2) and nitrous oxide (N_2O) and ammonia (NH_3) and their ionic derivatives, NO_3^- and NH_4^+. Nitric oxide (NO) is given off in the burning of fossil fuels in power stations and by motor vehicles, but is rapidly converted to nitrogen dioxide (NO_2) (Hornung, 1994). The NO_2 is itself converted to nitric acid (HNO_3). Thunderstorm activity can produce NO_X (NO and NO_2) naturally. On a global scale this natural production of NO_X is much greater than the anthropogenic sources, but the latter generally results in small areas having very high concentrations of NO_Xs, while natural sources are more widely dispersed (Galloway *et al.* 1994). Industrial sources of NH_3 include the manufacture of fertilizers, nitric acid and explosives. Other sources include the burning of coal in power stations and emissions from motor vehicle exhausts. Significant amounts of NH_3 are produced by animals such as pigs and cattle and high concentrations of these animals in Western Europe have lead to NH_3 being the dominant N pollutant. Ammonia is a weak base, easily soluble in water to form NH_4^+ and a hydroxyl ion (Nihlgård, 1985). On a global scale, N pollutants are exceeded only by sulphuric acid as the most important component of acid rain (Galloway *et al.* 1995).

Much of the anthropogenic N is deposited over various forest ecosystems in Europe and North America since forest vegetation covers roughly one-third of the Earth's land surface (Waring and Schlesinger, 1985). The major portion is natural forest, ranging from boreal forests in the arctic regions to tropical rainforests. In some countries natural forests are managed and exploited for fuel and as a resource for industry, while fast growing plantation forests have been established in various countries. With the demand for pulp and paper increasing in reaction to the increase in world population, the current emphasis is to grow trees to harvesting age in the shortest possible time. Over the last century huge tracts of land, especially in the Southern

Hemisphere, have been planted to softwood species such as *Pinus* spp. and hardwoods such as *Eucalyptus* spp. (Landsberg and Gower, 1997). In many southern hemisphere countries such as South Africa and Australia, significant climatological and pedological constraints limit land available for commercial forestry. The management of these short rotation forests is therefore highly intensive and rotations generally range from 6 to 9 years for *Eucalyptus* spp. to 40 years for some *Pinus* spp. (Landsberg and Gower, 1997).

The physiological basis underlying the high productivity of these forests is a shift in dry matter allocation to leaves, thereby increasing the photosynthetic capacity of the tree (Ericsson *et al.* 1992). One of the objectives of this highly intensive biomass production is to keep nutrient concentration, water supply and light exposure optimal balanced in order to optimise growth. Productivity of forest ecosystems, whether natural or cultivated, is often limited by a shortage of N (Waring and Schlezinger, 1985; Landsberg and Gower, 1997). This is because N is an essential element for plant growth and is a major component of amino acids, proteins and other biologically active compounds. In contrast to the normal situation, several cases of high N deposition to forest ecosystems have been recorded in Europe (Mohren *et al.* 1986; Van Breemen and Van Dijk, 1988; Heinsdorf, 1993) and North America (Johnson and Lindberg, 1992; Fenn *et al.* 1998). This leads to a state where water or light or nutrients other than N limit growth. This situation has been termed "N saturation" by Aber *et al.* (1989). Much debate surrounds the exact definition and significance of the term. Aber *et al.* (1989) define it as a state where N is in excess of plant and microbial demands, leading to leaching of NO_3^- from the rooting zone (Fig. 1.1). N saturation can also be defined as a state where primary production will decrease with an increase in N supply, growth being limited by other factors such as P, base cations and water availability (Nilsson, 1986). Nitrogen saturation as defined by Aber *et al.*

(1989) can already be seen in a number of forest ecosystems in North America (Aber *et al.* 1989; Bytnerowicz and Fenn, 1996; Williams *et al.* 1996) and Europe (Schulze, 1989; Katzensteiner *et al.* 1992; Dise and Wright, 1995) and will be used as a definition in this thesis.

Plants are not preadapted to N availability exceeding demand, hence excess N represents a unique stress on plants (McNulty *et al.* 1990). Recent studies and reviews have shed more light on the direct consequences of N excess in forestry ecosystems (Aber *et al.* 1989; Ågren and Bosatta, 1988; Schulze, 1989; Aber *et al.* 1993). Nitric acid contributes to weakening of the cuticle of leaves of conifers and this may predispose trees to other environmental stresses such as drought and other air pollutants (Bytnerowicz and Fenn, 1996). It is also known that N, in the form of NO_3^- and NH_4^+ can be taken up directly through the leaves, leading to elevated N concentrations in the leaves (Wilson and Skeffington, 1994b; Bytnerowicz and Fenn, 1996). Direct effects of high levels of NO_x and NH_3 in the long term lead to a loss of needle biomass (Boxman *et al.* 1998b), however, the indirect effects have led to most concern.

Hypotheses regarding changes in forest ecosystem functioning in response to elevated N deposition were first put forward by Aber *et al.* (1989), who recognised four stages of ecosystem response (Fig. 1.1). **Stage 0** represents an ecosystem that receives only background levels of N deposition. This is normally the case in coniferous ecosystems where the N-cycle is tightly controlled and no N is lost (Aber *et al.* 1989; Gundersen *et al.* 1998). In **stage 1** of ecosystem response small losses of N occurs through leaching of NO_3^-, although this is still mainly confined to the growing season (Aber *et al.* 1989; Gundersen, 1991). With further increases in N, the total N concentration in the leaves increases, similar to the response found after a single event fertilization of forest ecosystems with N.

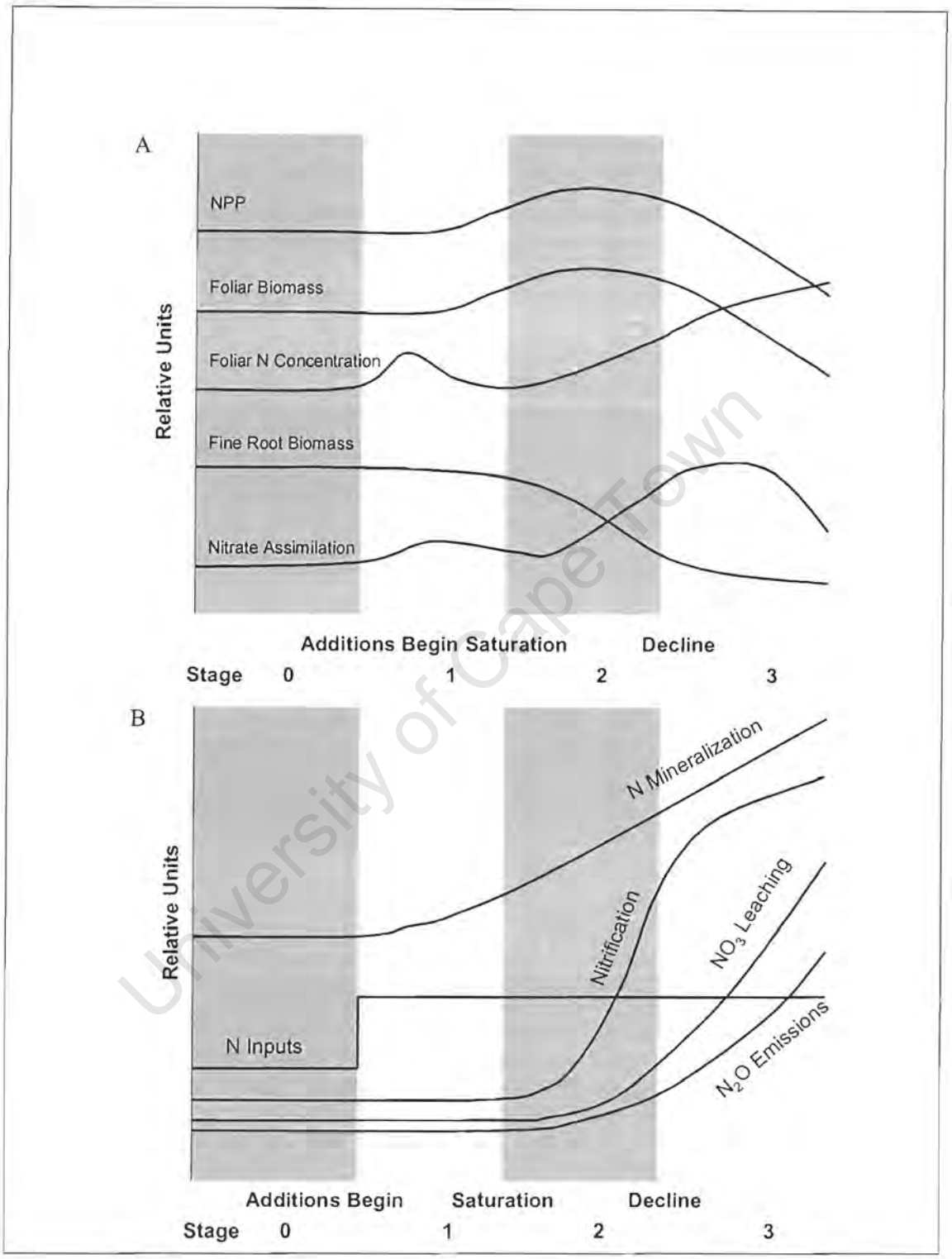

Fig. 1.1 *Hypothesized timecourse of N saturation in northern temperate forest ecosystems (modified from Aber* et al. *1989). Diagram A reflects changes in nitrogen cycling and nitrogen loss, while diagram B reflects plant reactions to the changing levels of N availability.*

This response to elevated N would be associated with increased foliar biomass and tree growth, leading to dilution of N and a subsequent return to pre-treatment N concentration in the foliage. These changes in nutrient dynamics are associated with an increase in biomass accumulation towards the end of stage 1, which will continue into and peak in stage 2.

A decline in the growth rate and foliar biomass is expected to take effect towards the end of **stage 2**, mainly due to a hypothesized reduction in the root biomass and consequent shortage of water and nutrients. A reduction in root biomass in reaction to increased N availability has been demonstrated for coniferous forests (Nadelhoffer *et al.* 1985; Sullivan, 1993) and is blamed for some instances of forest dieback where drought coincided with the advent of stage 3 N saturation. Many mycorrhizas have been shown to be sensitive to chronic N deposition. Both NO_3^- and NH_4^+ have been shown to be toxic to mycorrhizal growth and development (Termorshuizen and Schaffers, 1987; Jansen and Dighton, 1990), which has led to reduced mycorrhizal frequency and changes in mycorrhizal species composition such as those recorded in forests in the Netherlands (Termorzhuizen, 1993) and Sweden (Brandrud, 1998). The rate of mineralization and nitrification as well as gaseous losses of N are also hypothesized to increase at the end of stage 1, and even further in stages 2 and 3. A recent advance in the understanding of the consequences of N saturation was reported where it was found that below a certain ratio of C:N in the ecosystem the rate of nitrification is dramatically increased, leading to further NO_3^- leaching and loss of base cations (Gundersen *et al.* 1998). The C:N ratio of litter in the organic soil layers has been found to be a controlling factor that determines the rate of mineralization in coniferous ecosystems (Berg and Ekbohm, 1983; Gundersen *et al.* 1998). An increased rate of nitrification, induced by elevated N deposition, is one of the major reasons for an increased NO_3^- leaching seen in N saturated ecosystems (Emmett *et al.* 1995b; Gundersen *et al.* 1998). As a

consequence, leaching would not be confined to the growing season only, but will also happen in the non-growing season (Emmett *et al.* 1995b; Gundersen, 1991). Leaching of NO_3^- has been shown to lead to losses of base cations from the soil. K, Mg and Ca act as accompanying ions to NO_3^- leaching from the soil profile and in this way base cations are lost permanently form the soil pool. In forests growing in soils with low base cation status, this has been shown to lead to deficiencies of K, Mg and other nutrients (Katzensteiner *et al.* 1992).

Stage 3 of ecosystem response to elevated N is characterised by dramatic losses in foliar biomass, NPP and root biomass and have been demonstrated for coniferous forests in the Netherlands (Boxman *et al.* 1998b) and Germany (Schulze, 1989). Reduced root biomass and reduced mycorrhizal numbers contribute to reduced uptake of nutrients and water, predisposing forests to drought and other disturbances (Aber *et al.* 1989; Fenn *et al.* 1998). The mechanism that eventually triggers this forest decline and dieback appears to be different among different ecosystems. Base cation deficiencies (Köllig *et al.* 1997), P deficiency (Mohren *et al.* 1986) and aphid attacks (Flückiger and Braun, 1998) have been shown to contribute to forest decline. An increase in the N concentration in the leaves of deciduous and evergreen trees in Europe has been shown to be positively related to an increase in fungal attack as well as an increase in the frequency of aphid outbreaks (Fluckiger and Braun, 1998). It is also thought that increased N concentrations in the leaves of forest trees will lead to reduced frost hardiness (Nihlgård, 1985). Further negative responses in certain ecosystems may result from the interactive effects of N with other factors contributing to global change such as CO_2, O_3 and global warming, although some initial positive responses are also likely.

Aber *et al.* (1989) suggested that the change in N dynamics in the ecosystem from one of N limitation to one of saturation takes the form of a continuum (N saturation continuum).

However, recently Cannell and Thornley (2000) suggested that the continuum of N saturation be clarified to include states of equilibrium and disequilibrium with or without N saturation. Two possible states of equilibrium can exist: true N saturation where N inputs equal N outputs, and an unsaturated state where N inputs equal outputs. The former situation is of an ecosytem exposed to large amount of N, and the next step in the continuum is of a damaged ecosystem with changes to the soil and damage to the vegetation, which approximates stage 2 and 3 of the Aber *et al.* (1989) definition. For the sake of simplicity and comparison, the Aber *et al.* (1989) definition and stages of N saturation will be used in this study.

Along with several developed countries, emissions of S and N compounds are on the increase in many developing countries in Africa and East Asia (Galloway *et al.* 1994). It is estimated that by the year 2020, emissions from these countries will have increased beyond those of the developed countries in Europe and North America, where legislation introduced is expected to lead to a reduction of S and N emissions. Emission densities of sulphur dioxide (SO_2) and inorganic N compounds (NO_X; HNO_3, NH_X) on the Mpumalanga Highveld are relatively high and rival some of the worst cases of air pollution in Sweden, Britain and the USA (Tyson *et al.* 1988). Urban and industrial activities have been identified as the main sources of anthropogenic S and N compounds in the atmosphere over the Highveld (Tyson *et al.* 1988). The deposition rate of N species ranges from 15 kg N ha^{-1} yr^{-1} (wet deposition, NO_3^- and NH_4^+ combined) on the Highveld in Mpumalanga (Tyson *et al.* 1988) to an estimated 24 to 41 kg N ha yr^{-1} bulk (wet and dry deposition combined) at three sites on the Drakensberg escarpment adjacent to the Highveld (Olbrich and du Toit, 1993).

Tyson *et al.* (1988), in a review of air pollution in the Highveld and adjacent areas, concluded that N and S air pollutants could have a negative impact on the commercial forests of Mpumalanga (formerly known as the Eastern Transvaal Highveld). A number of studies investigated the direct effects of SO_2 on commercial pine species (Kelly, 1986) and indirect effects of SO_4^{2-} on pines stands in South Africa (Carlson, 1992) while virtually no work exists on the effects wet and dry deposition of N. Forest decline of *P. patula* stands has been noticed in Swaziland while chlorotic flecking and mottling of pine needles have been noticed for the past two decades (Tyson *et al.* 1988). None of these symptoms could be attributed to drought or disease. The effects of the increase in the acidity of rain due to the conversion of SO_2 to H_2SO_4 are dealt with by Carlson (1992). Her work indicated that excessive S *per se* is unlikely to cause harm to the ecosystem. It is recognized, however, that the acidifying effect of SO_4^{2-} ions can influence commercial pine stands via its effect on Ca and Mg availability, mycorrhizal populations and Al mobilization (Cronan and Grigal, 1995). Currently it is not known whether increased inputs of N will enhance the growth of plantation trees in the Drakensberg escarpment or whether it will eventually lead to nutrient imbalances and disease and subsequent mortality of the trees in the ecosystem.

Traditionally, N is limiting in coniferous forest ecosystems, but new evidence indicates that this is not the case in the plantations of the Eastern seaboard of South Africa (Dames, 1996; Nowicki, 1997) although responses to fertilizer N are varied (Schonau, 1983). While N availability is high, the availability of other nutrients such as P, K and Mg may be in short supply. In addition, soils in the area are highly leached and inherently low in base cations and therefore highly sensitive to acidification due to inputs of SO_3^- and NO_3^- (Olbrich, 1995). Quartzite and shale soils of the Timeball Hill series were identified as being particularly sensitive

to acid deposition (Olbrich, 1995). These factors may work together to create a situation where any additional N could reduce optimal functioning of the ecosystem, resulting in either an increase or a decrease in productivity, combined with changes in the pH of the soil substrate.

The overall objective of this thesis was to study changes that occur in ecological functioning of a *P. patula* plantation ecosystem during and after N availability in the ecosystem had been increased by applying NO_3^- and NH_4^+ to experimental plots. A simulated N deposition experiment was chosen over a longer-term experiment in order to speed up and track the effects of the ambient and anthropogenic levels of N deposition on the *P. patula* ecosystem. A field trial was set up to test the reaction of *P. patula* ecosystems on shale-derived soils to simulated N deposition. *Pinus patula* is the most important pine species in the region and was therefore chosen as subject for this study. There are indications that stands of different ages react differently to high levels of N deposition (Stevens *et al.* 1994), and this was accommodated by examining three sites that have different aged trees, but are on the same soil type and in the same geographical area. The reaction of the different components of the ecosystem, such as soil chemistry, litter decomposition, mycorrhizas, and ^{15}N of different ecosystem elements were tested against plots that received no additional N, apart from ambient imputs. Mechanisms of change in ecosystem functioning in ecosystems exposed to elevated N levels were tested in a seedling experiment with *P. patula* seedlings, similar to the method applied by Wilson and Skeffington (1994a; 1994b). Changes that may occur in the seedling experiment were compared to trends observed in the field experiment and the implications discussed.

The structure of this thesis is of a series of papers that describe different aspects of ecosystem functioning in reaction to simulated N deposition. Fertilized plots are compared to unfertilized plots to determine changes in ecosystem dynamics and identify mechanisms

whereby forest functioning may change. A site and species description is given in Chapter 2, while in Chapter 3, growth and nutrition of *P. patula* trees exposed to simulated N deposition is examined. Excess N effects on the soil and soil water chemistry are reported in Chapter 4. Decomposition and nitrification are also prone to changes with an increase in N and this is examined in Chapter 5. Root and mycorrhizal dynamics is documented in Chapter 6, while ^{15}N natural abundance of *P. patula* ecosystems and changes with increased N availability is examined in Chapter 7. Chapter 8 sums up work that deals with growth and nutrition of *P. patula* seedlings that have been exposed to high levels of N, with reference to similar changes in the field experiments. Chapter 9 is a synthesis of the main conclusions of this study, with recommendations for future research.

Chapter 2

Experimental site and species description

SITE AND SPECIES DESCIPTION

In 1997 approximately 1,4 million hectares of land in South Africa were planted to exotic forests (Forest Owners Association, 1997). Of that figure, 47% was under various *Pinus* species and 24% planted to *Eucalyptus grandis*, with the rest planted to hybrids of *E. grandis*, other *Eucalyptus* spp. and *Acacia mearnsii*. The majority of pine plantations consist of *P. patula*, *P. taeda*, *P. elliotii*, and *P. radiata*. Approximately 41 % of the total planted forestry area in South Africa is situated in the Drakensberg escarpment or foothills in Mpumalanga province (Fig 2.1). The Drakensberg escarpment runs in a North-South direction and separates the Highveld to the west from the Lowveld to the east. In terms of area planted, *P. patula* is the most important forest species in the escarpment area (Forest Owners Association, 1997). With a limitation on land suited to forestry, these plantations are managed intensively to optimise wood production. Most of the pine stands in Mpumalanga are in their second rotation, and a small number in the third rotation. The wood is used for pulp production and sawlogs (Forest Owners Association, 1997). Mining timber, for use as support beams in mine shafts, also forms a substantial portion of wood produced, although, due to the economic decline in the mining sector, this is on the decrease. Currently pine stands earmarked for sawwood is clearfelled at 25 - 32 years, depending on the forestry company involved and the growing conditions. For pulpwood, the

rotation is much shorter, ranging from 16 to 20 years. In the Usutu forest in Swaziland, where *P. patula* is grown for pulpwood, the rotations range from 16-18 years (Morris, 1993).

Pinus patula is native to Mexico, where it grows in a warm temperate climate (Wormald, 1975). It was introduced into South Africa in 1907. *Pinus patula* is used extensively for both sawwood and pulp and paper manufacturing purposes (Wormald, 1975). It is a straight cylindrical, occasionally forked tree, normally 20-30 m in height. Distinctive characteristics of the species are its slender pendulous leaves, rough, reddish orange bark on the upper bole and conical pedunculate cones. Leaves are persistent for 2 - 4 years and held in fascicles of 3 - 4, with fascicles of 2 and 5 rare (Wormald, 1975). Recent studies in the Drakensberg Escarpment area have found that leaf longevity varies between 1.5 and 2.5 years (Olbrich, 1993b).

Pinus patula grows in a wide variety of soils, but fertility is of great importance (Wormald, 1975). Soils favoured by *P. patula* are acid and have a good moisture supply (Wormald, 1975). *Pinus patula* will not grow well in sites that have a high pH, such as volcanic soils (Durham, 1965). Another factor that seems to be of importance is the rooting depth. It can grow in 15 cm of soil, provided that the roots can penetrate the underlying material, be it rock or soft laterite. However, soil depth is important when soil moisture becomes limiting to growth. *Pinus patula* is also planted in Swaziland, Zimbabwe, Kenya, Tanzania and other southern and eastern African countries as well as in New Zealand, Ecuador, Chile and Argentina. In South Africa, it is grown most successfully between 1000 and 1800 metres above sea level. The species appears to do well in the summer rainfall areas in South Africa where rainfall exceeds 750 mm per annum (Schutz, 1990).

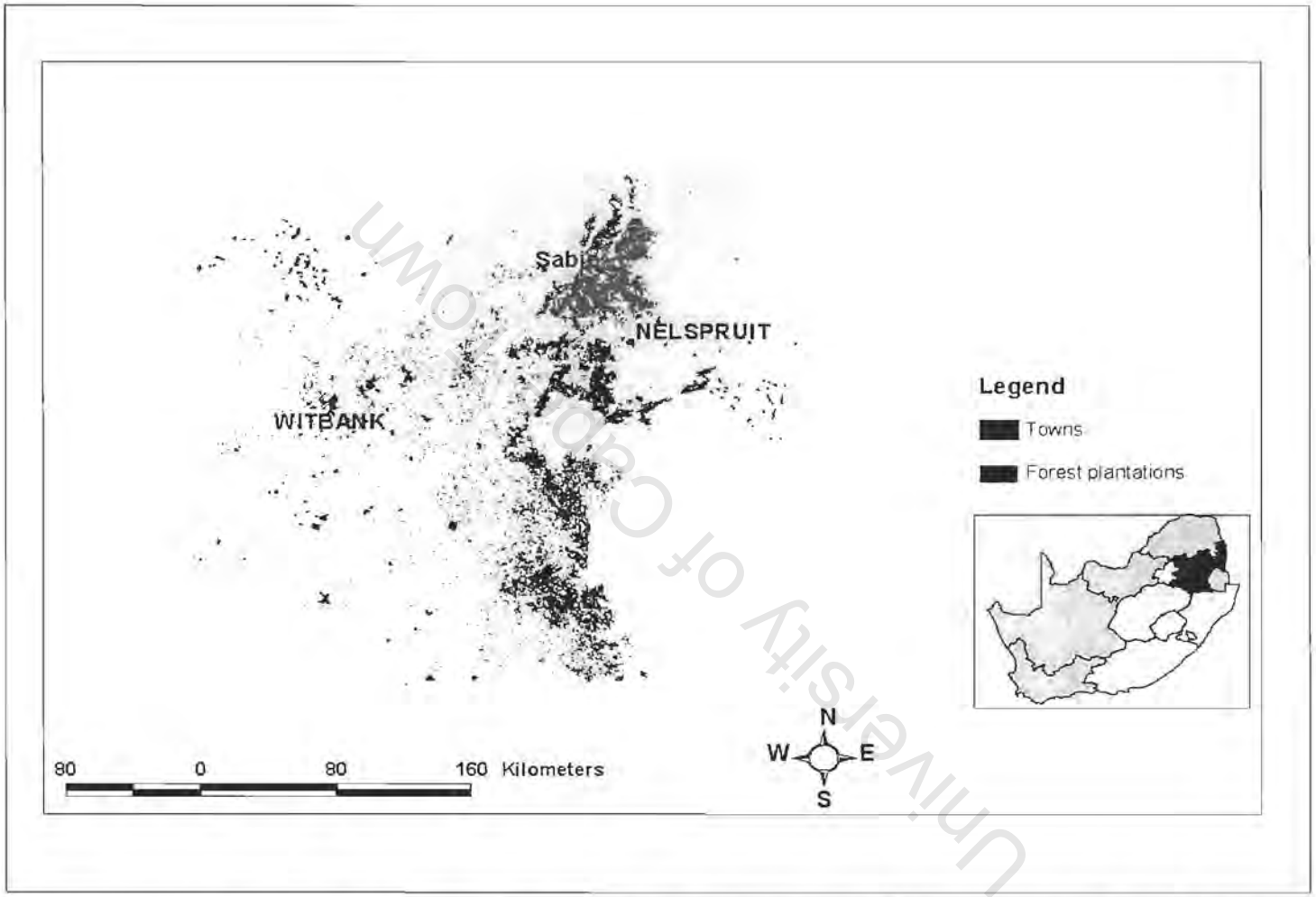

Fig. 2.1 *The distribution of some of the major towns and other urban areas in Mpumalanga area and the extent of afforested areas in Mpumalanga province. Most of the forestry area is situated in the Drakensberg escarpment area separating the highly industrialized Highveld to the west and the Lowveld to the east.*

The Drakensberg escarpment in Mpumalanga province is characterized by prime conditions for commercial forestry (Schutz, 1992). It is a summer rainfall area with January and February being the wettest months and June and July the driest (Fig. 2.2). Mean average temperature rarely falls below freezing in winter. In most of the forestry area the rainfall exceeds 800 mm per annum, which is sufficient for growing *P. patula*, *P. elliotti* and *P. taeda*.

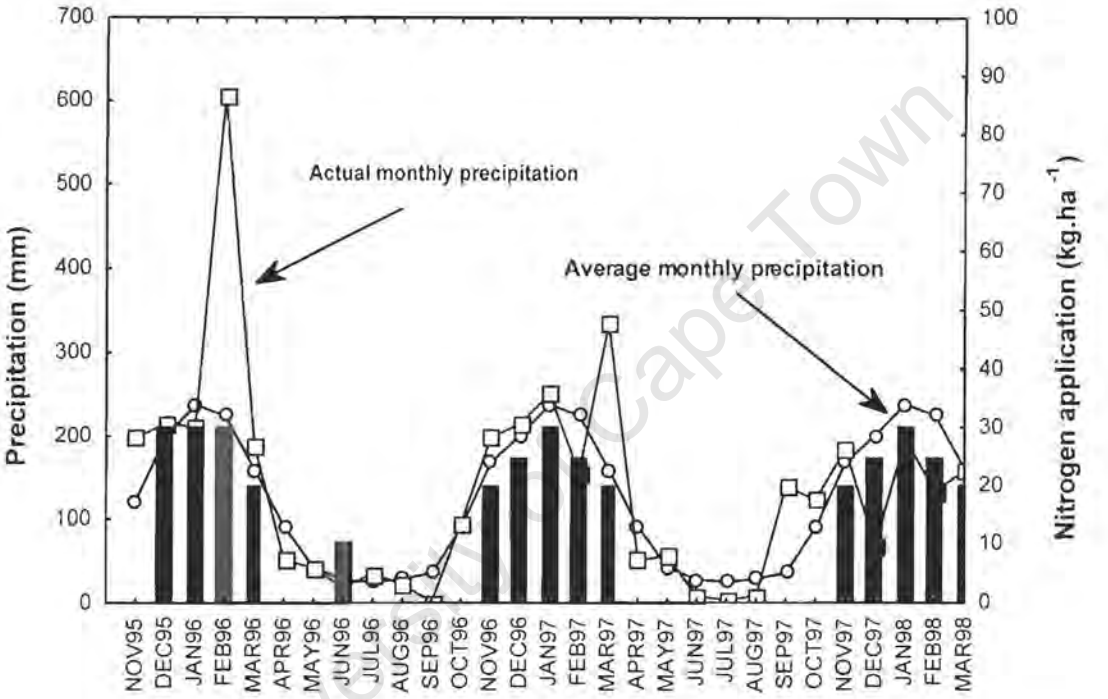

Fig. 2.2 *Average and actual monthly rainfall at Brooklands plantation office and the regime for application of N on the three sites. Rainfall is on the left hand side y-axis, and times and dates of N application on the right hand side y-axis. Brooklands plantation office represents the most proximate rainfall gauge with long-term rainfall data to the three sites. It is approximately 12 km away from the 4-year-old site, which is the furthest away. The long-term average monthly precipitation represents the mean of 46 years (Schutz, 1990).*

The soils underlying the afforested areas in Mpumalanga arise from a variety of parent materials, including shale, dolomite, granite, quartzite and diabase (Fig. 2.3). These soils are inherently low in fertility (Olbrich, 1995). Generally the soils are highly leached, relatively poor

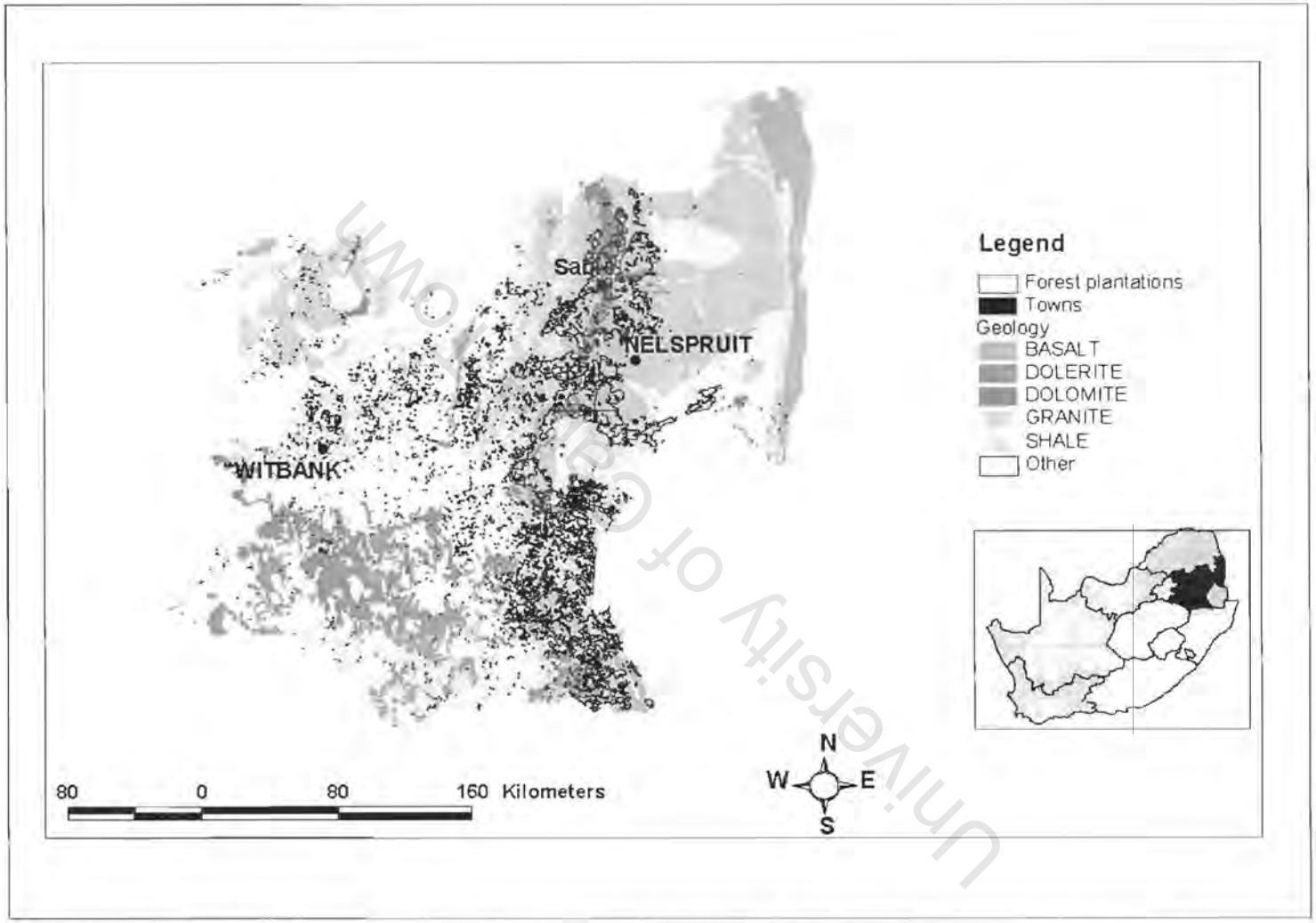

Fig. 2.3 *A depiction of the afforested areas in Mpumalanga Province, South Africa, superimposed on a depiction of the major geological formations in the province. The area under forests in the Drakensberg escarpment area coincides with areas where shale, dolomite and, granite dominate as soil parent material.*

in base cations and have moderate to high acidity. The low base saturation and hence relatively high acidity makes the soil of the area especially prone to damage from pollutants. Soils derived from quartzites and shales, which make up an important part of soils in the Escarpment area, are highly sensitive and moderately sensitive to pollution and acidification, respectively (Olbrich and du Toit, 1993). In addition to the low base cation content and the high acidity, a substantial portion of shale and quartzite soils are characterized by very shallow profiles. *Pinus patula* is frequently planted on marginal shale and quartzite soils and therefore forms the focus of this study. Portions of the forest are on areas where root growth is constrained due to limitations in the effective rooting depth, either because of shallow soil or a large percentage of stones or other obstructions in the soil horizon.

In the last decade it has become increasingly clear that the remaining land area in the escarpment is not suitable for afforestation (Louw, 1997; 1999). Therefore, the focus is shifting to strategies to optimise wood production in areas currently afforested. This strategy is taking the form of increased focus on tree breeding and forest management, including fertilization with N, phosphorus and potassium.

The impact of industrial activity on the Highveld and Drakensberg escarpment is reflected in the figures for actual wet and dry deposition of NO_3^- and NH_4^+ on the soil of the Highveld and the adjacent Drakensberg escarpment in Mpumalanga Province (Fig. 2.3). The rate of the deposition of N species range from 15 kg N ha^{-1} yr^{-1} (wet deposition, NO_3^- and NH_4^+ combined) (Tyson *et al.* 1988) on the Highveld in Mpumalanga to an estimated 24 to 41 kg N ha^{-1} yr^{-1} of N as NO_3^- (wet and dry deposition) at three sites on the Drakensberg escarpment adjacent to the Highveld (Olbrich and du Toit, 1993). The ratio of NO_3^- to NH_4^+ ranges from 3:1 to 6:1 (Tyson *et al.* 1988; Olbrich and du Toit, 1993).

EXPERIMENTAL DESIGN OF TRIALS

The field experiment was established in December 1995. Details of the sites are given in Table 2.1. Three sites were chosen to represent different ages in a sawwood rotation. Care was also taken to ensure homogeneity in terms of soil depth, soil type and climatic variables, where possible. All three sites were situated within a 20 km radius of each other near Sabie in Mpumalanga province (30°39'E, 25°12'S), on soil derived from the dominant geology in the immediate vicinity, namely shale. All three sites are situated above 1200 metres above sea level and receive mean annual precipitation in excess of 1000 mm. The soils are all highly dystrophic (Soil Classification Working Group, 1991). A summary of the most important chemical characteristics of the upper 10 cm of mineral soil on the unfertilized plots of the three sites, determined at the end of the experiment in March 1998, is given in Table 2.2. Organic C concentration was highest on the youngest site and lowest on the oldest site. A similar trend was evident for total base cation concentration and available P and total N concentrations. Although not significantly different ($p<0.05$; ANOVA, followed by Duncan's multiple range post hoc test), the 22-year-old site had lower pH (in 0.01M $CaCl_2$) than the two other sites.

The field experiment ran from December 1995 until March 1998. Each study site consisted of four fertilized plots and four unfertilized plots. More detail on the experimental design of the three sites is given in Table 2.3. As far as possible, trials were laid out in a randomised design. All plots consisted of a working area containing a measuring plot, where all measurements and sampling were carried out. Due to the low stocking rate in the 14- and 22-year-old compartments, the size of the outer plots and the measuring plots was increased in order to increase the number of measuring trees on each plot. A summary of the most important data concerning the layout of the field experiments is given in Table 2.3. The fertilized plots received

Table 2.1 *Geographical and silvicultural information for the three experimental sites at the time of commencement of the field experiment. The three sites were chosen to represent different ages in a* P. patula *sawwood rotation.*

	4-year-old	14-year-old	22-year-old
Slope (°)	3	7	15
Aspect of terrain	SW	NE	East
Altitude (m.a.s.l.)	1570	1234	1234
Mean annual precipitation (mm)	1853	1201	1201
Parent Material	Shale	Shale	Shale
Soil form	Mispah	Nomanci	Nomanci
Effective soil depth (cm)	30	40	60
Date of planting	Dec. 1991	Nov. 1983	Sept. 1973
Thinning status	Not been thinned	First thinning	Second thinning
Pruning status (height pruned in m)	1,5	7	11
Fertilizer regime	No fertilization	No fertilization	No fertilization
Rotation	Second	Second	Second
Current stocking (stems ha^{-1})	816	617	419

Table 2.2 *Soil chemistry of the unfertilized plots from the three sites at the end of the treatment period. Values are the means of the four plots per treatment. The values in parenthesis represent one standard error from the mean. Within each parameter, values followed by the same letter are not significantly different at* $p<0.05$ *determined by ANOVA followed by Duncan's multiple range post hoc test. In all cases* $n=4$.

	4-year-old	14-year-old	22-year-old
EC (mS)	0.27ab (±0.03)	0.31a (±0.04)	0.16b (±0.00)
pH ($CaCl_2$)	3.94a (±0.05)	3.82a (±0.07)	3.73a (0.06)
Acidity (me%)	5.99a (±1.7)	7.52a (±0.61)	6.41a (±0.43)
Na (meq $100mg^{-1}$)	0.18a (±0.05)	0.14ab (±0.02)	0.13b (±0.01)
K (meq $100mg^{-1}$)	0.14a (±0.03)	0.19a (±0.07)	0.06b (±0.01)
Ca (meq $100mg^{-1}$)	0.31a (± 0.05)	0.28a (± 0.02)	0.26a (±0.03)
Mg (meq $100mg^{-1}$)	0.19a (±0.04)	0.14a (±0.02)	0.08b (± 0.01)
Total bases (meq $100mg^{-1}$)	0.81a (±.17)	0.75ab (±0.12)	0.53b (±0.04)
Total bases, excluding K (meq $100mg^{-1}$)	0.68a (±0.15)	0.56ab (± 0.05)	0.48b (±0.04)
Cu ($mg\ kg^{-1}$)	4.13b (±0.53)	3.95b (±0.51)	5.89a (±0.87)
Zn ($mg\ kg^{-1}$)	1.47a (±0.42)	0.77a (±0.10)	1.32a (±0.53)
Mn ($mg\ kg^{-1}$)	1.93a (±0.58)	2.38a (±0.96)	2.06a (± 0.32)
Fe ($mg\ kg^{-1}$)	214.25b (±86.82)	244.75b (±69.59)	908.75a (±256.30)
Organic C (%)	3.78a (±0.88)	4.16a (±0.63)	2.09b (±0.05)
Total P ($\mu g\ g^{-1}$)	9.63a (±1.43)	10.18a (±0.47)	8.73a (±0.56)
Total N ($mg\ g^{-1}$)	1.09a (±0.08)	0.86b (±0.02)	0.41b (±0.04)

Table 2.3 *Design of the field experiment. Within each of the three sites, four plots received no N (controls) ,while four other plots received 120 kg N ha^{-1} yr^{-1} (treatments).*

	4-year-old	14-year-old	22-year-old
Number of plots	8	8	8
Size of outer plot (tree rows)	8 x 8	9 x 9	9 x 9
Size of measuring plot (tree rows)	5 x 5	6 x 7	6 x 7
Total area in use (ha)	0,7	0,9	0,9

a total of 320 kg N ha^{-1} over the course of the experimental period (Fig. 2.2). Nitrogen was applied as a mixture of KNO_3 and NH_4NO_3 dissolved in water and applied with backpack sprayers. The ratio of NO_3^-:NH_4^+ in the application mixture was 3:1, reflecting the ratio in the precipitation over the Drakensberg escarpment area. Ammonium nitrate in crystal form and KCl were mixed with water from a nearby river to give a final volume of 0.01 litres m^2. This mixture was sprayed onto the litter layer of the fertilized sites with backpack sprayers. To simulate the periodicity of bulk N deposition inputs, N was applied in five batches of 20 - 30 kg N ha^{-1} in the summer (November to March of each year), starting in the summer of 1995/1996 and ending in the summer of 1997/1998 (Fig. 2.2).

N inputs were restricted to a maximum of 30 kg N ha^{-1} at any single application event. Unfertilized plots received no additional water or N, bar the annual input from rain. Detailed methods and sampling strategies are given in the relevant chapters.

Chapter 3

The effect of simulated N deposition on growth and nutrition of three *P. patula* plantation ecosystems

INTRODUCTION

Nitrogen in the form of NH_4^+ or NO_3^- is essential for plant growth and regulates production, structure and functioning of many terrestrial ecosystems (Tamm, 1985). Nitrogen is, however, frequently in short supply in terrestrial ecosystems, effectively limiting productivity. Most forest ecosystems are adapted to low levels of N and many trees are often adapted to scavenge for N in different forms (Waring and Schlesinger, 1985). In such ecosystems the N cycle is closed – very little N is lost from the ecosystem. Efficient and conservative cycling of N is disturbed under conditions of high N deposition to forest ecosystems, and this eventually culminates in the ecosystem becoming saturated with N (Aber *et al.* 1989). Many European forest ecosystems are already saturated with N and deposition of N to forest ecosystems is also increasing in Africa and East Asia (Galloway, 1998). It is therefore essential to study the effect of high N deposition on N cycling in ecosystems in these regions to gain insight into long-term trends in economic and ecological sustainability of ecosystems.

Current hypotheses regarding the effects of increased N deposition on N cycling in forest ecosystems predict that the N concentration in the foliage would increase after the ecosystems have reached N saturation (Aber, 1992; Fenn *et al.* 1998; Gundersen *et al.* 1998). A good correlation has been found between the level of N deposition and the concentration of N in the

foliage of coniferous trees (Boxman *et al.* 1998b; Gundersen *et al.* 1998). High levels of N in the needles are frequently accompanied by changed inter-element relationships in the needles, which may also impact negatively on growth (Oren *et al.* 1988). The N:P ratio is especially crucial to growth of forest trees; P is also frequently limiting to growth (Waring and Schlesinger, 1985; Mohren *et al.* 1986). High levels of N in the foliage, if not augmented by increased uptake of P, will disrupt optimal N:P ratios for growth, eventually leading to P deficiency (Koerselman and Meuleman, 1996; Mohren *et al.* 1986). A unilateral increase in N availability in the ecosystem due to high N deposition will also disrupt N:base cation ratios. In the Northern Hemisphere, needle loss has been correlated to imbalances in the N:Mg ratio and low Mg and Ca levels in foliage and soil (Katzensteiner *et al.* 1992; Oren *et al.* 1988).

In many areas affected by high N deposition rates, growth of trees has declined after N saturation has been reached (Schulze, 1989; Gundersen *et al.* 1998), eventually reaching a stage of tree decline where dead or dying trees are common. In the stage following N saturation any N in excess of plant demand will not lead to increases in growth (Aber *et al.* 1989; Gundersen *et al.* 1998). Instead, luxury uptake of N may take place where the N content of the foliage increases, without a concomitant increase in growth. However, any changes in growth and nutrition of trees following N application usually take place over several years to decades, depending on the degree of predisposition to N saturation (Emmett *et al.* 1995a). The extended development of these primary symptoms usually then give way to an array of secondary symptoms that are specific to certain circumstances. Tree decline in Europe has been linked to decreased frost hardiness and decreased resistance to fungal pathogen attacks, which has been linked to excess N in foliage (Nihlgård, 1985; Skeffington, 1990; Fluckiger and Braun, 1998). Excess N has also been shown to reduce drought resistance of some plants (Radin, 1981; Radin and Parker, 1979).

Older stands of coniferous trees usually reach N saturation sooner than younger stands, probably because of a reduced demand for N during this stage (Miller, 1981; Stevens *et al.* 1994; Fenn *et al.* 1998) coupled with higher N capture due to higher filtering effect of mature canopies as opposed to younger, open canopies (Stevens *et al.* 1994). This aspect is especially important in commercial forest plantations exposed to high N deposition, which are normally a tapestry of stands of varying ages.

In South African commercial pine forests N is generally not strongly limiting to growth of commercial forests, although strong responses to fertilizer N are sometimes encountered (Morris, 1986). This is mainly due to immobilization of N in thick litter layers in certain high lying areas. In contrast, in the Drakensberg escarpment forestry area application of P usually leads to significant growth increases, although these are generally relatively small (Morris, 1992). Wienand and Stock (1995) found that application of P to stands of *P. radiata* in the Southern Cape region of South Africa, a region with sandy soils and low P reserves, leads to substantial growth increases, which in turn, leads to a deficiency of N in mid-rotation, suggesting an imbalance in the N:P ratio. In contrast, nutritional problems in *P. patula* stands have been mainly related to second rotation decline (Evans, 1978; Morris, 1986). This has been attributed to P and K deficiency on gabbro-derived soils. A decrease in growth also resulted due to the immobilisation of N, P and Ca in the forest floor, especially evident at higher elevation (Morris, 1986).

A review of available literature suggests that high levels of N are present in the *P. patula* plantations in the Drakensberg escarpment area and that growth will not be affected by additional N, suggesting that the second phase of N saturation may be present (Aber *et al.* 1989). The aim of this study was therefore to determine the responsiveness of *P. patula* plantations of different

ages growing in the Drakensberg escarpment area to high applications of simulated N deposition. At the same time the effects of high N inputs on N:P ratios, N:base cation ratios as well as retranslocation of N and P in young, middle rotation and old *P. patula* trees were evaluated. The relationship between element content, concentration and leaf (needle) growth of the fertilized and unfertilised plots of the three sites was also evaluated (vector analysis). It is expected that simulated N deposition will change the N:P and N:cation ratios in the foliage such that this may impact on growth. In addition, the effect of similar doses of fertilizer will provide the opportunity to study the effect of simulated N deposition on different aged stands in a *P. patula* plantation ecosystem.

MATERIAL AND METHODS

Experimental sites (25°10'S 30°40'E) were located in the Drakensberg escarpment forestry area, approximately 20 km from Sabie, Mpumalanga Province, South Africa. All three sites were situated on shale-derived soils. Detailed soil descriptions, climate and characteristics of each site are given in Chapter 2. At the start of the experiment the sites contained 4-, 14- and 22-year-old *P. patula* stands and all three sites were in the second rotation of planting. Olbrich (1995) showed that the higher elevation *P. patula* forests in the Drakensberg escarpment receive in excess of 20 kg N ha^{-1} yr^{-1}. Nitrogen in the simulated N deposition sites was applied as a mixture of KNO_3 and NH_4NO_3 dissolved in 70 ℓ of water and applied with backpack sprayers (fertilized plots), totalling 320 kg N ha^{-1} over three years. The ratio of NO_3^-:NH_4^+ in the application mixture was 3:1, reflecting the ratio in the precipitation over the Drakensberg escarpment area (Olbrich, 1995). To simulate current deposition patterns, N was applied in five batches of 20 -

30 kg N ha^{-1} in the summer, starting in the summer of 1995/1996 and ending in the summer of 1997/1998 (See Fig. 2.2). The unfertilized plots received no additional N or water.

Growth

Diameter at breast height (DBH) and height of the trees were taken as the main measures of growth. Diameter at breast height was taken at the start of the experiment and thereafter three times a year in February, June and December from 1995 to 1998. A diameter tape was used to measure the DBH at 1.3m above the level of the forest floor. Where possible, height measurements were taken at the same time as DBH measurements. Rods were used to measure height of trees on the 4- and 14-year-old sites, while a Blume-Liess hypsometer was used to measure the height of trees on the 22-year-old site. Three replicates were taken for every tree on the latter site and the average used in data analyses. The mean volume per tree was calculated according to Kotze (1995), using the Max and Burkhart equation (Max and Burkhart, 1976), adapted for *P. patula*.

Nutrient relations

Needles were sampled in May 1996 and again in May 1997 for nutrient analysis, in accordance with the recommendations of Payn *et al.* (1989). They recommended sampling of coniferous foliage in South Africa during the winter season because of the relative stability of the nutrient content during this period. Samples were taken form the current year's needles (Y0) and the previous year's needles (Y1) in the upper third of the canopy. Olbrich (1993b) showed that the longevity of needles for *P. patula* is approximately 18 months. Needles older than two years were a rarity and were not sampled. The needles were dried at 60°C and 10 fascicles per tree

counted off. These were combined for each plot and the mass (in grams) determined to four decimal points. The combined samples were then ground and total N, total P, K, Ca, Mg, Al, Mn and Fe concentrations determined. Total P was determined using the method of Murphy and Riley (1962) after acid digestion, while total N was determined using the Kjeldahl method followed by colorimetric determination of the NH_4^+ formed. Cation concentrations were determined using the inductively coupled plasma spectrometer facility at the Chemistry Department of the University of Cape Town. Freshly fallen litter was taken from the litter traps (methodology described in Chapter 5, under Material and Methods, subsection Litterfall and mass) in June 1997 and analysed for total N, total P, K, Ca, Mg, Al, Mn and Fe using the methods described above.

Graphical vector analysis represents changes in the relationship between elemental content, elemental concentration and growth of needles (represented by weight). This relationship is used to depict trends in growth and nutrient relations, the effect of the understory vegetation and forest sustainability (Proe *et al.* 1999). Timmer and Morrow (1984) found a good correlation between graphical vector analysis predictions (Fig. 3.1 and Table 3.1) and actual growth and nutritional responses in subsequent years. Interpretations of the direction of responses (Table 3.1) are based on Timmer and Stone (1978) and Timmer and Morrow (1984). For the vector analysis the nutrient content, concentration and mass of the needles were plotted on a vector graph. Only data from the current needles of the three sites was used in the vector analyses.

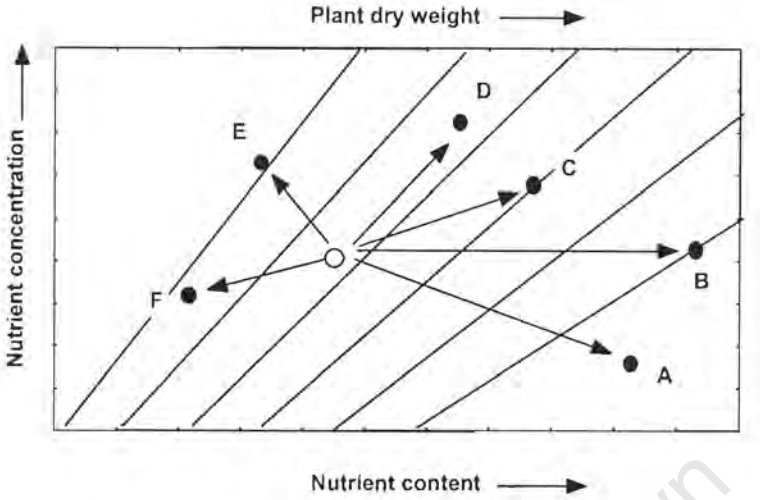

Fig. 3.1 *Schematic representation of relationship between nutrient concentration, nutrient content and dry weight of needles following nutrient application (from Timmer and Stone, 1978). The open circles represent the unfertilized and the solid circles the fertilized plots. Each of the solid circles represents a possible direction shift, which represents changes in nutrient concentration, nutrient content and leaf growth.*

Table 3.1 *Interpretation of directional differences in nutrient concentration, nutrient content and dry weight between fertilized and unfertilized plots (Timmer and Stone, 1978; Timmer and Armstrong, 1987). The interpretation also includes a diagnosis based on the interpretation of the shift.*

Direction of shift	Change in plant weight	Change in nutrient concentration	Change in nutrient content	Interpretation	Possible diagnosis
A	+	-	+	Dilution	Non-limiting
B	+	0	+	Sufficiency	Non-limiting
C	+	+	+	Deficiency	Limiting
D	0	+	+	Luxury consumption	Non-toxic
E	-	++	+ / -	Excess	Toxic
F	-	-	-	Excess	Antagonistic

Statistical analysis of data

Mean values for fertilized and unfertilized sites were compared using the students t-test expressed at the $p<0.05$ value unless otherwise stated. Analysis of Variance (ANOVA),

followed by Duncan's post hoc test was used where more than two mean values were compared. The Wilcoxon rank sum test was used to compare nutrient concentrations, nutrient contents and leaf growth of fertilized and unfertilized plots (Stevens *et al.* 1994). Summarized ANOVA tables can be found in Appendix I.

RESULTS

Growth

DBH increment (the difference between the first and last measurement) over the three growing seasons was largest on the 4-year-old site, the trees on both unfertilized and fertilized plots nearly doubling their mean diameter at 1.3m height (Fig. 3.2). The two older sites exhibited a much lower diameter growth rate than the 4-year-old site over the course of the three growth seasons. There was a significant difference ($p<0.05$; student's t-test) between the total DBH increment of the unfertilized and fertilized trees on the 4-year-old site. This is the result of a significant increase in annual DBH increment in 1996, while no increase occurred in 1997. No significant increases in annual or total DBH increment were found at any of the other sites. N treatment in 1996 also resulted in significantly shorter trees on the fertilized than on the unfertilized plots of the 4-year-old site (Fig. 3.3). No differences were evident in 1997, but the total incremental difference in height was again significantly smaller on the fertilized than the unfertilized plots. Neither the 14- nor the 22-year-old sites reacted significantly in terms of tree height in 1996 or 1997. Height increment on the 14-year-old site was higher than on the 22- or the 4-year-old site, both in terms of annual increment and total height increment. No significant volume increments were evident from any of the three sites (Fig. 3.4). Although the 14-year-old

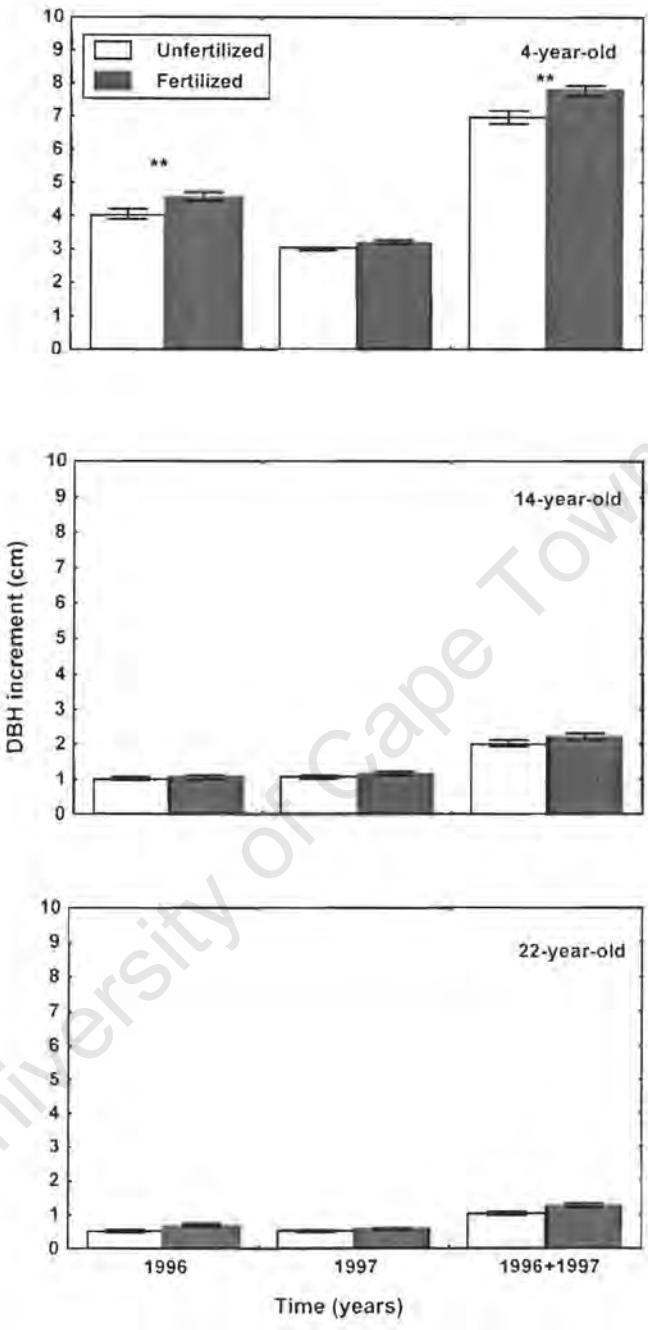

Fig. 3.2 *Mean DBH increment on the unfertilized and fertilized plots of the 4-, 14- and 22-year-old* P. patula *sites. Points plotted are the mean of all four the plots per treatment or control. Error bars represent one standard error from the mean. Asterisks are used to indicate significant differences between treatments at a specific sampling date and ** denotes significance at the* $p<0.05$ *level by Student's t test.*

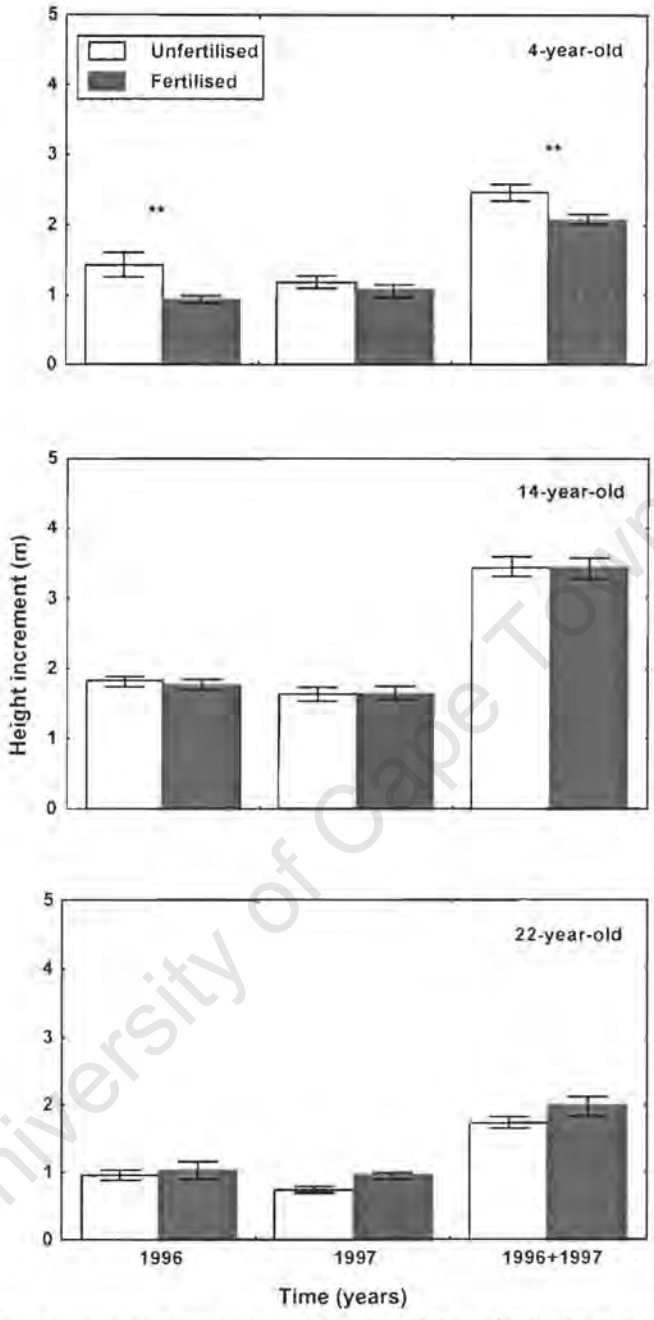

Fig. 3.3 *Mean height increment on the unfertilized and fertilized plots of the 4-, 14- and 22-year-old* P. patula *sites. Points plotted are the mean of all four the plots per treatment or control. Error bars represent one standard error from the mean. Asterisks are used to indicate significant differences between treatments at a specific sampling date and denotes significance at the* $p<0.05$ *level by Student's t test.*

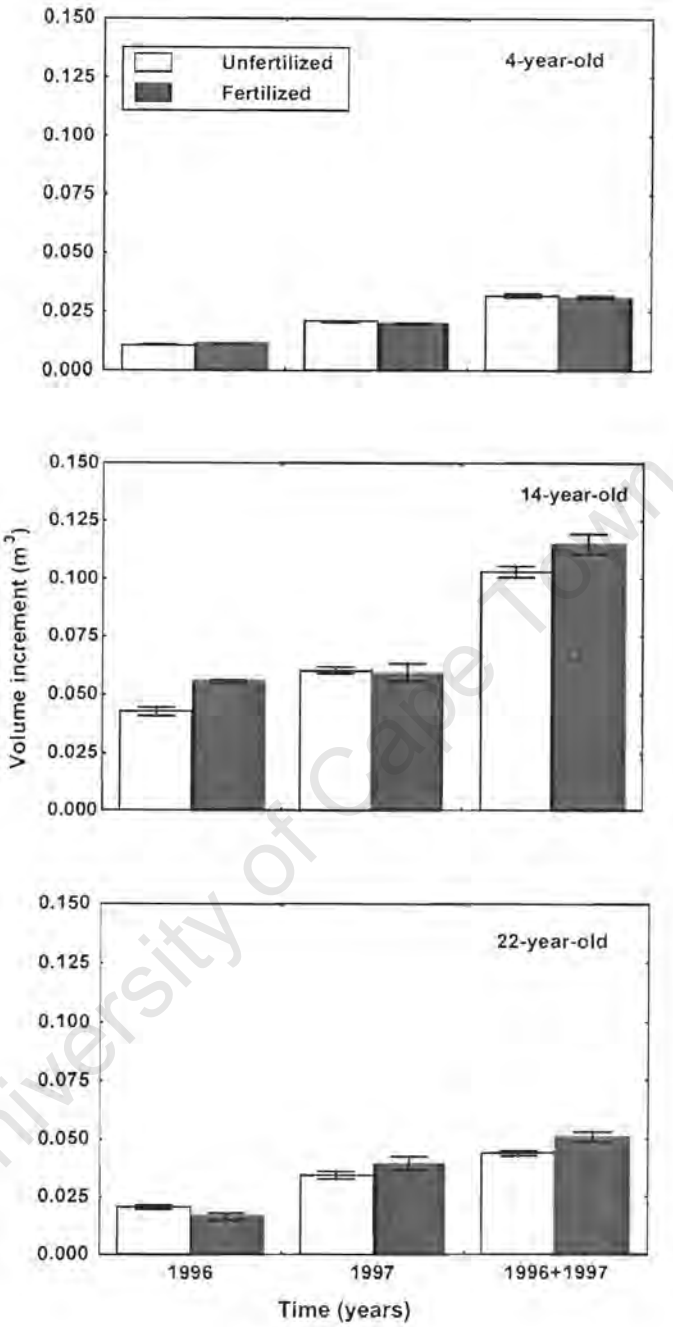

Fig. 3.4 *Mean volume increment on the unfertilized and fertilized plots of the 4-, 14- and 22-year-old* P. patula *sites. Points plotted are the mean of all four the plots per treatment or control. Error bars represent one standard error from the mean. Asterisks are used to indicate significant differences between treatments at a specific sampling date and denotes significance at the* $p<0.05$ *level by Student's t test.*

site showed a volume increment that was of the same order the 4-year-old site, both sites showing significantly higher mean volume increment than the 22-year-old site.

Nutrient relations

Mean N concentrations in the needles of *P. patula* range from 2.1% in needles sampled from unfertilized plots to 2.7% in needles sampled from fertilized plots of the 4-year-old site (Table 3.2). Mean N concentrations in current needles sampled from the fertilized plots of all three sites were significantly higher (only at $p<0.1$) than the mean concentration in the needles sampled from the unfertilized plots. This trend was also seen in the one-year-old needles. No significant differences were observed between one-year-old needles and current needles. Simulated N treatment resulted in a significantly lower P concentration in the current leaves sampled from the 4-year-old site.

Calcium and Mg concentrations generally showed decreases from the unfertilized to the fertilized leaves, but this was significant ($p<0.05$) only between the Mg concentration of the unfertilized and the fertilized plots of the three sites. There were no significant differences between the Mg and Ca concentrations of the current and the one-year-old needles. Potassium was the carrier ion in the N mixture that was sprayed onto the plots and therefore shows significant increases in concentration from the unfertilized to the fertilized plots in both the current and the one-year-old needles.

The microelements Al, Fe, Cu and Mn did not show any significant changes due to simulated N treatment, with the exception of a significant ($p<0.05$) increase in Cu concentration from the unfertilized to the fertilized plots of the 22-year-old site. The current needles showed a

general decrease in concentrations of Al, Fe and Cu from the 4-year-old to the 22-year-old site, while a decreasing trend in Mn concentrations was evident.

Mean N:P ratios in the current and one-year-old leaves of fertilized plots of all three plots were higher than those of the unfertilized plots, although, like the total N results, this was significant only at the $p<0.1$ level when the ANOVA was applied across ages, treatments and leaf ages (Table 3.3). This trend was, however, significant only at the 22-year-old site. Nitrogen:Ca and N:Mg ratios generally increased significantly from the unfertilized to the fertilized sites, except in the one-year-old needles where the increasing trend was not significant ($p<0.05$). Simulated N treatment resulted in significant decreases in the N:K ratio on the 14-year-old site, while mean Ca:Al ratios decreased significantly from the unfertilized to the fertilized plots of the 14- and the 22-year-old sites.

Leaf litter taken from the fertilized plots of the 4- and 14-year-old sites contained significantly higher N concentrations than the unfertilized plots (Table 3.4). A similar trend was evident at the 22-year-old site, but this was not significant. The level of N in the litter of the 4-year-old site was significantly higher than at both the 14-year-old and the 22-year-old sites. Phosphorus concentration in the leaf litter of the fertilized plots of the 4-year-old site were significantly lower than that of the fertilized plots. Phosphorus concentration in the leaf litter also showed a significant decreasing trend from the 4-year-old to the 22-year-old site. Potassium concentration in the leaf litter of all three sites was significantly higher in the fertilized plots than the unfertilized plots. No significant differences between fertilized and unfertilized plots were evident in Ca and Mg concentrations and microelements did not show any significant differences between unfertilized and fertilized plots, with the exception of a significant increase in Fe on the 4-year-old site. The Ca concentrations in the leaf litter were higher than that of the current and

Table 3.2 *Means of nutrient concentrations in current and one-year-old needles of unfertilized and fertilized plots of the three sites investigated. Leaves were sampled in June 1997. Values are the mean of the four plots per treatment. Within each parameter values followed by the same letter are not significantly different at the p<0.05 level of significance (three-factor ANOVA followed by Duncan's multiple range post hoc test).*

Nutrient	Site	Current needles		One-year-old needles	
		Unfertilized	Fertilized	Unfertilized	Fertilized
N	4-year-old	2.31 (± 0.07)bc	2.59 (± 0.11)ab	2.19 (± 0.02)c	2.49 (± 0.09)abc
(%)	14-year-old	2.38 (± 0.10)abc	2.66 (± 0.08)a	2.41 (± 0.11)abc	2.59 (± 0.12)ab
	22-year-old	2.21 (± 0.04)cd	2.55 (± 0.08)ab	2.14 (± 0.12)c	2.52 (± 0.16)ab
P	4-year-old	0.17 (±0.012)a	0.16 (±0.005)b	0.14 (±0.007)bc	0.13 (± 0.004)c
(%)	14-year-old	0.16 (±0.005)b	0.17 (±0.004)b	0.16 (±0.004)b	0.15 (±0.007)bc
	22-year-old	0.17 (±0.007)b	0.17 (±0.014)b	0.13 (±0.005)c	0.13 (±0.012)c
K	4-year-old	1.35 (±0.05)ab	1.64 (±0.07)a	0.84 (±0.25)d	1.24 (±0.05)cb
(%)	14-year-old	1.00 (±0.07)cd	1.56 (±0.04)ab	0.97 (±0.07)cd	1.27 (±0.04)bc
	22-year-old	0.96 (±0.07)cd	1.34 (±0.09)ab	0.92 (±0.08)d	1.25 (±0.11)bc
Ca	4-year-old	0.36 (±0.04)a	0.33 (±0.05)abc	0.23 (±0.07)b	0.31 (±0.02)abc
(%)	14-year-old	0.35 (±0.02)a	0.21 (±0.01)abc	0.27 (±0.06)abc	0.24 (±0.04)c
	22-year-old	0.34 (±0.04)a	0.26 (±0.04) bc	0.29 (±0.01)a	0.26 (±0.04)abc
Mg	4-year-old	0.21 (±0.01)a	0.17 (±0.01)b	0.13 (±0.04)bc	0.15 (±0.01)ab
(%)	14-year-old	0.20 (±0.01)a	0.19 (±0.03)b	0.16 (±0.02)ab	0.13 (±0.01)b
	22-year-old	0.21 (±0.02)a	0.19 (±0.01)b	0.20 (±0.04)a	0.17 (±0.02)ab
Al	4-year-old	1761.25 (±196.89)ab	1547.91 (±50.88)b	1389.57 (±406.93)b	1705.67 (±137.58)ab
(mg kg^{-1})	14-year-old	1459.37 (±72.56)b	1530.63 (±214.71)b	1653.21 (±106.61)ab	1346.42 (±104.48)b
	22-year-old	1367.41 (±55.72)b	1402.80 (±66.30)b	2123.55 (±167.73)a	1668.31 (±212.64)ab

Table 3.2 (continued) *Means of nutrient concentrations in current and one-year-old needles of unfertilized and fertilized plots of the three sites investigated. Leaves were sampled in June 1997. Values are the mean of the four plots per treatment. Within each parameter values followed by the same letter are not significantly different at the p<0.05 level of significance (three-factor ANOVA followed by Duncan's multiple range post hoc test).*

Nutrient	Site	Current needles		One-year-old needles	
		Unfertilized	Fertilized	Unfertilized	Fertilized
Fe	4-year-old	349.81 (±43.74)a	366.88 (±25.09)a	326.18 (±27.54)ab	343.77 (±6.77)a
(mg kg^{-1})	14-year-old	310.05 (±37.25)ab	302.35 (±50.77)ab	315.99 (±38.56)ab	298.12 (±20.36)ab
	22-year-old	220.83 (±15.22)b	283.57 (±26.44)ab	305.58 (±50.45)ab	295.23 (±30.16)ab
Cu	4-year-old	20.86 (±3.25)a	20.93 (±2.38)a	16.79 (±5.22)a	20.46 (±1.86)a
(mg kg^{-1})	14-year-old	16.59 (±0.47)a	19.21 (±2.85)a	17.58 (±2.27)a	18.37 (±1.13)a
	22-year-old	16.00 (±2.02)b	23.76 (±1.65)a	20.53 (±3.27)a	17.69 (±1.45)a
Mn	4-year-old	1222.66 (±195.63)b	1001.66 (±192.70)b	1019.75 (±303.28)b	1171.61 (±87.02)b
(mg kg^{-1})	14-year-old	1439.59 (±220.22)b	1200.10 (±263.34)b	1177.87 (±313.75)b	1049.01 (±180.83)b
	22-year-old	2228.82 (±438.92)a	2372.13 (±610.07)a	1800.31 (±320.01)ab	2219.96(±568.04)a

Table 3.3 *Means of N:nutrient ratios in current and one-year-old needles of unfertilized and fertilized plots of the three sites investigated. Leaves were sampled in June 1997. Values are the mean of the four plots per treatment. Within each parameter values followed by the same letter are not significantly different at the* $p<0.05$ *level of significance (three-factor ANOVA followed by Duncan's multiple range post hoc test).*

Ratio	Site	Current needles		One-year-old needles	
		Unfertilized	Fertilized	Unfertilized	Fertilized
N:P	4-year-old	12.58 (±0.83)c	16.43 (±0.36)bc	15.34 (±0.69)b	19.45 (±1.01)ab
	14-year-old	14.95 (±0.92)b	15.92 (±0.54)bc	14.90 (±0.59)b	17.15 (±0.84)ab
	22-year-old	13.35 (±0.59)c	15.47 (±1.23)b	16.31 (±0.70)b	20.11 (±2.63)a
N:K	4-year-old	1.72 (±0.10)de	1.59 (±0.07)e	2.01 (±0.06)bcde	2.04 (±0.15)bcde
	14-year-old	2.43 (±0.21)ab	1.71 (±0.08)de	2.54 (±0.20)a	2.06 (±0.14)bcd
	22-year-old	2.36 (±0.21)abc	1.94(±0.17)cde	2.36 (±0.07)abc	2.08 (±0.26)abcd
N:Mg	4-year-old	6.64 (±0.73)d	8.18(±0.90)bcd	7.62 (±0.06)bcd	8.18 (±0.96)bcd
	14-year-old	6.92 (±0.43)cd	12.73 (±0.76)a	9.60 (±1.18)abcd	11.65 (±2.23)a
	22-year-old	6.65 (±0.72)d	10.77(±1.84)ab	7.50 (±0.45)bcd	10.08 (±1.30)abc
N:Ca	4-year-old	11.03 (±0.64)c	15.23 (±0.46)bc	13.01 (±1.03)bc	16.31 (±0.99)b
	14-year-old	11.72 (±0.38)c	15.17 (±1.92)bc	15.22 (±0.87)bc	20.66 (±1.82)a
	22-year-old	11.08 (±1.31)c	13.41 (±1.14)bc	11.31 (±1.47)c	15.58 (±2.14)b
Ca:Al	4-year-old	2.08 (±0.16)abcd	2.19 (±0.43)abc	1.57 (±0.14)de	1.90 (±0.24)abcde
	14-year-old	2.41 (±0.21)ab	1.43 (±0.3)e	1.62 (±0.22)cde	1.79 (±0.23)cde
	22-year-old	2.51 (±0.20)a	1.81 (±0.23)bcde	1.37 (±0.11)e	1.57 (±0.05)de

Table 3.4 *Means nutrient concentration in leaf litter of unfertilized and fertilized plots of the three sites investigated. Litter was sampled in June 1997. Values are the mean of the four plots per treatment. Within each parameter values followed by the same letter are not significantly different at the p<0.05 level of significance (two-factor ANOVA followed by Duncan's multiple range post hoc test).*

Nutrient	Site	Unfertilized	Fertilized
N (%)	4-year-old	0.85 (±0.05)b	1.08 (±0.03)a
	14-year-old	0.62 (±0.08)c	0.83 (±0.09)b
	22-year-old	0.53 (±0.03)c	0.65 (±0.02)c
P (%)	4-year-old	0.07 (±0.01)a	0.05 (±0.01)b
	14-year-old	0.05 (±0.00)b	0.04 (±0.00)bcd
	22-year-old	0.02 (±0.00)d	0.03 (±0.00)cd
K (%)	4-year-old	0.33 (±0.02)b	0.56 (±0.04)a
	14-year-old	0.29 (±0.04)b	0.53 (±0.05)a
	22-year-old	0.11 (±0.01)c	0.35 (±0.02)b
Ca (%)	4-year-old	0.59 (±0.05)a	0.60 (±0.08)a
	14-year-old	0.57 (±0.10)a	0.55 (±0.03)a
	22-year-old	0.37 (±0.04)b	0.42 (±0.01)ab
Mg (%)	4-year-old	0.14 (±0.02)b	0.14 (±0.01)c
	14-year-old	0.22 (±0.02)a	0.19 (±0.00)abc
	22-year-old	0.20 (±0.03)a	0.17 (±0.0 1)abc
Al ($mg\ kg^{-1}$)	4-year-old	2099.05 (±144.88)a	2110.93 (±52.78)a
	14-year-old	1506.94 (±114.24)b	1554.40 (±87.42)b
	22-year-old	1674.88 (±157.08)b	1584.21 (±71.33)b
Fe ($mg\ kg^{-1}$)	4-year-old	699.17 (±23.17)b	888.32 (±76.89)a
	14-year-old	353.07 (±38.10)d	409.62 (±23.60)cd
	22-year-old	469.63 (±22.52)cd	491.34 (±41.37)c
Cu ($mg\ kg^{-1}$)	4-year-old	20.38 (±4.73)a	17.29 (±1.45)a
	14-year-old	12.15 (±0.75)a	16.27 (±2.48)a
	22-year-old	15.33 (±1.62)a	16.58 (±0.85)a
Mn ($mg\ kg^{-1}$)	4-year-old	1170.48 (±320.71)b	1345.64 (±297.50)b
	14-year-old	2307.11 (±585.57)ab	2096.27 (±368.89)ab
	22-year-old	2351.39 (±345.63)ab	2554.18 (±152.77)a

one-year-old needles. Magnesium concentrations in the leaf litter were generally comparable with Mg concentrations in the current and the one-year-old leaves, except at the 4-year-old site, where it was lower.

Vector analysis

The vector analyses technique was used to illustrate the relationships between nutrient content, nutrient concentration and growth of the current needles of the three sites in 1997. This was only done on nutrients that showed significant differences in terms of at least two of the stated parameters at the $p<0.1$ level. Results showed that the N content and N concentration increased in the leaves of the 4-year-old site, while the leaf weight did not change significantly (Fig. 3.5; Table 3.5). This is interpreted as luxury consumption (Timmer and Morrow, 1984; Weetman, 1989). The 14-year-old site also showed a significant increase in needle N concentration and content, while the increase in the leaf weight was not significant ($p<0.1$), which is interpreted as luxury consumption. The 22-year-old site showed significant increases in N concentration and N content, while the leaf weight decreased, although this was not significant. This trend is interpreted as a toxic reaction (Weetman, 1989).

Vector analyses of P dynamics revealed that N application to the 4-year-old site induced a significant reduction in the P concentration and content, while the leaf weight increased, but not significantly, indicating antagonism (Fig. 3.6; Table 3.5). On the 22-year-old site, the application of N acted antagonistically to Ca nutrition in the tree (Fig. 3.7). No evidence could be found that this was the case with Mg on any of the sites.

A significant increase in the K concentration and content and an increase in needle weight (not significant) revealed luxury consumption (Fig. 3.8 and Table 3.5) on both the 4-year-

Table 3.5 *Results from statistical analyses on needle weight, nutrient content and nutrient concentration. Values are the means of the four plots per treatment per site. Statistical analyses were performed using the Wilcoxon test, comparing fertilized to unfertilised treatment. * denotes significant differences on the* $p<0.1$ *scale and **significant differences on the* $p<0.05$ *scale. Where vector relationships were not determined, the symbol n.d. is used.*

Nutrient	Site	Needle weight mg needle^{-1}	Nutrient concentration % dry weight	Nutrient content µg needle^{-1}
N	4	-	**	*
	14	-	**	*
	24	-	**	**
P	4	-	**	**
	14	n.d.	n.d.	n.d.
	24	n.d.	n.d.	n.d.
K	4	-	**	*
	14	-	**	*
	24	-	**	**
Ca	4	n.d.	n.d.	n.d.
	14	-	**	**
	24	n.d.	n.d.	n.d.
Mg	4	n.d.	n.d.	n.d.
	14	n.d.	n.d.	n.d.
	24	n.d.	n.d.	n.d.

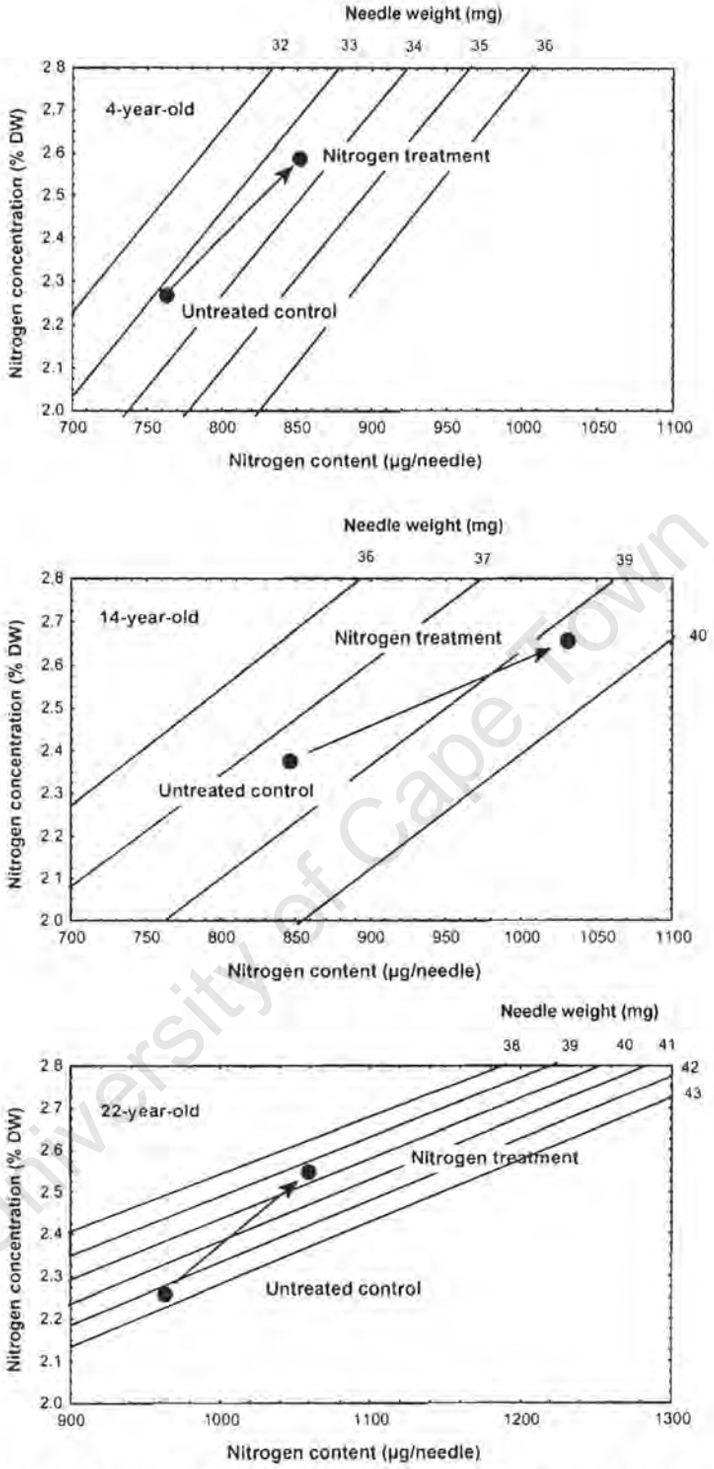

Fig. 3.5 *Shift in the relationship between N content, N concentration and unit needle weight of samples taken from the three sites investigated. The data points represent the means of the four plots per treatments. Differences between treatments were determined with the Wilcoxon rank sum test and* $p<0.01$ *was taken as a significant difference.*

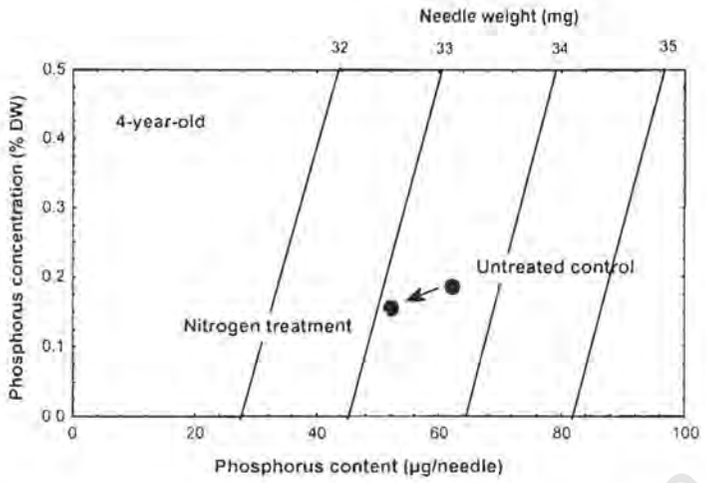

Fig. 3.6 *Shift in the relationship between needle P content, needle P concentration and unit needle weight of samples taken from the 4-year-old site. The data points represent the means of the four plots per treatments. Differences between treatments were determined with the Wilcoxon rank sum test and* $p<0.01$ *was taken as a significant difference.*

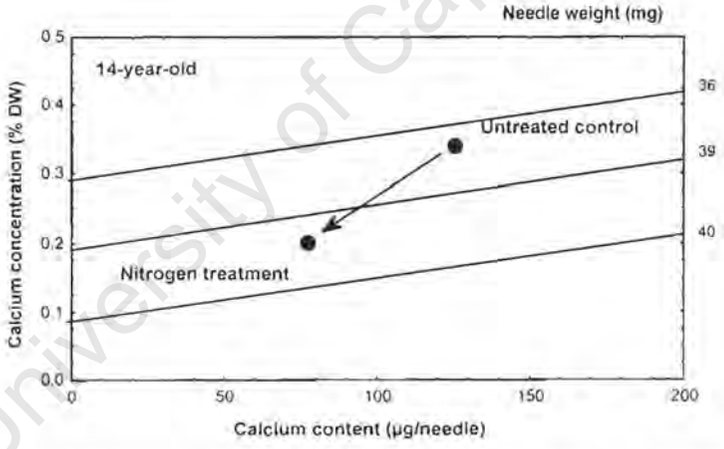

Fig. 3.7 *Shift in the relationship between needle Ca content, Ca concentration and unit needle weight of samples taken from the 14-year-old site. The data points represent the mean of the four plots per treatments. Differences between treatments were determined with the Wilcoxon rank sum test and* $p<0.01$ *was taken as a significant difference.*

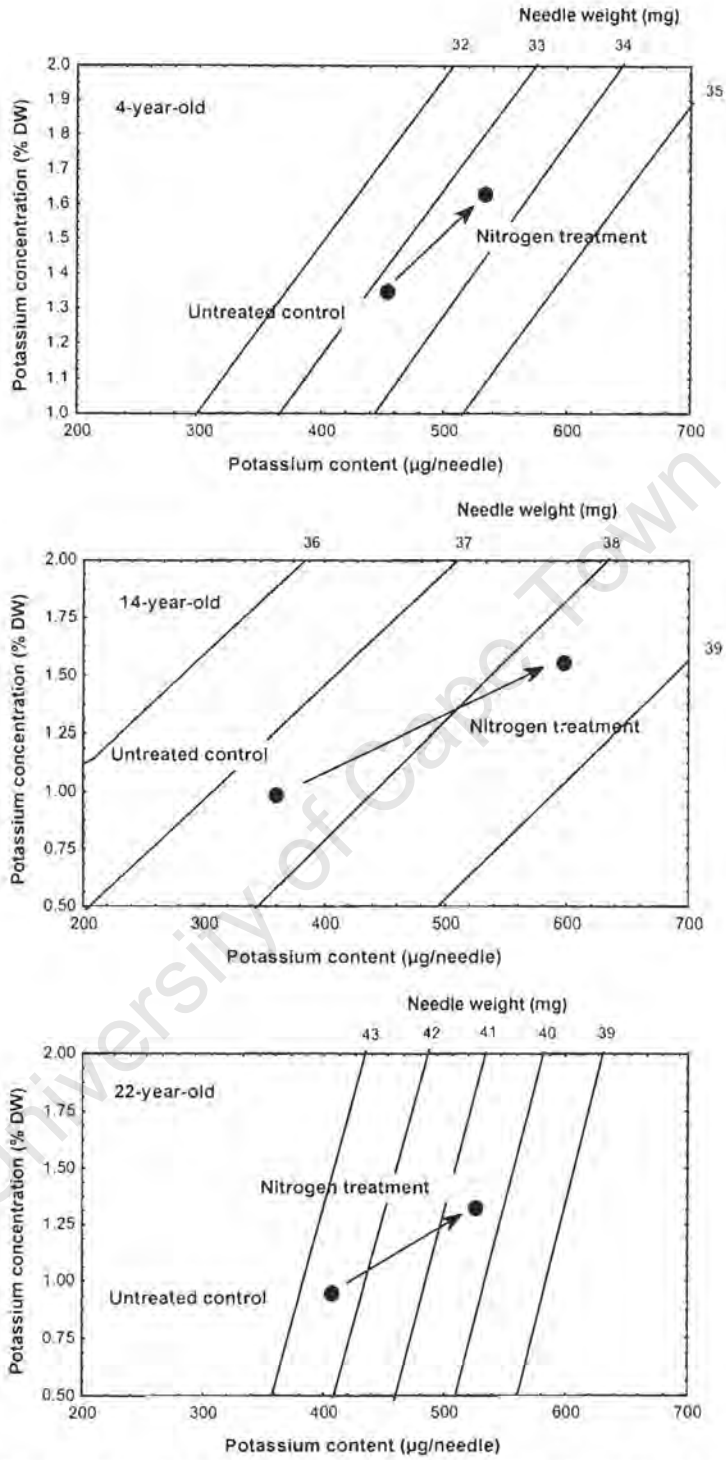

Fig. 3.8 *Shift in the relationship between needle K content, K concentration and unit needle weight of samples from the three sites investigated. The data points represent the means of the four plots per treatments. Differences between treatments were determined with the Wilcoxon rank sum test and* $p<0.01$ *was taken as a significant difference.*

old and the 14-year-old sites. The 22-year-old site showed a trend that can be interpreted as antagonism.

DISCUSSION

Simulated N deposition resulted in a significant diameter growth increase on the 4-year-old site only in the first growing season after N addition started. The second growing season showed no significant diameter growth increase, suggesting that N is no longer limiting to growth of the 4-year-old trees. These conclusions are borne out by the vector analyses, which showed a significant increase in the N concentration and N content in the foliage in 1997. The leaf weight did not show any significant increases and this is interpreted as luxury consumption (Weetman, 1989). The absolute direction of the shift can, however, also be interpreted as N deficiency (Kiefer and Fenn, 1997). The 4-year-old site received an unscheduled 1.5 m pruning in January 1997. This would increase demand for nutrients such as N, P and cations (Miller, 1981) and as such the direction of shift exhibited by this site should be treated with caution. The demand for N is highest in young trees, when the needle biomass is still being constructed (Miller, 1981) and the lack of a response to N in the second year of application suggests other nutrients are limiting to growth. Furthermore, Aber *et al.* (1989) predicted that an increase in N deposition would initially lead to increase growth in forest ecosystems. After the system has reached N saturation, other nutrients such as P and cations become limiting to growth.

Height growth on the 4-year-old site was significantly higher on the unfertilized plots than the fertilized plots in the first growing season. Seen in conjunction with the significant increase in DBH, this suggests that there is a shift in the allocation of C in the aboveground tree elements due to simulated N deposition. This resulted in a change in the taper of the trees, and thus,

aboveground allometry. This shift in allocation of biomass is not well described in the literature, but may be caused by a significant increase in the N:P ratio of the treated plots that was observed in 1997. It has been shown that the N:P ratio is crucial to the optimal growth of plants (Koerselman and Meuleman, 1996). In this study there was a significant decrease in P concentration and content in the current needles of the 4-year-old site, suggesting possible antagonism of N with P uptake. The net result of the changes in diameter and height growth on the 4-year-old site is that volume increment did not show any significant difference between the fertilized and the unfertilized plots of the 4-year-old site in the first year of the field experiment.

Neither of the two older sites showed any significant diameter or height increases in reaction to simulated N deposition, suggesting that N is not limiting to growth of these two sites. As is the case with the 4-year-old site, the direction of the shift in N content, N concentration and leaf weight on the 14-year-old site could be interpreted as N deficiency. However, continuing N fertilization in the 1997/1998 growing season did not lead to any significant growth increases, suggesting that N is not in short supply, but rather that other nutrients are limiting to growth of these sites. Normal growth of plants is characterised by a narrow range of N:P ratio that is optimal for growth (Koerselman and Meuleman, 1996). This range is from 14-16 for terrestrial plants; above this range N is short supply, while below the range P is deficient. On both the older sites an increase in the N concentration was not accompanied by a concomitant increase in P concentration and consequently the N:P ratio in the current needles of *P. patula* fell outside the range suggested by Koerselman and Meuleman (1996). The foliage of the 22-year-old site showed a P-shift that is interpreted as a toxic reaction to N fertilization. This suggests that growth of the 22-year-old trees on the fertilized plots will be negatively influenced by future N inputs by impacting on the P level in the leaves.

The 4-year-old site showed the highest diameter growth increment of the three sites, but height growth was highest on the 14-year-old site. This resulted in the 4-year-old site having the smallest annual volume increment. The 22-year-old site exhibited slower diameter and height growth than the 14-year-old site. This is possibly due to the lower MAI on the older site, suggesting lower site fertility, but can also be ascribed to a decline in MAI in older forest trees due to nutrient constraints that are found in some plantation forests (Miller, 1981).

The N concentrations in both the current and one-year-old needles of *P. patula* are higher than for other conifers. They are also higher than those found in most other studies involving *P. patula* in South Africa (Morris, 1986), although N concentrations of more than 2% (dry weight) were found in the Drakensberg escarpment area (Schutz, 1992). Leaf growth during the initial period of increased N availability will dilute the higher N concentration, but sustained high N availability will eventually increase absolute concentrations in the leaves of N saturated ecosystems. High levels of N in the leaves of *P. patula* found in this study and high growth rates in the Drakensberg escarpment area (Schutz, 1990) are an indication of an N-rich ecosystem. Application of N increased the N levels in both the current and one-year-old leaves of the fertilized sites of all three sites, suggesting that these trees benefited from the increased availability of N, either through luxury uptake, or by increased biomass.

A decrease in base cations in simulated N experiments has been recorded for red spruce in Eastern North America (Schaberg *et al.* 1997) and spruce in Germany (Oren *et al.* 1988). Decreases in base cations following N saturation are thought to be one of the causal factors that lead to forest decline in some forests ecosystems (Schulze, 1989). However, in this thesis, simulated N deposition did not have a consistently significant impact on the foliar concentrations of other ions, except for a significant decrease in Ca concentrations on the 14-year-old site and a

decrease in the P concentration of the current needles of the 4-year-old site. The decrease in the P level following increased N availability on the 4-year-old site could be the result of decreased uptake of P in the presence of high levels of N, a trend that has previously been described in coniferous forests in Britain (Thomas and Miller, 1992).

Along with an increase in the N:P ratio following N fertilization, the N:Ca and N:Mg ratios also increased. Although Ca and P have seldom been shown to be limiting to growth of *P. patula* in the Drakensberg escarpment (Morris, 1986; Schutz, 1990), these results suggest that N saturation could induce a deficiency in base cations and reduce growth of trees in that way. The Ca:Al ratio in the foliage was low, suggesting that Al is readily available in the soil of all three sites. The pH of forestry soils in the region is generally low, a prerequisite for high levels of soluble Al in the soil. The low Ca:Al ratios found in this study can be ascribed mainly to high Al concentration in the foliage, although it is still within the range for shale soils found by Schutz (1990). The Ca:Al ratios reported in this study are at the lower end of the range for the foliage of coniferous trees and could be an indication that these ecosystems are under stress from acidification and consequent Al damage to ecosystem elements such as mycorrhizas (Cronan and Grigal, 1995).

Significant trends between the three sites in terms of nutrient dynamics were detected in the leaf litter that was collected in litter traps in June 1997. The concentration of N in the leaf litter of unfertilized plots was less than 50% of that of the current needles in the 4-year-old site. The two older sites had significantly lower N concentrations that the 4-year-old site, while no significant difference in current foliage is evident between the two older sites and the youngest site. This suggests that the older trees reabsorb more N from the senescent needles than in the younger trees. This trend is consistent with older forests that satisfy a large part of nutrient

demand by retranslocation of nutrients from older needles rather than uptake (Miller, 1981). This trend is also evident in P retranslocation, which is significantly higher on the 22-year-old site than the 4- and 14-year-old sites. The leaf litter shed from the treated plots of the 4- and the 14-year-old sites had significantly higher N concentrations than that of the unfertilized plots of the two sites. A similar trend was observed on the 22-year-old site, although this trend was not significant. This is consistent with the increase in foliar N concentration that was found in current and one-year-old needles of the three sites. Leaf litter quality will thus increase as *P. patula* plantations become N saturated and the N content of the foliage increase. This could increase litter decomposition and reduce litter accumulation, a trend similar to that found by Gundersen *et al.* (1998), but also increase release and leaching of N.

The Ca concentration in the leaf litter was higher than that of the current and one-year-old needles. This suggests accumulation of Ca in the cell walls of the older needles (Waring and Schlesinger, 1985). Calcium is also a very immobile ion in the foliage and accumulates in the older needles over time (Waring and Schlesinger, 1985). Magnesium concentrations in the litter were generally similar to Mg concentrations in the current leaves, except the 4-year-old site, where they were lower. This may suggest that Mg is actively retranslocated from the older needles, although this is significant only on the 4-year-old site. The current needles on the unfertilized plots of the three sites did not show any significant differences between the three sites. Active retranslocation is an indication that Mg might become limiting to growth of the 4-year-old site.

Potassium is the carrier ion for the N mixture that was applied to the fertilized plots and was taken up into the foliage of all three sites. This is evident from the significantly higher K concentrations in the leaves of the treated trees on all three sites. It is clear that K was not in

short supply on any of the three sites. This conclusion is confirmed by results from the vector analyses that were performed on leaves sampled after the second season of N application. K was not used for growth, but was rather taken up and stored in large amounts in the current and one-year-old leaves of *P. patula*. K is generally not limiting to growth in the Drakensberg escarpment forestry area and Schutz (1992) has found K levels in excess of 0.90% in current foliage. None of the three sites show any toxicity symptom that could be ascribed to the increased availability of K.

For a non-added nutrient, dilution may progress to the point where a deficiency is induced or aggravated (Timmer and Morrow, 1984; Weetman, 1989). Dilution is not evident in any of the three sites investigated. Instead, two years of simulated N deposition resulted in significant reductions of P on the youngest site and reductions of Ca on the 14-year-old site. Reduced P concentration in the soil after simulated N and S deposition has been shown in European coniferous forests (Carriera *et al.* 1997). Base cation deficiency is one of the factors recognized as a contributor to forest decline in areas exposed to high N deposition. In the Drakensberg escarpment base cations are generally not limiting to growth of *P. patula*, although soils are classified as dystrophic (Schutz, 1990). However, this situation may change with continuing high inputs of N.

This study show that increased levels of N deposition, simulated by fertilising with N, can alter nutrient relations in the foliage. This is especially critical for older or less vigorous stands that might already have limitations in cations or P. N deposition will increase N:cation and N:P ratios, resulting in reduced growth rates in the manner suggested by Aber *et al.* (1989). Phosphorus is known to be a limiting nutrient in some forestry soils in the Drakensberg escarpment and increased N depositions to P limited plantations has the potential to induce N

saturation in these forests and reduce growth of *P. patula*. Although base cations are not a critical resource in forestry soils in the Escarpment area, continued N inputs, N saturation and subsequent N loss will reduce available base cations, possibly inducing cation deficiency, but more likely an imbalance in N:cation ratios (Aber *et al.* 1989). Nitrogen induced P and cation deficiencies are likely to be exacerbated by management practices such as pruning, which temporarily slow down growth by reducing foliar biomass and then later increase demand for limiting nutrients to rebuild the removed foliar biomass.

Chapter 4

The effect of simulated N deposition on soil chemistry in three *P. patula* plantation ecosystems

INTRODUCTION

Low levels of N deposition are considered to be an important part of the N cycle in many forest ecosystems, while a small amount of leaching of NO_3^- from the ecosystem during the growing season also takes place, mainly due to the flush of nitrification when water and temperature reach optimal conditions at the start of the growing season (Waring and Schlesinger, 1985; Landsberg and Gower, 1997). The N cycle is, however, closed and a minimum of N is lost from the cycle. The N cycle is altered in ecosystems that receive high levels of anthropogenic N deposition (<15 kg ha^{-1} yr^{-1}). The total amount of N in the system gradually increases, eventually culminating in leaching of NO_3^-, one of the early measurable symptoms of N saturation (Ågren and Bosatta, 1988; Aber *et al.* 1989; Gundersen, 1991). It has become clear that N deposition and subsequent chemical and biological changes contribute to incidents of forest decline as seen in parts of Europe and North America (Van Dijk and Roelofs, 1988; Schulze, 1989; Aber *et al.* 1995).

Nitrogen deposition and subsequent N saturation have the potential to acidify the soil via several chemical and biological processes. Increased leaching of NO_3^- will lead to soil acidification due to the concomitant loss of base cations (Reuss *et al.* 1987; Skeffington, 1990; Gundersen, 1991). Ammonium has an even greater potential for acidification due to the extra proton produced during the process of nitrification (Arnold, 1992). Conceptual models predict, and recent experimental results demonstrate, increased mineralization and nitrification following

N saturation (Aber *et al.* 1989; Gundersen *et al.* 1998). In a previous study, application of N to forest soils reduced pH by up to one pH unit (Nohrstedt, 1992), the result of a change in soil ionic strength due to increased nitrification rates (Nilsson *et al.* 1980). However, most simulated N deposition experiments have failed to show significant changes in soil pH following N saturation (Emmett *et al.* 1995b). It has been suggested that this is due to the natural buffering capacity of soils and the processes of ion exchange and dissolution on the soil exchange complex (Tyson *et al.* 1988).

The symptoms of N saturation are usually measurable in the soil before the vegetation starts showing any effects. Aber *et al.* (1989) hypothesised that leaching of NO_3^- would be one of the first signs of a forest ecosystem that is N saturated. This hypothesis has since been validated in many forest ecosystems with high anthropogenic N inputs (Dise and Wright, 1995; Fenn *et al.* 1996) and by simulated N deposition experiments (Aber *et al.* 1993; Emmett *et al.* 1995a; Emmett *et al.* 1995b; Gundersen *et al.* 1998). The magnitude of NO_3^- leaching depends upon the N retention capacity of the forest ecosystem (Johnson, 1992; Aber *et al.* 1998; Fenn *et al.* 1998). Initially, the plant and microbial biomass, as well as the soil can immobilise a large part of the incoming N with minor leaching of NO_3^-. Once an ecosystem has moved to the stage 2 or 3, and depending on the characteristics of the system, a substantial portion of incoming NO_3^- is leached (Dise and Wright, 1995; Gundersen *et al.* 1998). Nitrogen taken up by the plant and microbial biomass will be lost through the increase in nitrification, which is another symptom of N saturation (Aber *et al.* 1998; Gundersen *et al.* 1998). This will contribute to the overall pool of NO_3^- that is leached. In contrast to NO_3^-, NH_4^+ is usually retained in forest ecosystems, mainly due to the lower mobility of NH_4^+ and exchange with Al on the cation exchange complex (Wright *et al.* 1995). Ammonium is also immobilized by the plant and

microbial biomass, and is indeed preferentially taken up by the conifers (Holopainen and Heinonen-Tanski, 1992). However, in some severely saturated forests with very low N retention capacity, NH_4^+ also leaches from the rooting zone (Emmett *et al.* 1995b). In some N saturated coniferous ecosystems, outputs of N as NO_3^- is greater than inputs of N, suggesting conversion of organic and soil-bound N to soluble forms of N such as NO_3^-. An increase in nitrification is one of the characteristics of N saturated ecosystems (Aber *et al.* 1989; Gundersen *et al.* 1998). Older and taller stands are likely to become N saturated earlier than younger ones because of reduced demand for N and the larger filtering effect in taller stands (Stevens *et al.* 1994; Fenn *et al.* 1998). This predisposition effect in older stands may, however, also be present in younger stands, even when N is not present in large amounts. This situation may develop where another nutrient, such as P, is limiting to growth. Additional N will be used for luxury uptake, while a relatively large proportion may leach from the mineral soil. It is known that N is present in large amounts in the soil of pine plantations in Mpumalanga (Nowicki, 1997), while P is occasionally limiting to productivity (Schonau, 1983).

Anthropogenic N deposition can increase or decrease soil nutrient concentrations, depending on site history, inherent nutrient availability and the level and form of N in precipitation. In a simulated N deposition experiment where a *Picea abies* plantation was fertilized with NH_4SO_4, higher available base cations in the rhizosphere and bulk soil was found, possibly due to replacement of the base cations with NH_4^+ in the cation exchange complex (Majdi and Persson, 1995). However, many studies investigating the causes of forest decline in Europe have shown a reduction in base cation saturation of forest soils following N saturation (McNulty *et al.* 1990; Wilson and Skeffington, 1994b). This manifested in the trees as nutrient deficiencies, most notably, Mg^{2+} deficiency (Köllig *et al.* 1997; Binkley and Högberg, 1997). In

addition NO_3^- has been shown to compete with the phosphate ion for plant uptake (Carriera *et al.* 1997; Wilson and Skeffington, 1994b) while NH_4^+ competes with base cations (Wilson and Skeffington, 1994b).

In this study, the effects of increased N deposition on NO_3^- and NH_4^+ dynamics in the soil, availability of base cations and soil pH were studied to predict the effect of continuing N deposition on soil chemistry in *P. patula* plantation ecosystems in high N input sites in Southern Africa. It is expected that NO_3^- will start leaching rapidly, mostly due to the high levels of N already present in the forests, while NH_4^+ may not leach at all.

MATERIALS AND METHODS

Sites and treatment

The experimental sites were located in the Drakensberg escarpment forestry area, 20 km from Sabie, Mpumalanga Province, South Africa (25°10'S 30°40'E). All three sites were situated on shale-derived soils (see Chapter 2, Fig. 2.1). Details of the climate are given in Chapter 2. At the start of the experiment in 1995 the sites contained 4-, 14- and 22-year-old *P. patula* and all three sites were in the second rotation of planting. A complete site description and full details of the treatments are given in Chapter 2 and the dates and amounts of N added are given in Table 4.1.

Fluxes of mineral N in soil solution

In November 1995, one porous ceramic cup lysimeter (Soil Moisture Equipment, USA) was installed in each plot. A suction system, modified from de Clerq *et al.* (1993), was used to

extract soil solution from the soil. The ceramic cup was installed at a 60° angle with the horizontal plane, with the top of the cup 20cm below the surface of the mineral soil.

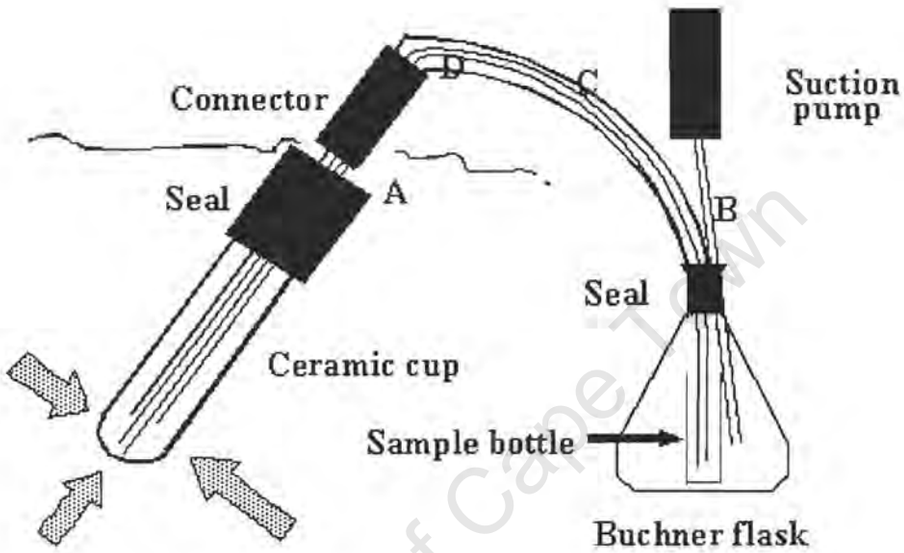

Fig. 4.1 Diagram showing the suction system used to extract soil solution using porous ceramic cups and a suction system. The ceramic cups were inserted in the topsoil and negative pressure applied at the time of sampling soil moisture.

The open end of the ceramic cup was sealed with a rubber stopper (Fig. 4.1). A polythene tube, with an inside diameter of 0.8cm (A), ran from the inside of the ceramic cup, through the rubber stopper, to the forest floor. A suction pump was connected to the Buchner flask with a rubber tube, 0.8cm inside diameter (B). A second tube (C), with an inside diameter of 0.8cm, ran from the inside of the Buchner flask. At time of sampling, pipe A was connected to pipe C, while a thin tube (D), with an inside diameter of 1mm, was inserted inside pipes A and C. This connected the ceramic cup to a sample collection bottle in the Buchner flask. A suction pump was used to create a negative pressure on the suction system and soil solution was sucked into a

sample collection bottle through tube D. Soil solution was collected in McCarthy bottles, which were sealed and placed in an icebox. Samples were stored at 4°C until further analyses.

Samples were collected from the suction cup lysimeters in summer, during the high rainfall period, when the soil solution content allowed for effective sampling.

Resin-available N

Resin-available NO_3^- and NH_4^+ were measured using ion exchange resin bags buried in the soil (Lajtha, 1988). Cation and anion exchange bags were constructed from polythene printers mesh with a mesh size of 0.2mm. Cation bags contained 8g AR400 cation exchange resin (20 - 50 mesh) and the anion bags contained 8g AR100 anion exchange resin (20 - 50 mesh; UniVar). The ion exchange bags had an area of 40 cm^2.

Cation exchange bags and anion exchange bags were activated by shaking in a 5% HCl solution for 30 min. This was followed by three distilled water rinses of 30 min. each. The bags were put in a large cotton bag and spun by hand until most of the liquid had been removed. The bags were sealed in a plastic bag and kept at 4°C until used. One of each of the cation and anion exchange resin bags were buried at the interface between the F-layer and the mineral soil at a random spot within each plot. The bags were removed after six weeks and replaced by a second set of bags. Used bags were eluated by shaking in a 5% HCl solution for 30 min, followed by analyses for NO_3^- and NH_4^+.

Soil pH

Samples for soil pH analyses were taken from the top 20cm of the mineral soil. A tube with a sharpened edge was used to take nine samples per plot. The three samples from one third of the

plot were combined to form one composite sample. The same procedure was followed for the rest of the plot and the process repeated with the rest of the plots, ending up with three composite samples per plot. The soil was sieved through a 2mm mesh, air dried and stored until analyses could be performed. Soil pH was measured in 0.01M $CaCl_2$ according to Smit and Hauptfleisch (1970) in which 20g of sieved and air-dried soil were added to 50ml of a 0.01M $CaCl_2$ solution and the pH measured after agitation with a magnetic stirrer.

Soil chemical analyses

Samples for soil chemical analyses were taken from the top 20 cm of the mineral soil in December 1997. Samples were sieved through a 2mm mesh, air dried and stored until analyses could be performed.

Soil texture analysis (three fractions) was undertaken by dispersion of soil particles with sodium hexametaphosphate [$(NaPO_4)_6$]. The sand, silt and clay fractions were determined according to the Bouyoucos method (The Non-Affiliated Soil Analysis Work Committee, 1990). Electrical conductivity was measured on a soil paste (20g soil to 50ml distilled water) using a conductometer (Metrohm, Switzerland). Exchangeable acidity was measured after extraction with 0.5M K_2SO_4 followed by titration with 0.1N NaOH. Available Ca, Na, P, K and Mg concentrations were determined on a Direct Stream Emission Plasma Spectrometer, after extraction with 1% citric acid. An Inductively Coupled Plasma Spectrometer was used to determine Cu, Zn, Mn and Fe concentrations, after extraction with 0.02M di-ammonium-EDTA. Organic carbon was analysed by the Walkley-Black dichromate oxidation method (Nelson and Sommers, 1982). The NO_3^- and NH_4^+ concentrations were determined after extraction of 20g of soil with 50ml 1M KCl (Stock, 1983).

Statistical analysis

Analysis of variance was used to determine statistical significance between the means, followed by Duncan's post hoc tests to determine the ranking of the different means, where necessary. In all cases the 5% level of significance was used to indicate a statistically significant difference. Summarized ANOVA tables can be found in Appendix I.

RESULTS

Fluxes of mineral N in soil solution

Soil solution sampled with the aid of ceramic suction cups, buried at 20cm depth in the mineral soil showed measurable NO_3^- and NH_4^+ levels present in the unfertilized plots of all three sites during the summer of 1996 and 1997 (Fig. 4.3 and 4.4).

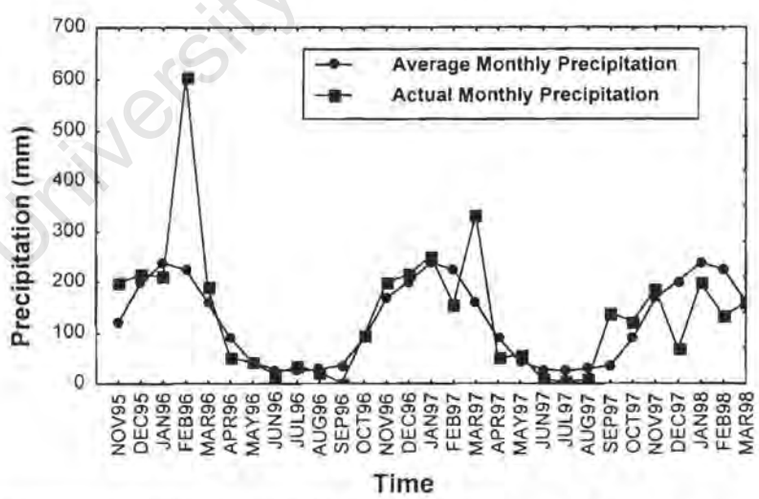

Fig. 4.2 *Mean monthly precipitation and actual precipitation at Brooklands plantation office, situated close to Sabie, Mpumalanga Province, South Africa. The rainfall gauge at the plantation office was 12km away from the 4-year-old site, with the other two sites situated within a 12km radius from the office. Mean monthly precipitation was calculated over a period of 46 years (Schutz, 1990).*

Although the 4-year-old site had higher concentrations of NO_3^-, this was not significantly different from the 14- and 22-year-old sites (ANOVA, followed by Duncan's post hoc test, $p<0.05$). There were no significant differences between the NH_4^+ concentrations in the soil solution of the unfertilized plots of the three sites ($p<0.05$).

Simulated N deposition treatment induced a rapid increase in NO_3^- concentration in the mineral soil solution at all three sites (Fig. 4.3). At all four sampling dates the 4-year-old site had significantly higher ($p<0.05$) NO_3^- concentration in the in the soil solution of the fertilized plots than the 22-year-old site.

Table 4.1 *Dates of N application to fertilized plots of the three sites. The application mixture was dissolved in water from a nearby stream and applied with backpack sprayers, mostly during the summer, when annual rainfall was highest.*

Date of N application	Amount of N added (kg N ha^{-1})
14 December 1995	30
18 January 1996	30
22 February 1996	30
19 March 1996	20
10 June 1996	10
20 November 1996	20
19 December 1997	25
21 January 1997	30
24 February 1997	25
27 March 1997	20
11 November 1997	20
17 December 1997	25
20 January 1998	30
23 February 1998	25
19 March 1998	20

The NO_3^- concentration in the 4-year-old site was also higher than the 14-year-old site on two sampling dates, namely 10 January 1996 and 25 January 1997, but not on the other sampling occasions. Significant differences between the unfertilized and the fertilized values were observed for the 4-year-old site, although the increase on the first date of sampling was significant only at $p<0.1$. Significant differences between the mean fertilized and unfertilized NO_3^- concentrations were restricted to the first year of sampling, while the increases in NO_3^- concentration were not significant on the 22-year-old site.

The NH_4^+ concentrations in the soil solution were up to 10 times lower than the NO_3^- concentration at the same date (Fig. 4.4). The NH_4^+ concentrations were higher in the fertilized plots of all three sites, but these were not consistently significant at the $p<0.05$ level (Fig. 4.3). Only on one occasion did the level of NH_4^+ in the soil solution of the 22-year-old site rise significantly higher ($p<0.05$) than those of the other sites, namely on the sampling occasion of 25 January 1997. The concentration of NH_4^+ in the soil solution on January 1997 was also higher than the fertilized plots of the 22-year-old site sampled on other dates. There were no significant differences between the NH_4^+ concentrations in the soil solution sampled from the unfertilized plots on any occasion.

Resin available N

Resin available NO_3^- concentrations increased in the fertilized plots of the three sites and appeared to be highest in the 4-year-old site (Fig. 4.5). This is in contrast to the trend observed for resin available NH_4^+. The increases were, however, not consistently significant at the $p<0.05$ level of significance (ANOVA followed by Duncan's post hoc test). Resin available NO_3^- concentrations appeared to be highest during the summer months.

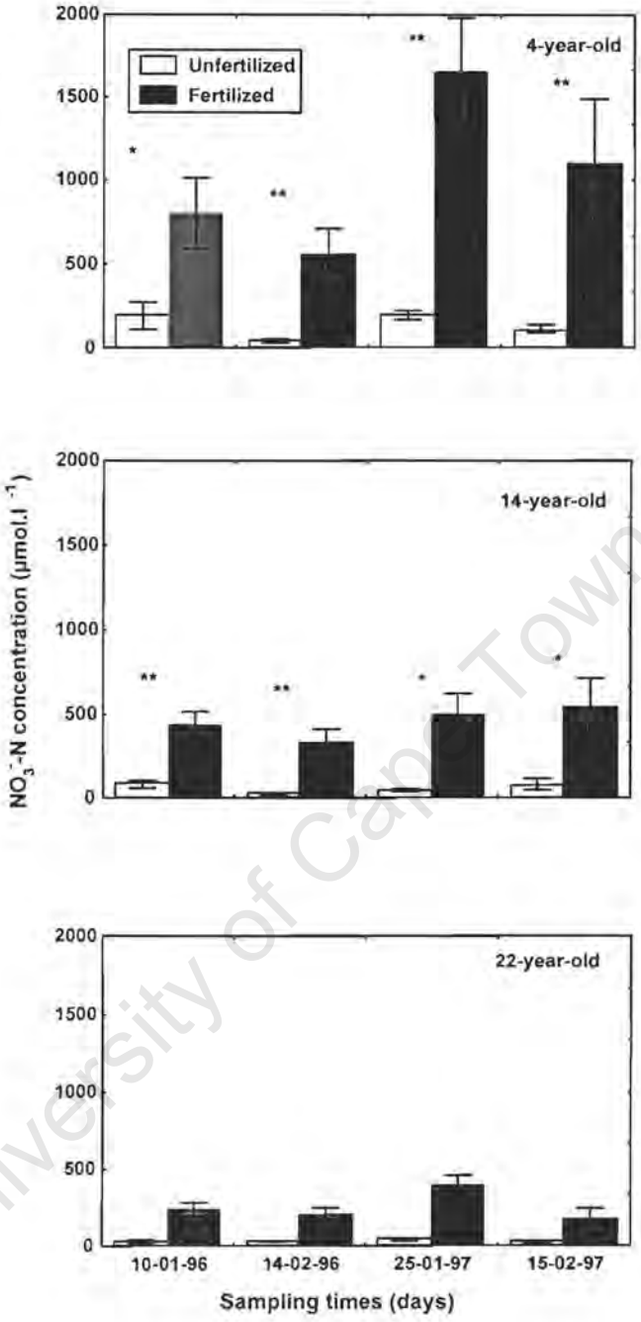

Fig. 4.3 *Mean NO_3^--N concentration in the mineral soil solution of the fertilized and unfertilized plots of the 4-, 14- and 22-year-old sites in the summer of 1995/6 and 1996/7. Asterisks are used to indicate significant differences between treatments at a specific sampling date. * denotes significance at p<0.1 and ** denotes significance at p<0.05 using Student's t-test. Each of the bars represents the mean of the four plots per treatment and error bars represent one standard error from the mean.*

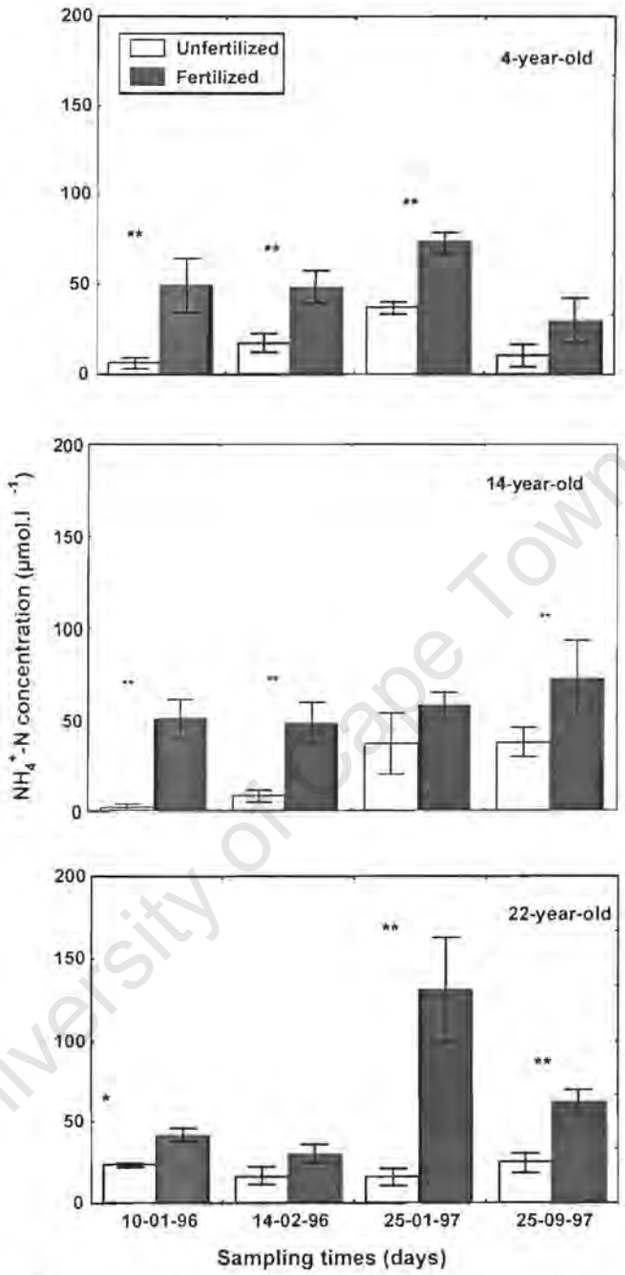

Fig. 4.4 *Mean NH_4^+-N concentration in the mineral soil solution of the fertilized and unfertilized plots of the 4-, 14- and 22-year-old sites in the summer of 1995/6 and 1996/7. Asterisks are used to indicate significant differences between treatments at a specific sampling date. * denotes significance at p<0.1 and ** denotes significance at p<0.05 using Student's t-test. Each of the bars represents the mean of the four plots per treatment and error bars represent one standard error from the mean.*

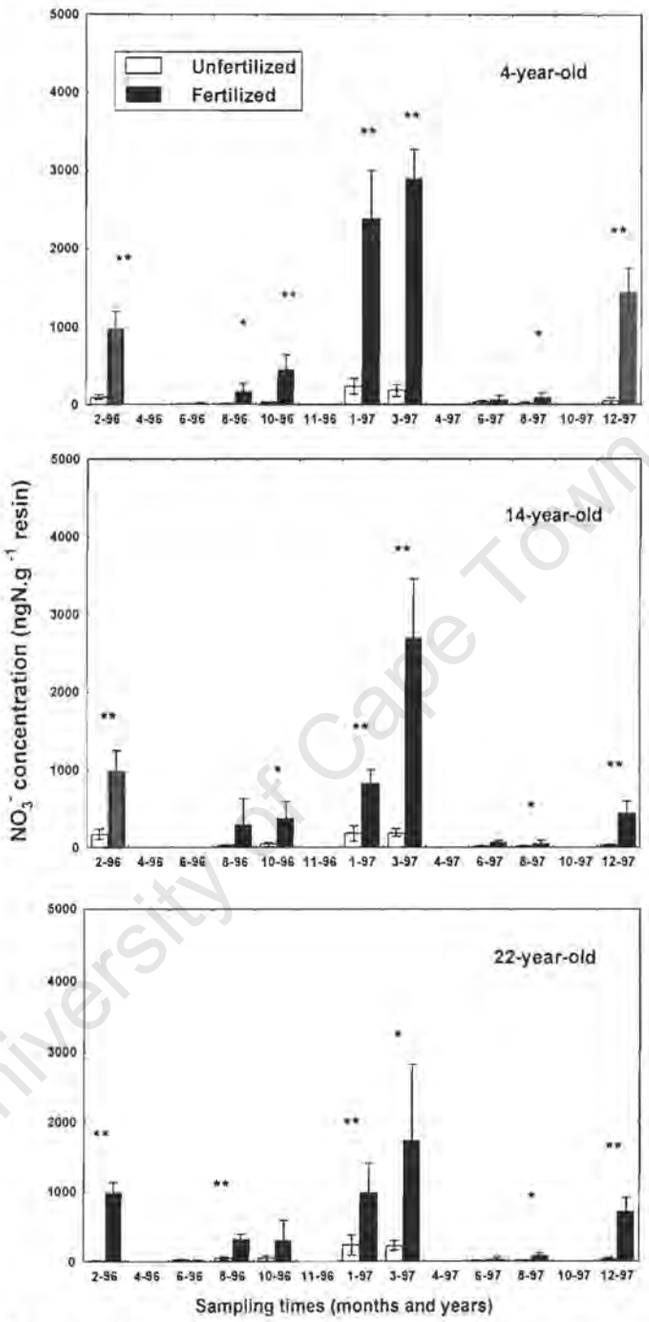

Fig. 4.5 *Mean NO_3^--N accumulation in field-placed resin bags from the fertilized and unfertilized plots of the 4-, 14- and 22-year-old sites during the experimental period, starting in 1995 and ending in 1998. Asterisks are used to indicate significant differences between treatments at a specific sampling date. * denotes significance at* $p<0.1$ *and ** denotes significance at* $p<0.05$ *using Student's t-test. Each of the bars represents the mean of the four plots per treatment and error bars represent one standard error from the mean.*

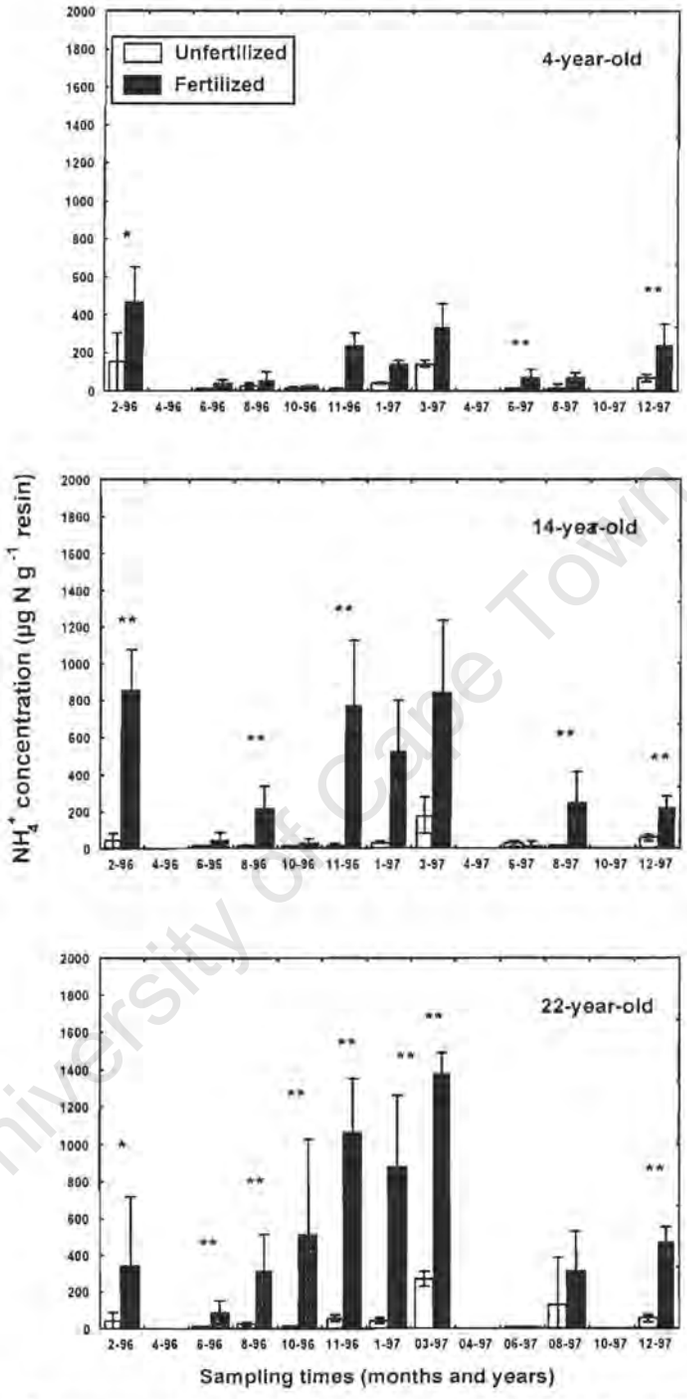

Fig. 4.6 *Mean NH_4^+-N accumulation in field-placed resin bags from the fertilized and unfertilized plots of the 4-, 14- and 22-year-old sites during the experimental period, starting in 1995 and ending in 1998. Asterisks are used to indicate significant differences between treatments at a specific sampling date. * denotes significance at* $p<0.1$ *and ** denotes significance at* $p<0.05$ *using Student's t-test. Each of the bars represents the mean of the four plots per treatment and error bars represent one standard error from the mean.*

Resin available NH_4^+ (Fig. 4.6) increased in litter layers of the fertilized plots, although the increases were not consistently significant at $p<0.05$. A highly consistent trend is that high absolute values for resin available NH_4^+ in the unfertilized plots were observed during the summer months. In the fertilized plots of the 4-year-old site absolute concentrations of resin available NH_4^+ increased, but this was significant at $p<0.05$ only during June 1997 and December 1997. Significant differences ($p<0.05$) between unfertilized and fertilized plots of the 14-year-old site were observed throughout the fertilized period, except on the first sampling occasion (February 1996) and during the winter of 1997 (June 1997 and August 1997). The 22-year-old site also exhibited significantly higher resin available NH_4^+ concentrations than the 4-year-old site. This is especially evident during the winter of 1996 and the summer of 1996/7.

Soil pH

Soil pH remained relatively constant throughout the treatment period (Fig. 4.7). No significant differences were found between the control plots of the three sites. Simulated N deposition did not significantly alter the pH of the mineral soil of any of the sites. .

Soil chemical analyses

Soil texture analyses showed high clay contents in all soils, ranging from 17 to 39% (Table 4.2). The 22-year-old site has the highest clay content, while the organic carbon on this site is significantly lower than the remaining sites (Table 4.3).

There is a trend of decreasing base cation concentration in the top 10cm of the mineral soil with an increase in age of the trees (Table 4.3). The 22-year-old site had significantly less

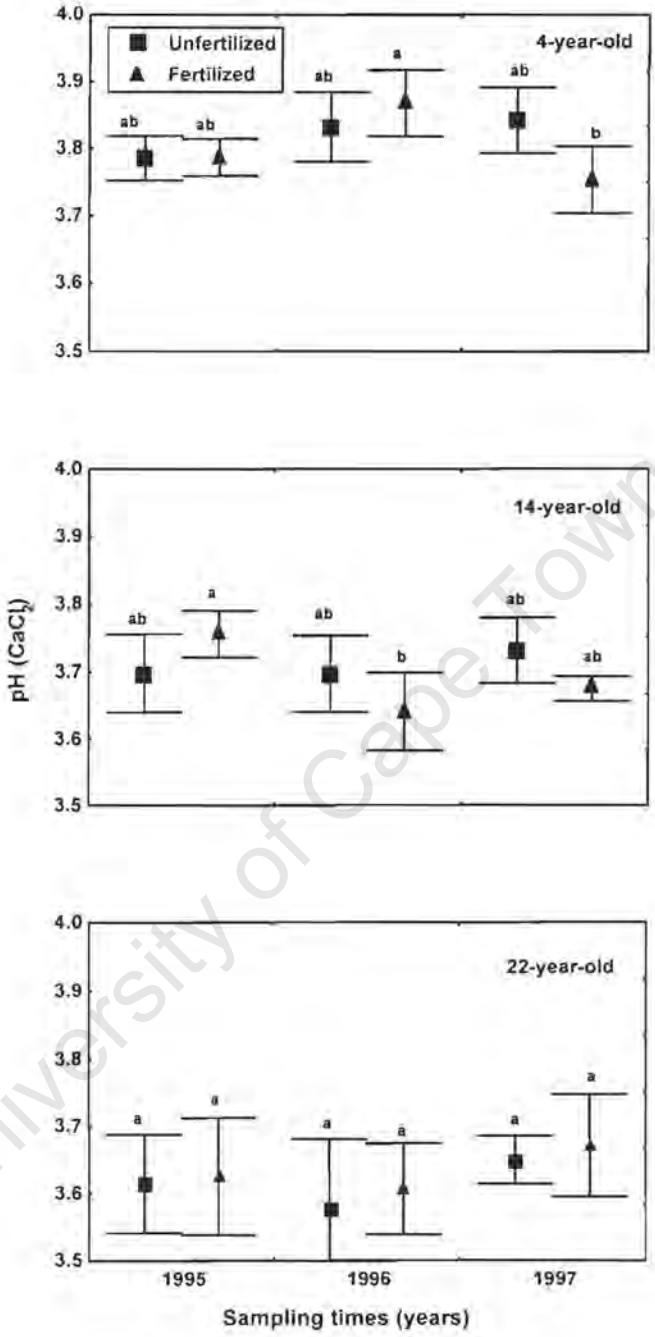

Fig. 4.7 *pH($CaCl_2$) of top 10cm of mineral soil of the fertilized and unfertilized plots of the 4-, 14- and 22-year-old sites. Within each site values with the same letter are not significantly different at* $p<0.05$ *(two way ANOVA, followed by Duncan's multiple range post hoc test). Points plotted are the means of the 12 measurements and error bars represent one standard error from the mean.*

($p<0.05$) Na, K and Mg than the 4-year-old site and also had significantly lower K and Mg concentration than the 14-year-old site. Total bases were significantly lower in the 22-year-old site than on the 4-year-old site, but were not significantly different from the 14-year-old site. Simulated N treatment did not change the concentrations of any of the base cations in the top 10cm of the mineral soil significantly, except for an increase in K, which was the carrier ion in the N mixture that was applied to the fertilized plots. None of the three sites show any significant change in total bases after N addition, when K was excluded from the sum of base cations.

After three years of simulated N treatment, exchangeable acidity, including both H^+ and Al, was unchanged from the unfertilized plots. The electrical conductivity (EC) was also unchanged, while organic C was significantly lower on the 22-year-old site than the 4- and 14-year-old sites. The organic C content did not change significantly after simulated N treatment.

Copper and Fe showed an inverse trend to that exhibited by the base cations. The 22-year-old site had significantly higher Cu and Fe concentrations than the 4- and the 14-year-old sites. Neither Zn nor Mn concentrations were significantly different between the sites. Simulated N deposition had no significant effects on any of the trace elements, except Fe, which decreased significantly after simulated N deposition treatment. Available P on the 22-year-old site was significantly lower than at the 4- and 14-year-old sites (Table 4.4). Available P remained unaffected by simulated N treatment.

The Pearson correlation coefficients of soil chemical characteristics calculated with and without the values from the fertilized plots showed very similar values (Table 4.5). In the unfertilized plots, total base cations, when K was excluded from calculations, was well correlated with organic C ($r = 0.72$) and with available P ($r = 0.68$). This relationship increased when all

Table 4.2 *Results of three-fraction soil texture analyses of samples from the top 20cm of the mineral soil from the 4-, 14- and 22-year-old sites. Values are the means of the four plots per treatment and the values in parentheses represent one standard error from the mean.*

	4-yr-old (n = 4)	14-yr-old (n = 4)	22-yr-old (n = 4)
Clay (%)	29.50 (± 0.87)	16.67 (± 1.23)	39.09 (± 0.58)
Silt (%)	23.51 (± 3.18)	28.33 (± 0.6)	24.15 (± 1.15)
Sand (%)	47.26 (± 4.04)	54.33 (± 1.41)	37.59 (± 0.58)

Table 4.3 *Soil chemistry of the top 20cm of the fertilized and unfertilized plots from the 4-, 14- and 22-year-old sites at the end of the treatment period, in December 1997. Values are the mean of the four plots per treatment. Within each parameter, values followed by the same letter are not significantly different at* $p<0.05$ *determined by two-factor ANOVA followed by Duncan's multiple range post hoc test. Value in parenthesis represents one standard error from the mean.*

	4-yr-old		14-yr-old		22-yr-old	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized
EC (mS)	0.27bc (±0.03)	0.51a (±0.08)	0.31b (±0.04)	0.39b (±0.03)	0.16c (±0.00)	0.30b (±0.01)
Acidity (me%)	5.99 b (±1.70)	6.99 b (±1.01)	7.52 a (±0.61)	8.05 a (±0.28)	6.41b (±0.43)	6.53 b (±1.01)
Na (meq $100mg^{-1}$)	0.18a (±0.05)	0.16ab (±0.02)	0.14ab (±0.02)	0.16ab (±0.02)	0.13b (±0.01)	0.12b (±0.02)
K (meq $100mg^{-1}$)	0.14c (±0.03)	0.33a (±0.07)	0.19bc (±0.07)	0.24b (±0.03)	0.06d (±0.01)	0.14c (±0.01)
Ca (meq $100mg^{-1}$)	0.31a (± 0.05)	0.34a (±0.05)	0.28ab (± 0.02)	0.33a (±0.06)	0.26c (±0.03)	0.24c (±0.02)
Mg (meq $100mg^{-1}$)	0.19a (±0.04)	0.19a (±0.05)	0.14b (±0.02)	0.17b (±0.01)	0.08c (± 0.01)	0.07c (±0.01)
Total bases (meq $100mg^{-1}$)	0.81b (±.17)	1.02a (±0.19)	0.75bc (±0.12)	0.89ab (±0.06)	0.53c (±0.04)	0.58c (±0.03)
Total bases , excluding K (meq $100mg^{-1}$)	0.68a (±0.15)	0.68a (±0.18)	0.56ab (± 0.05)	0.65a (±0.07)	0.48b (±0.04)	0.45b (±0.03)
Cu (mg kg^{-1})	4.13a (±0.53)	3.52a (±0.30)	3.95a (±0.51)	4.33a (±0.39)	5.89b (±0.87)	5.46b (±0.80)
Zn (mg kg^{-1})	1.47a (±0.42)	0.90b (±0.33)	0.77b (±0.10)	1.18ab (±0.31)	1.32ab (±0.53)	1.00ab (±0.15)

Table 4.3 (Continued) *Soil chemistry of the top 20cm of the fertilized and unfertilized plots from the 4-, 14- and 22-year-old sites at the end of the treatment period, in December 1997. Values are the means of the four plots per treatment. Within each parameter, values followed by the same letter are not significantly different at* $p<0.05$ *determined by two way ANOVA followed by Duncan's multiple range post hoc test. Value in parenthesis represents one standard error from the mean.*

	4-yr-old		14-yr-old		22-yr-old	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized
Mn (mg kg^{-1})	1.93a (±0.58)	2.35a (±0.97)	2.38a (±0.96)	4.27a (±4.09)	2.06a (± 0.32)	2.42a (±10.15)
Fe (mg kg^{-1})	214.25c (±86.82)	191.25c (±49.66)	244.75bc (±69.59)	274.25bc (±58.55)	908.75a (±256.30)	538.00b (±304.32)
Organic C (%)	3.78b (±0.88)	3.93b (±0.62)	4.16b (±0.63)	4.67b (±0.23)	2.09a (±0.05)	2.17a (±0.34)
C:N ratio	18.08a (±0.66)	18.60a (±0.21)	18.29a (±0.36)	20.29a (±0.31)	20.11a (±0.19)	22.93a (±0.75)

Table 4.4 *Nitrogen and P chemistry of the top 20cm of the unfertilized and fertilized plots from the 4-, 14- and 22-year-old sites at the end of the treatment period. Values are the means of the four plots per treatment. Within each parameter, values followed by the same letter are not significantly different at* $p<0.05$*, determined by two-factor ANOVA followed by Duncan's multiple range post hoc test. Values in parenthesis represent one standard error from the mean.*

	4-yr-old		14-yr-old		22-yr-old	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized
Available P (µg g^{-1})	2.23a (±1.16)	2.93a (±0.33)	2.13a (±0.54)	2.50a (±0.49)	0.35b (±0.24)	0.50b (±0.20)
Total P (µg g^{-1})	9.63a (±1.43)	9.56ab (±0.53)	10.18a (±0.47)	9.89a (±0.87)	8.73b (±0.56)	9.25ab (±0.25)
Total N (mg g^{-1})	1.09b (±0.08)	1.31a (±0.08)	0.86b (±0.02)	1.03b (±0.04)	0.41c (±0.04)	0.50c (±0.01)

the plots, including the fertilization treatment, were included in calculations. Individual base cations were negatively correlated with Cu and Fe, irrespective of whether the fertilized plots were included or excluded. Total base cations were also negatively correlated with the aforementioned microelements. The concentration of Cu and Fe in the top 10cm of the mineral soil was positively correlated ($r = 0.75$ when fertilized plots were excluded, and $r = 0.74$ when

Table 4.5 *Pearson correlation coefficients for different soil parameters after analysing soil taken from the field sites. Soil was sampled from the top 20cm of the mineral soil at the end of the experimental period (December 1997). All coefficients below the highlighted numbers represent calculations based on all sites and all treatments. Values above the highlighted values excludes results from the fertilized plots.*

	pH	EC	Acid	Na	K	Ca	Mg	Total Bases	Total Bases (-K)	Cu	Zn	Mn	Fe	Organic C	Available P	Total P	Total N
pH	1.00	-0.02	-0.53	0.17	0.20	0.08	0.42	0.27	0.26	-0.55	0.04	-0.21	-0.63	0.22	0.23	0.18	0.69
Electrical Conductivity	0.10	1.00	-0.30	-0.15	-0.45	-0.32	-0.39	-0.42	-0.32	0.48	0.35	-0.20	0.43	-0.42	-0.40	-0.56	-0.37
Total Acidity	-0.58	-0.39	1.00	0.30	0.57	0.39	0.28	0.49	0.34	-0.07	-0.30	0.35	-0.03	0.64	0.49	0.46	0.03
Na	0.12	-0.14	0.33	1.00	0.28	0.89	0.77	0.79	0.94	-0.10	0.35	-0.36	-0.25	0.52	0.46	0.58	0.37
K	0.05	-0.60	0.51	0.31	1.00	0.37	0.64	0.78	0.49	-0.71	-0.32	0.48	-0.70	0.87	0.79	0.57	0.68
Ca	-0.02	-0.41	0.46	0.73	0.60	1.00	0.77	0.83	0.94	-0.30	0.38	-0.17	-0.28	0.59	0.50	0.41	0.44
Mg	0.23	-0.44	0.43	0.71	0.72	0.79	1.00	0.94	0.93	-0.59	0.15	0.11	-0.67	0.83	0.86	0.66	0.81
Total Bases	0.10	-0.54	0.53	0.68	0.88	0.87	0.93	1.00	0.93	-0.59	0.07	0.12	-0.63	0.89	0.83	0.67	0.74
Total Bases (-K)	0.12	-0.39	0.45	0.86	0.63	0.93	0.93	0.93	1.00	-0.39	0.30	-0.12	-0.46	0.72	0.68	0.60	0.62
Cu	-0.29	0.52	-0.23	-0.24	-0.65	-0.46	-0.67	-0.65	-0.54	1.00	0.30	-0.35	0.75	-0.64	-0.68	-0.30	-0.84
Zn	0.04	0.41	-0.12	0.33	-0.23	0.09	0.10	-0.01	0.16	0.24	1.00	-0.37	0.01	-0.17	-0.14	-0.30	0.02
Mn	-0.43	-0.11	0.26	-0.16	-0.01	-0.00	-0.14	-0.07	-0.10	0.02	-0.12	1.00	-0.09	0.40	0.42	-0.12	0.19
Fe	-0.38	0.48	-0.20	-0.21	-0.59	-0.35	-0.64	-0.58	-0.47	0.74	0.07	-0.17	1.00	-0.67	-0.69	-0.58	-0.83
Organic C	0.05	-0.43	0.72	0.57	0.68	0.69	0.85	0.82	0.79	-0.63	-0.04	0.03	-0.64	1.00	0.92	0.66	0.73
Available P	0.14	-0.51	0.52	0.44	0.77	0.60	0.89	0.83	0.74	-0.73	-0.14	-0.10	-0.70	0.86	1.00	0.65	0.80
Total P	0.20	-0.46	0.39	0.59	0.40	0.47	0.55	0.55	0.58	-0.23	-0.24	-0.03	-0.47	0.60	0.54	1.00	0.45
Total N	0.57	-0.38	0.09	0.49	0.62	0.54	0.76	0.72	0.68	-0.78	-0.04	-0.17	-0.73	0.67	0.78	0.49	1.00

included). Only a relatively weak positive relationship between pH ($CaCl_2$) and total acidity and pH and total bases was found. When the fertilized plots were excluded from the correlation analysis, pH and Fe and pH and Cu were negatively correlated. This coefficient decreased when all plots were included in calculations. Organic C and available P were highly correlated in both sets of calculations, while organic C was also negatively correlated with Fe and Cu.

DISCUSSION

Application of N to the fertilized plots of the three sites resulted in elevated levels of N in the soil solution, with NO_3^- as the dominant N ion. The ratio of NO_3^- to NH_4^+ in the application mixture was 3:1, but the ratio of NO_3^- - to NH_4^+ in the soil solution of the fertilized sites was higher than 10:1, indicating an imbalance between applied N and N leaching from the rooting zone. This could be due to the high mobility of the NO_3^- ion in soil solution relative to the NH_4^+ ion (Emmett *et al.* 1995b; Wright *et al.* 1995), but could also be due to a number of other N transformation interactions or uptake from the plant. This trend is consistent with results obtained from other simulated N deposition experiments where NH_4NO_3 was applied to coniferous ecosystems (Emmett *et al.* 1995b; Aber *et al.* 1993). Nitrate dominates outputs from N saturated ecosystems, while leaching of NH_4^+ from the rooting zone is seen in rare circumstances where the ecosystem is saturated with N and the ability of the soil to retain N is low (Emmett *et al.* 1995b).

Although there were detectable levels of NO_3^- in the soil solution of the unfertilized plots, this could be due to elevated nitrification and mineralization of N during summer, as suggested by Powers (1990). Measurable NH_4^+ was also present in the unfertilized plots of the three sites. All four sampling occasions were during summer, when the rate of N mineralization is highest

(Powers, 1990). This suggests that NH_4^+ produced in the organic soil during that period and driven downward in the soil profile contribute to significant levels of NH_4^+ in the soil solution from unfertilized plots. There is also a trend of significantly higher NH_4^+ in the 22-year-old site, while the NO_3^- is significantly lower on the 22-year-old site than the 4-year-old site. This is because nitrification makes a greater contribution to the soil solution NO_3^- concentration on the 4-year-old site than on the other sites and is consistent with results obtained from *ex situ* nitrification experiments conducted in this thesis (Chapter 5). The 4-year-old site has a higher rate of nitrification and soil temperature is higher, partially due to a more open canopy than the other sites.

Soil incubated resin bags were used to gain a longer-term perspective on the dynamics of NH_4^+ and NO_3^- in the organic layers of the three sites. The overall trend in resin available mineral N in the fertilized plots on the sites reflected the pattern of N application, i.e. high during summer and low during the winter months. Simulated N deposition increased the NH_4^+ and NO_3^- concentrations in the litter layer of all three sites, but resulted in disproportionately higher levels of NO_3^- on the youngest site. This is the result of the lack of a uniformly thick litter layer on this site, reducing the immobilization of NO_3^- in the organic soil layer. The soil on this site is more exposed than the other sites, resulting in the site exhibiting higher levels of nitrification, mainly due to higher soil temperatures and pronounced wet-dry cycles during summer. This conclusion is borne out by higher nitrification rates found here as opposed to the other sites (Chapter 5).

The 14-year-old site showed a significant increase in the concentration of NH_4^+ on the resin bags following the first application of N to the fertilized plots in December 1995. This could be attributed to a higher ability of the other sites to retain N in the organic soil horizons

through strong competition by nitrifying microorganisms or adsorption on the cation exchange sites (Johnson, 1992). Continued application of mineral N reduced the ability of the 14-year-old site to retain NH_4^+ and NO_3^- in the litter layer and the concentrations of these ions increased at subsequent sampling occasions, demonstrating the low N retention capabilities of these sites. The concentration of resin available NH_4^+ increased significantly with age of the site. This could be due to increased nitrification of added NH_4^+ on the youngest site, while relatively higher ammonification contributed to the high NH_4^+ concentrations on the 22-year-old site. Resin available NH_4^+ on the 22-year-old site was also high after application of N during June 1996 (Table 4.1). Lajtha (1988) concluded that field water regime is very important in results obtained from resin bags. This conclusion is not applicable to the present situation, where application of N during the relatively dry month of June did lead to a significant increase in resin available NH_4^+. Relatively more NH_4^+ and NO_3^- accumulate on ion exchange resin inserted in the litter layer of the unfertilized plots during the summer months. This is due to low water availability during the dry winter months as opposed to summer (Lajtha, 1988) as well as increased rates of nitrification and mineralization during the summer.

Two seasons of simulated N fertilization did not change the pH($CaCl_2$) of the top 10 cm of the mineral soil of any of the sites significantly, despite the addition of more than 320 kg N ha^{-1}. This is in contrast to relatively pristine forests in Scandinavia, where application of a single dose of 150 kg N ha^{-1} resulted in a one-unit drop in pH (Nilsson *et al.* 1980; Nohrstedt, 1992). This was attributed to acidification due to increases in the nitrification rate after N application. However, the lack of change in the pH after N fertilization in this study complements results from other simulated N deposition experiments that have previously been exposed to moderate levels of N input (NcNulty *et al.* 1996; Gundersen *et al.* 1998). It is possible that the addition of

K, as part of the application mixture, masked any changes in pH due to N transformations in the soil. Soils from all three sites investigated are acid, but comparable to other studies on the same parent material and in the same area. Schutz (1990) found pH(H_20) values of approximately 4.6, while Nowicki (1997) found pH(KCl) values for all types of geology in the Drakensberg escarpment area ranging from 3.8 to 4.5.

Application of N did not change the level of available cations in the mineral soil of the plots significantly, with the exception of K, the carrier ion in the application mixture, which increased significantly ($p<0.05$) on the 4-year-old and 22-year-old sites, but only at $p<0.1$ on the 14-year-old site. This increased the total bases significantly, which is not the case when K was excluded from total bases. Na, Ca and Mg was unaffected by simulated N treatment. This is in contrast to some studies where base cations have been found to increase in soil solution after fertilization with N (Majdi and Persson, 1995), while it is consistent with other studies (Emmett *et al.* 1995b).

The concentration of Na, K and Mg in the unfertilized plots decreases significantly from the 4- to the 22-year-old site. This is consistent with increased lockup of base cations in the standing biomass and litter layers on older relative to younger forest trees (Morris, 1986), increasing the sensitivity of the older sites to N saturation because of the limitation low levels of cations may place on growth (Fenn *et al.* 1998).

Acid anions such as Cu, Zn, Mn and Fe were unaffected by fertilization, while exchangeable acidity ($H^+ + Al^{3+}$) showed a trend of increasing concentration with fertilization. This is the result of increased availability of Al due to exchange with NH_4^+ on the soil exchange complex, as was suggested by Emmett *et al.* (1995b). Both Cu and Fe concentration increased significantly with an increase in age of the site, showing a negative correlation with organic C.

This could be related to inherent soil properties of the soil, but also to the trend of decreasing pH with age of the site. It is expected that increased nitrification will decrease the pH on these sites, releasing more heavy metals such as Fe, Mn, and Al, which are dependant on pH for solubility.

None of the three sites was fertilized at planting, and the decreasing trend in available P with age in the unfertilized plots may reflect uptake and immobilisation of P by the older trees on the 22-year-old site. From results presented here it can be concluded that older *P. patula* stands get most of their P from remobilisation and uptake of P from the organic soil layer, as suggested by Miller (1981). Plant available P was unaffected by simulated N deposition treatment, although foliage P levels decreased significantly due to N treatment in the first year of N addition (Chapter 3). Over the longer term this suggests that the addition of N to the soil could lead to P deficiencies by reducing P uptake.

Aber *et al.* (1989) and Fenn *et al.* (1998) suggested that older, less vigorous stands maybe more predisposed to N saturation than younger sites that can absorb the additional N more readily through higher rates of N uptake and growth. Results presented in this study indicate that all three *Pinus patula* stands have low retention capacity for incoming NO_3^- and this is lowest on the young sites with little accumulated litter and thin L- and F-layers. However, with faster growth rates, the younger sites will absorb incoming N more efficiently, although the addition of inorganic N may lead to increased mineralization and nitrification rates, assisted by the open canopy and higher temperatures. In contrast, seemingly higher retention capacity of older sites in this study could be the result of lower nitrification rates and slower vertical movement of NO_3^- through the mineral soil. These sites may be relatively sensitive to N saturation due to nutrient limitations, a predisposition suggested by Nilgård (1985) and recently, by Fenn *et al.* (1998). In the current study the 22-year-old stand have considerably lower P and base cation levels in the

soil than the other sites, making it a prime candidate for N saturation when exposed to high N inputs. This change in soil fertility will be further exacerbated by a reduction in the pH of the soil, but only after this has led to increased nitrification rate and lower base cation levels.

Application of NO_3^- and NH_4^+ increased the leaching of NO_3^- significantly, suggesting that a significant portion of additional N would not be utilized by the phytomass. Only a small amount of NH_4^+ moved through the mineral soil to the deeper soil layers.These results are consistent with stage 2 of N saturation (Aber *et al.* 1989) indicating that these ecosystems have reduced capacity to retain inorganic N. Increased N deposition will increase leaching of NO_3^- and may affect water quality of streams draining affected ecosystems. The lack of any effect of simulated N deposition on soil nutrients other that N indicates that these shale soils are well buffered and will not be affected in the short to medium term. The decreasing trend in base cations and plant available P with age, however, suggests that older sites may be first to show nutrient deficiencies due to elevated N inputs.

Chapter 5

The effect of simulated N deposition on litterfall, decomposition and soil microbial N transformations of three *P. patula* plantation ecosystems

INTRODUCTION

Litter production usually refers to both above- and belowground litter produced in an ecosystem (Waring and Schlesinger, 1985). Litterfall refers to aboveground litter production, a major path whereby nutrients are returned to the soil to be eventually reabsorbed by the plant for biomass accumulation. A general increase in forest floor mass with altitude in coniferous forests has been found to correlate well with temperature and rainfall, indicating slower decomposition of these mor organic layers at high altitudes (McNulty *et al.* 1990). Similar relationships have been found with *P. patula* in South Africa (Morris, 1986; Schutz, 1990; Dames *et al.* 1999b). Increases in growth of *P. patula* forests following N fertilization correlated positively with forest floor mass in high lying areas in Swaziland, although increases were generally small (Morris, 1992). Nitrogen deposition is expected to initially increase growth of N limited forests by increasing the photosynthetically active foliage, in turn leading to increased litterfall and forest floor mass (Aber *et al.* 1989; Gundersen *et al.* 1998). Continued input of N to forest ecosystems will lead to N saturation and reduce growth and litter production. In the final stage of N saturation litterfall is hypothesized to increase as tree growth begins to decline due to N-induced nutrient deficiencies.

Decomposition refers to a number of interrelated processes by which organic matter is broken down to smaller particles and nutrients that are available for plant uptake (Waring and Schlesinger, 1985). The rate of decomposition depends on litter quality (lignin content, N

content; lignin:C ratio) and environmental factors such as rainfall and temperature as well as types and numbers of microflora and fauna. It is expected that N loading into coniferous ecosystems will gradually increase the N content in foliage and forest floor, thus lowering the C:N ratio and improving litter quality (Gundersen *et al.* 1998). In ecosystems that are saturated with N, the C:N ratio of the forest floor has been found to decrease, although these changes take place over several decades. In European coniferous forests the forest floor mass was found to decrease over an increasing N deposition gradient, indicating an increased rate of litter decomposition, probably due to improved litter quality (Gundersen *et al.* 1998). The simple addition of N to the forest floor, however, frequently has no effect or even a negative effect on decomposition (Fog, 1988; Prescott, 1995), although positive short-term changes can occur if N is assimilated by decomposing litter of high C and low N content. Although N deposition has been shown to be beneficial to litter decomposition, it is frequently accompanied by high S deposition. It has been shown previously that S reduced microbial activity (measured as respiration) in both coniferous and deciduous litter (Wookey *et al.* 1991).

The mineralization component of decomposition includes processes in which inorganic compounds such as CO_2, H_20 and NH_4^+, are released (Waring and Schlesinger, 1985). This is followed by nitrification, which is the oxidation of NH_4^+, with NO_2^- as first and NO_3^- as eventual product. Net nitrification refers to formation of NO_3^- only, while net mineralization refers to formation of both NO_3^- and NH_4^+. Soil microorganisms are good competitors for N, especially in situations where N inputs are continually elevated such as pollution sites or fertilized sites (Johnson, 1992). This suggests that the population of nitrifiers would increase as more N becomes available in the ecosystem. Mineralization and nitrification rates in forest ecosystems are positively related to several indicators of N status of the ecosystem, such as N deposition and

NO_3^- leaching (McNulty *et al.* 1990; Gundersen *et al.* 1998) and negatively to forest floor C:N ratio. Long-term addition of N stimulates nitrification and mineralization in coniferous and deciduous forests (van Miegroet *et al.* 1990, Gundersen *et al.* 1998). Increased nitrification under high levels of N deposition can be reversed by removal of N from precipitation, indicating dependence on external sources of N for maintaining high nitrification rates (Boxman *et al.* 1998a). An increase in net nitrification is expected to be one of the early signs of N saturated ecosystems (Gundersen *et al.* 1998).

In this study, the effect of increased N deposition on three crucial processes in the N cycle, litterfall, decomposition and N mineralization was examined by addition of N to gain insight into how these processes will be affected by continual high N inputs to *P. patula* plantations of different ages.

MATERIALS AND METHODS

Sites and treatment

The study was conducted on *P. patula* plantations of three different ages, which in 1995, at the start of the study, had been planted 4-, 14- and 22 years previously. The sites will be referred to by their ages in 1995. The sites were located in two adjoining plantations in the Drakensberg escarpment forestry area, namely, Brooklands (14- and 22-year-old sites) and Ceylon (4-year-old site). The fertilized plots received 120 kg N ha^{-1} yr^{-1} (as NH_4NO_3 and KNO_3 in a NO_3^-:NH_4^+ ratio of 3:1) additional to that present in precipitation, while the control plots received no additional N (unfertilized plots). A complete description of the sites and treatments is given in Chapter 3.

Litterfall and mass

In December 1995 four littertraps were installed on each plot. Litter traps consisted of a metal ring, 65 cm in diameter, with shade net to hold litter falling into the trap. The trap itself was raised 1.5m off the ground to prevent litter from trees outside the plot from falling into the traps and allow for easy identification of freshly fallen material. In the case of the trees on the 4-year-old site, the traps were raised about 30cm from the forest floor and were situated 30cm from the trunk of the tree. This was done because the canopy is still open and litterfall is restricted to the area under the canopy of each tree. Litter was collected once every month, and in some cases once every two months. No distinction was made between different types of litter. The litter was dried at 60°C for three days after which the weight was determined (Gower and Son, 1992) and litterfall is expressed in kg ha^{-1}.

Decomposition

A cotton strip assay was carried out according to the recommendations of Harrison *et al.* (1988), using commercially available calico (100% cotton). Cotton strips (45 x 10cm) were inserted in the organic and mineral soil by using a sharp spade to make an incision through the litter layer and into the mineral soil. Care was taken to disturb the litter layer as little as possible. A flat metal plate (50cm x 18cm) was used to insert the cotton strip lengthways into the incision. The sides of the incision were closed around the strip by making two other incisions 20cm from the original on both sides. At each of the three sites six control strips were taken, three on unfertilized plots and three on fertilized plots. Plots were randomly chosen, the strips inserted in the soil in the same way as the test strips and removed immediately. The strips were then washed under a cold stream of water, air-dried, sealed in plastic bags and stored until the tensile

strength loss (TSL) could be determined. The test strips were removed after six weeks and replaced with the next set of samples. On removal, the exact position of the top of the forest floor, as well as the mineral soil was marked on each strip with a permanent marker. The test strips were washed under a tap to remove soil, air-dried and sealed in plastic bags and stored at room temperature away from light until analysis could be performed. Analysis was usually done within 6-8 weeks of removal. The tensile strength (TS) of the cotton strips (fertilized and unfertilized) was determined on an INSTRON Tensiometer at the materials laboratory of the Faculty of Forestry at the University of Stellenbosch. The cotton strip was cut crosswise into 4.0cm strips and the individual fibres teased out to reduce the width down to 3.0cm. The tensile strength of the individual strips was then determined. TSL is defined as follows (Smith *et al.* 1993):

$$TSL = TS_{control} - TS_{test} / TS_{control} * 100\%$$

Decomposition bags were made from glass fibre mesh with a mesh size of 2cm. The size of the litterbags was 15 x 15cm with the sides sealed by double stitching with nylon string. Freshly fallen litter was taken from the littertraps of each plot in October, November and December 1996, and oven dried (60°C; 72h). Ten grams of this litter was sealed inside a decomposition bag. In December 1996 four bags were buried in the F-layer of every plot, the organic layer with the highest microbial activity in fertilized and unfertilized treatments. Decomposition bags were returned to the plot from where the litter originated. The litterbags were removed in December 1997 and the mass loss after one year determined.

Net N mineralisation and nitrification

A long-term, aerobic, laboratory-based incubation method (Kandeler, 1996) was used to determine net mineralisation and nitrification rates in samples taken from the top 5cm of the mineral soil. Samples were taken from unfertilized and fertilized plots of all three sites in December 1997. Three bulked samples per plot were sieved through a 2mm grid, air-dried and stored.

The NO_3^- and NH_4^+ content of water-saturated soil samples were assessed after incubating for 28 days at 25°C (Kandeler, 1996). For every sample, 10g of air-dried soil was weighed into five Erlenmeyer flasks and 3ml of distilled water added. This was done very slowly to avoid puddling of the soil. The flasks were capped and pre-incubated for 12 h at 2°C to eliminate the lag-phase associated with air-dried and re-wetted soils (Priha and Smolander, 1996). Three tubes were incubated at 25°C (samples). The other two were stored at -20°C until analyzed (controls, Day 0). The samples were removed on day 28 and stored at -20°C until analysis. After incubation, 15ml of 2M KCl was added to the samples and the control and the tubes shaken for 30 minutes. Samples and controls were filtered and the NO_3^- and NH_4^+ content determined. The NO_3^- concentration was determined after reduction to NO_2^- using copperized cadmium granules (Bate and Heelas, 1975). The indolphenol blue method (Novozamski *et al.* 1974) was used to measure the NH_4^+ concentration.

The rate of net mineralisation was calculated according to the following formula:

$$Min_{net} = \frac{(NH_4^+\text{-}N + NO_3^-\text{-}N)_A - (NH_4^+\text{-}N + NO_3^-\text{-}N)_B}{n}$$

where,

A = N_{MIN}-content of samples after incubation ($\mu g\ N\ g^{-1}dm$)

B = initial N_{MIN}-content (t_0) of samples ($\mu g\ N\ g^{-1}dm$)

n = incubation time (d)

The rate of net nitrification was calculated according to the following formula:

$$Nit_{net} = \frac{(NO_3^-\text{-}N)_A - (NO_3^-\text{-}N)_B}{n}$$

where,

A = N_{NITR}-content of samples after incubation ($\mu g\ N\ g^{-1}dm$)

B = initial N_{NITR}-content (t_0) of samples ($\mu g\ N\ g^{-1}dm$)

n = incubation time (d)

Analysis of variance was used to determine statistical significance between the means, followed by Duncan's post hoc tests to determine the ranking of the different means, where necessary. In all cases the 5% level of significance was used as a statistically significant difference. Summarized ANOVA tables can be found in Appendix I.

RESULTS

Litterfall

Figure 5.1 illustrates litterfall at two-monthly intervals. It is clear that all three sites have the same seasonal pattern of litterfall, although differences between two-monthly totals were less pronounced on the younger site than on the other sites. The 14- and 22-year-old sites also

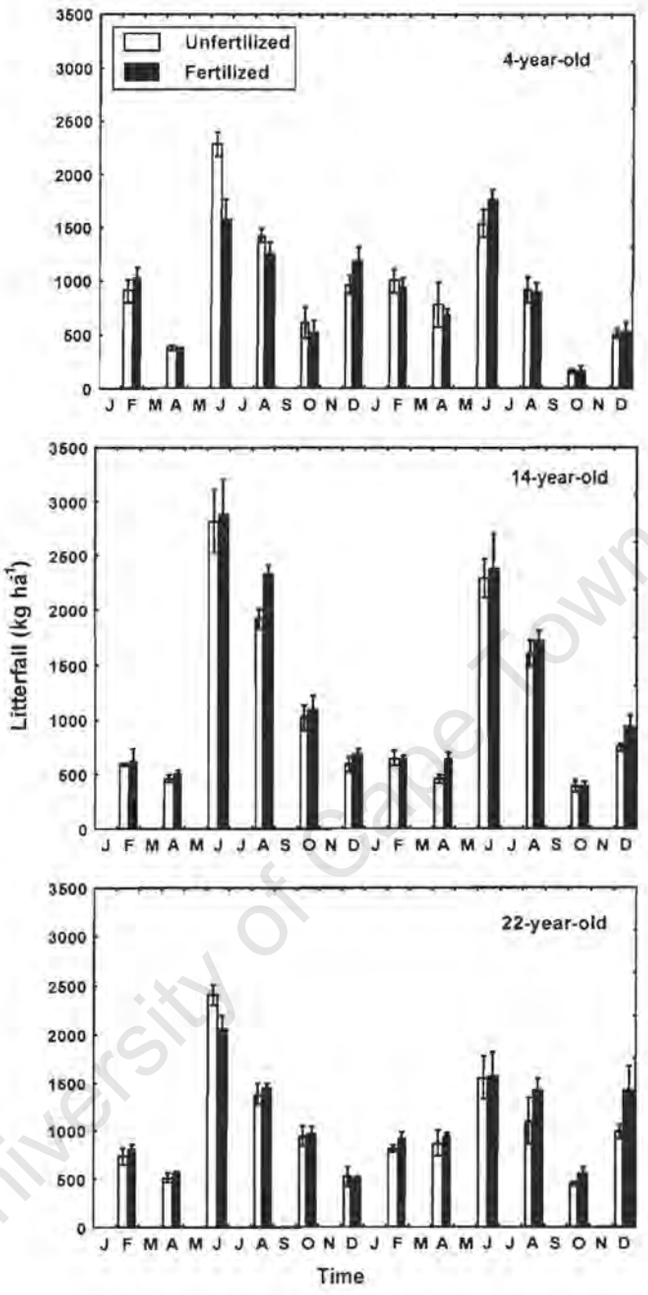

Fig. 5.1 *Mean litterfall into littertraps in fertilized and unfertilised plots of 4-, 14-, and 22-year-old* P. patula *stands, expressed on a two monthly basis, starting in 1996 and ending in 1997. Bars represent the mean of at least 12 samples and error bars represent one SE from the mean.*

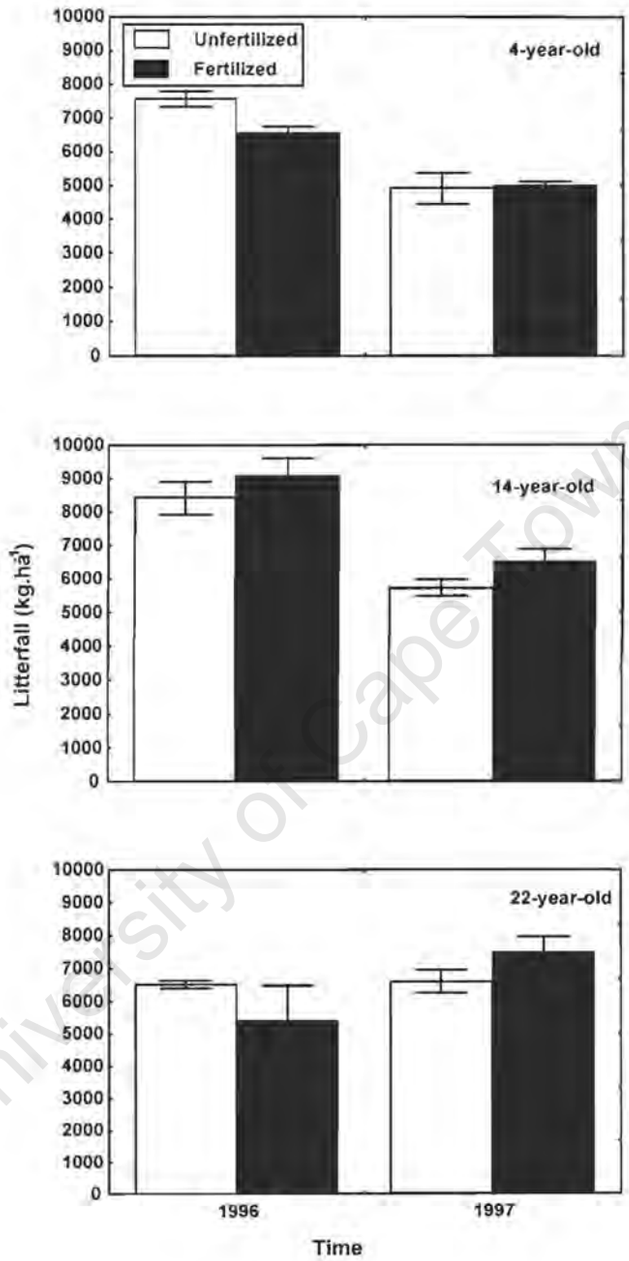

Fig. 5.2 *Means of total litterfall (n=4) on fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *stands during the period 1996-1997. Error bars represent one SE from the mean.*

showed higher litterfall in response to simulated N deposition. The trend is for treated plots of the 14- and 22-year-old sites to have higher litterfall than unfertilized plots.

Litterfall of all three sites was highest during the winter period, the season of least precipitation (Fig. 5.2) and lowest temperatures. The long term average minimum and maximum temperatures for the area in July (winter) is 4.8°C and 17.4°C for January (summer) 13.9°C and 24.2°C respectively (Louw, 1995). During the winter of 1996, more litter was produced on the 14- and 22-year-old sites than during the same period in the next year (Fig. 5.3). This was not the case with the 4-year-old site.

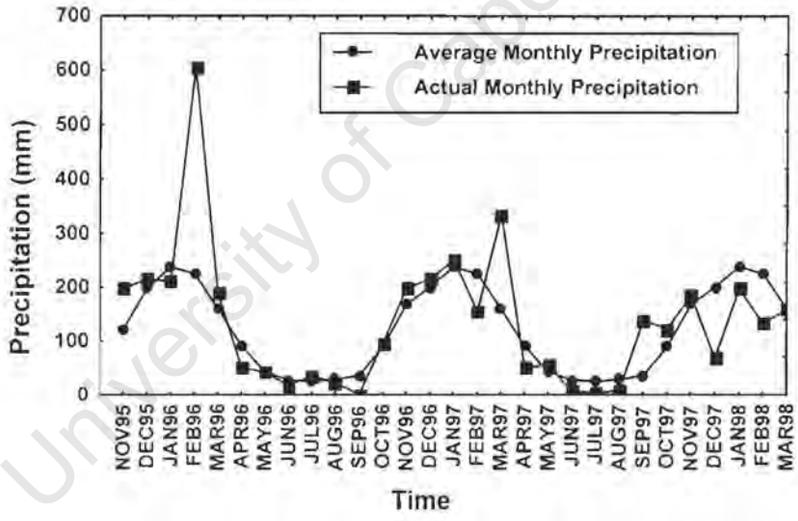

Fig. 5.3 *Average monthly precipitation plotted against actual monthly precipitation at Brooklands Plantation office during the experimental period. Brooklands plantation office represents the closest rainfall gauge with long-term rainfall data to the three sites. The long-term average monthly precipitation represents the means of 46 years (Schutz, 1990).*

The precipitation at Brooklands plantation office during the summer of 1996 was higher than the long-term monthly averages for this particular rain station (Fig. 5.3). In the summer of 1997, the rainfall returned to approximately average numbers and the amount of litter produced by sites the 14- and 22-year-old sites during this period was lower than the same period during the previous year. The total amount of litter produced was also lower in 1997 than 1996 (Fig. 5.2).

Decomposition

The cotton strip assay was used as an index of decomposition on a seasonal basis. Generally decomposition of cotton strips over a six-week incubation period was lower in the late winter (Fig. 5.4) than in autumn and summer. This seasonal trend was found on all three sites. All three sites have TSL lower than 70% during winter, while TSL higher than 70% was the norm during summer and fall.

Fertilized plots of all three sites generally had higher TSL than unfertilized plots. This trend is repeated at most soil depths. Tensile strength loss was highest near the forest floor surface during summer and fall, while decomposition seems to increase with depth during the winter. Tensile strength loss is thus higher in the organic horizons than the mineral soil horizons in all three sites. This was the case with fertilized as well as unfertilized plots. It is clear that incubated cotton strips decompose more readily during the wetter summer period than during the winter. However, this seasonal trend appeared dependant on incubation depth. At 24cm below the forest floor (in the mineral soil), differences between summer or fall and winter periods were less pronounced. The decrease in TSL with depth is evident even on a seasonal basis (Fig. 5.4). At 24cm below the forest floor, TSL is lower than closer to the surface.

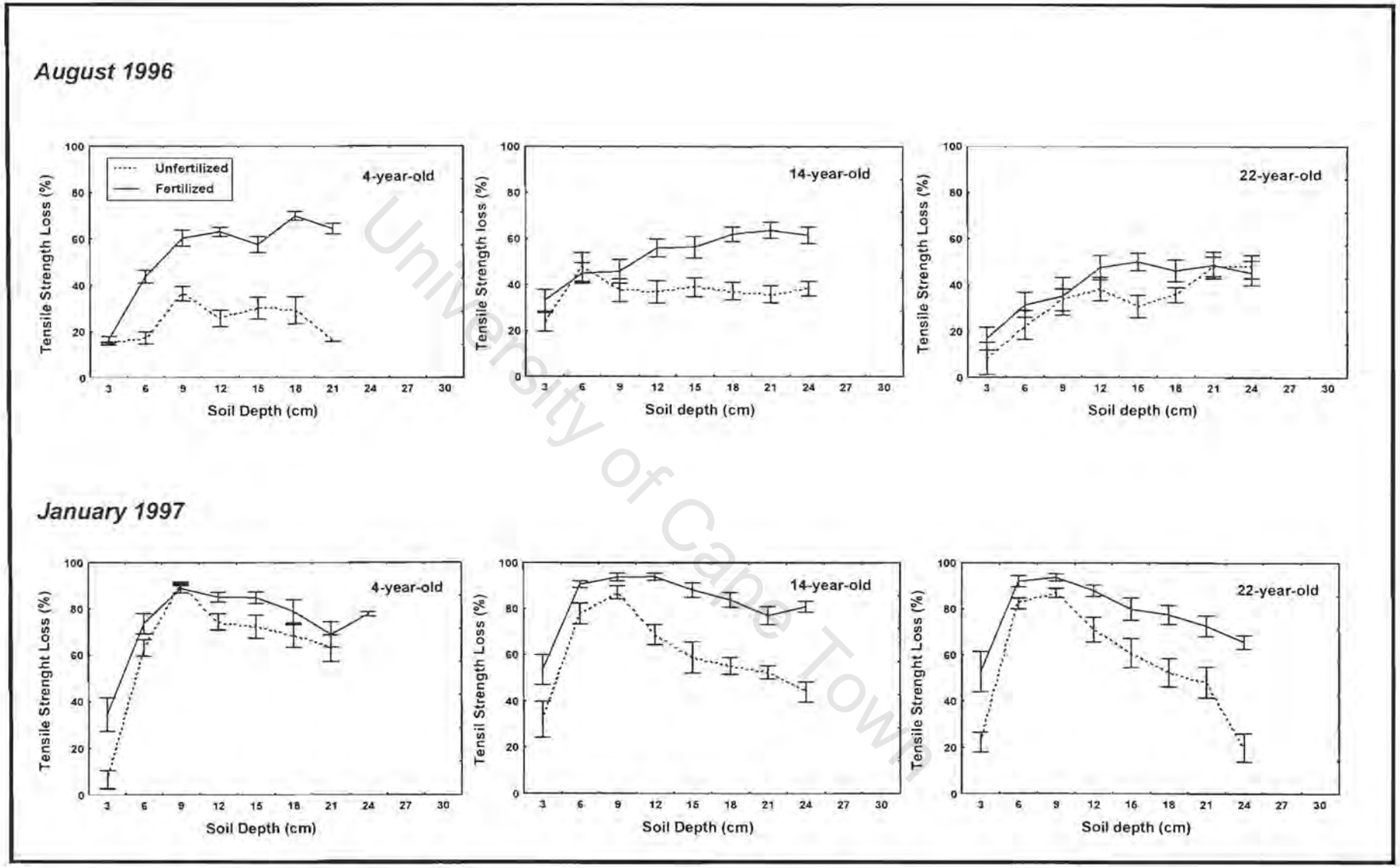

Fig. 5.4 *Mean Tensile Strength Loss (TSL) of cotton strips inserted in soil on fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *stands. The incubation period was six weeks, after which each cotton strip was cut into smaller strips and the TSL determined. Data points represent means of at least 12 cotton strips across the four unfertilized or fertilized plots for each site. Error bars represent one SE from mean*

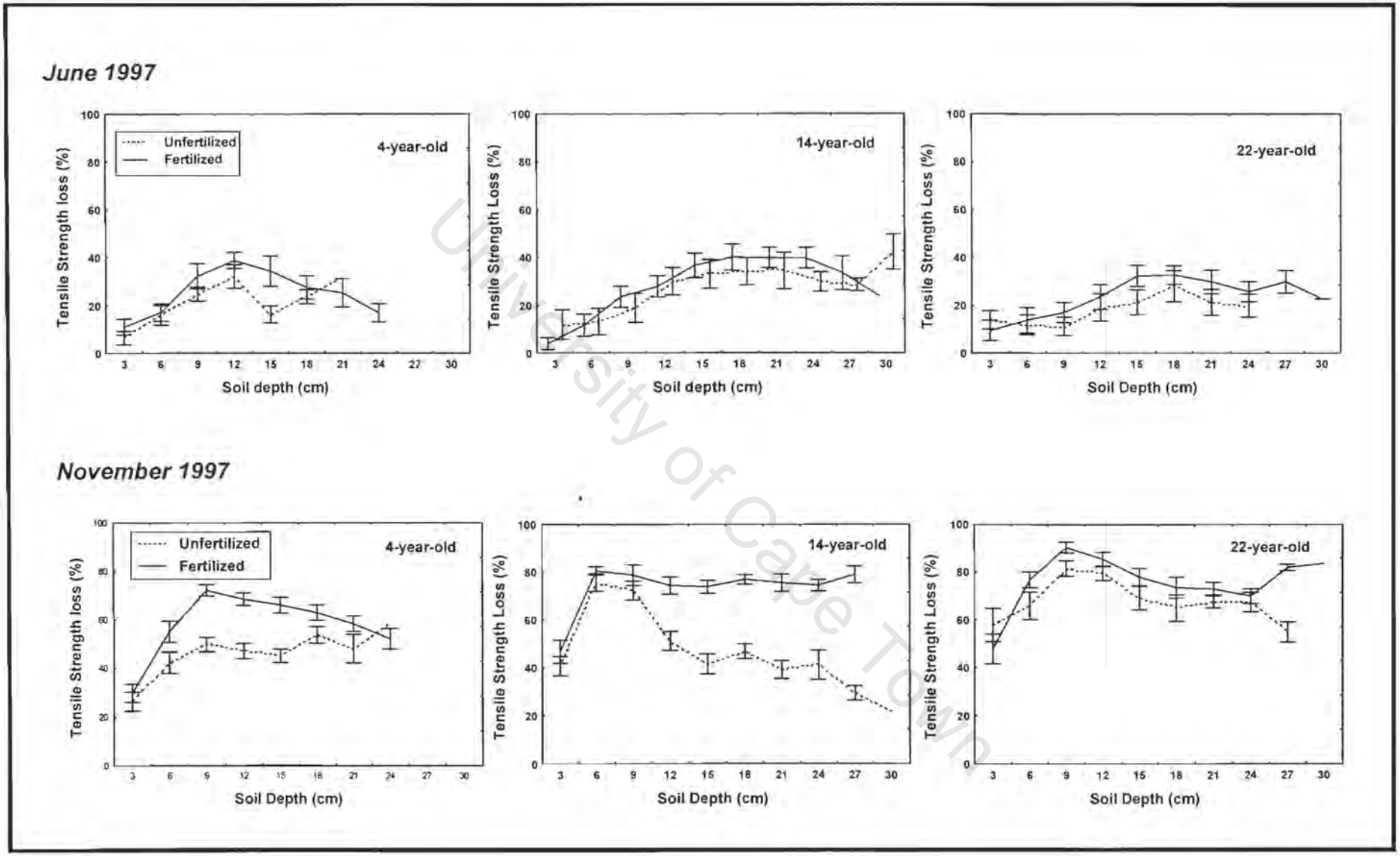

Fig. 5.4 *(continued) Mean TSL of cotton strips inserted in soil on fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *stands. The incubation period was six weeks, after which each cotton strip was cut into smaller strips and the TSL determined. Data points represent means of at least 12 cotton strips across the four unfertilized or fertilized plots for each site. Error bars represent one SE from mean..*

Decomposition of litter in litter decomposition bags inserted in the fermentation layer on each plot revealed no significant differences between fertilized and unfertilized plots in terms of mass of litter remaining after one year (Fig. 5.5). Between 50 and 60% of initial litter mass remained after field incubation for one year. Means of litter mass remaining in litterbags from the unfertilized plots of the three sites were not significantly different ($p<0.05$) and the same trend was exhibited by the fertilized plots.

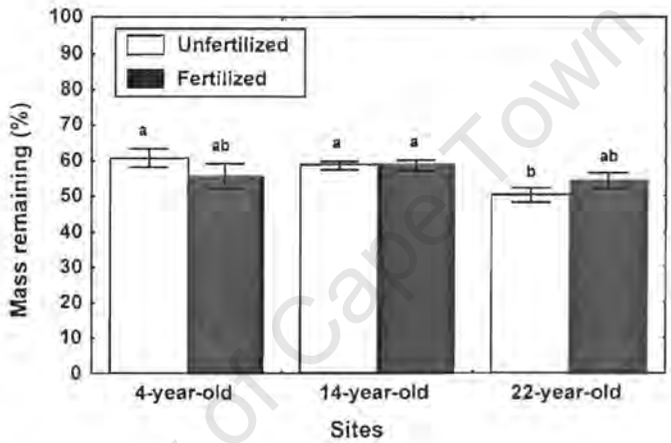

Fig. 5.5 *Mean percentage of litter mass remaining in litter decomposition bags after incubation in the fermentation layer for 12 months. The litterbags were inserted on fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *stands. Bars with similar letters are not significantly different (two-factor ANOVA followed by Duncan's post hoc test; $p<0.05$). Each data point represents 16 litterbags. Error bars represent one SE from mean.*

Nitrification and mineralization

Net N mineralization and net nitrification was measured in samples taken in June 1997 and December 1997. Both mineralization and net nitrification increased in the mineral soil on all three sites (Fig. 5.6). There were no large differences in mineralization between the unfertilized treatments of the three sites, although the 4-year-old and the 14-year-old sites tended to have higher mineralization rates than the 22-year-old site.

Table 5.1 *Nitrification as a percentage of mineralization in mineral soil samples taken from fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *stands in December 1997. The samples were incubated in a controlled environment for 28 days and analyzed for NO_3^- and NH_4^+.*

	4 year old		14 year old		22 year old	
	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized
%	89.26	88.90	65.28	62.00	57.17	28.93

The net formation of NH_4^+ was lower on the 4-year-old site than on the other sites.. The transformation of NH_4^+ to NO_3^- formed by far the greatest proportion of microbial turnover on the 4-year-old site (Table 5.1). Nitrification also accounted for more than 50% of mineralization on the 14-year-old site, but only 29% on the oldest site (Table 5.1). As a consequence of the increase in nitrification due to simulated N deposition, this proportion increased to 57% on the fertilized plots of the oldest site.

Table 5.2 *Mean C:N ratios in the different litter layers of fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *stands, which were sampled in April 1997. The different layers were the fermentation layer (F), the humus layer (H) and the mineral soil to a depth of 10cm (M10). Values are the means of four samples.*

	4 year old		14 year old		22 year old	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized
F	23.76 (± 2.38)	18.72 (± 1.96)	26.82 (± 1.4)	27.10 (± 0.55)	28.88 (± 0.44)	26.91 (± 1.18)
H	24.29 (± 3.88)	19.52 (± 1.83)	21.05 (± 0.83)	19.89 (± 0.67)	18.77 (± 0.7)	20.17 (± 0.53)
Mean (F+H)	25.81	19.12	23.93	23.89	23.82	24.38
M10	18.59 (± 0.21)	18.08 (± 0.66)	20.30 (± 0.31)	18.29 (± 0.36)	22.93 (± 0.75)	20.11 (± 0.19)

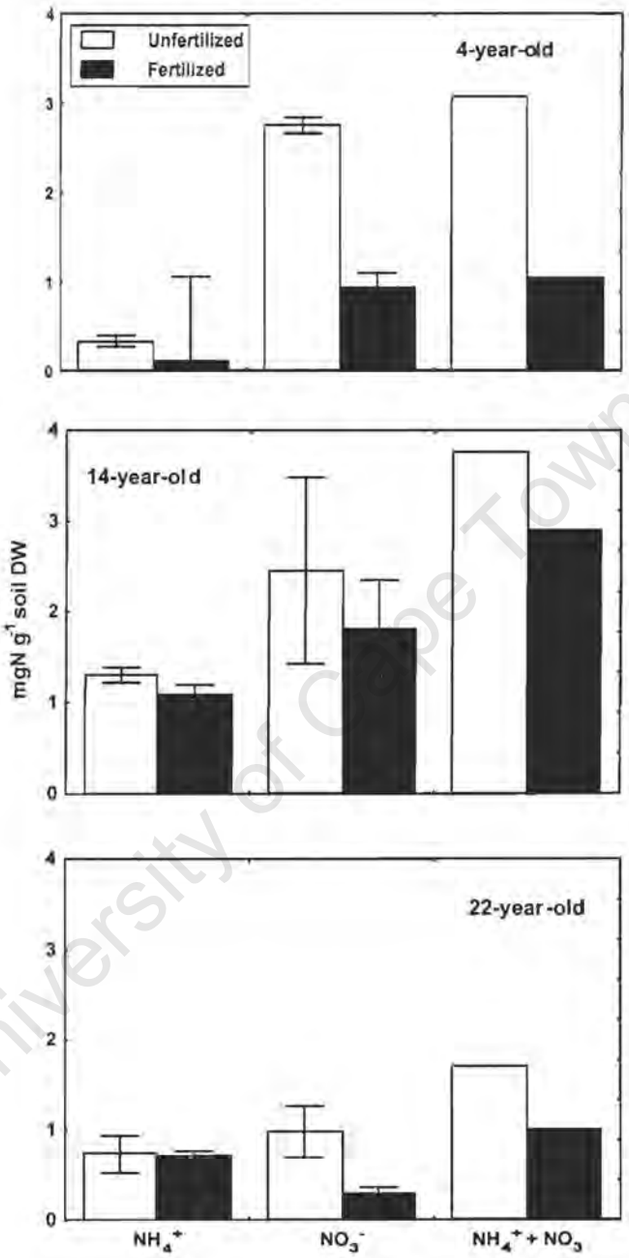

Fig. 5.6. *Means for net nitrification (NO_3^-) and net mineralization (NO_3^- + NH_4^+) of the top 10cm of the mineral soil taken from fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *stands. The samples were incubated at 25°C for 28 days and analyzed for NO_3^- + NH_4^+. Error bars represent one SE from the mean. Means represent eight samples.*

Table 5.1 shows the C:N ratios of the litter layers of the three sites in April 1997. There were no substantial differences in C:N ratio (Unfertilized) between the sites (Table 5.2). There was also a significant decreasing trend in C:N ratio only for the youngest site.

DISCUSSION

Simulated N deposition did not have any significant effect on litterfall. No diameter and height growth responses have been found after application of N for three years (Chapter 3). Considering the relationship between foliage biomass, growth and litterfall, it is not surprising that litterfall did not increase or decrease with N deposition. In cases where N fertilization did lead to increased biomass production of conifer forests, litterfall increased only after a lag phase of 2-4 years (Malkönen *et al.* 1992).

Litterfall in the *P. patula* plantations examined in this study followed the same seasonal pattern found in other studies on *P. patula* in South and Eastern Africa. Substantially more litter is shed in the winter and spring period than in the other seasons (Lundgren, 1978; Dames *et al.* 1999b). *Pinus patula* thus tends to shed litter during the cooler, drier periods of winter and spring, although it has been found that environmental factors such as drought can increase litterfall independently of this seasonal pattern (Dames*et al.* 1999b). She found that *P. patula* tends to retain the older needles on the tree for longer periods during drought events. Although drought did not occur during the experimental period of this study, the 1995-1996 rainy season was much wetter than the norm. In the next season (1996-1997) the precipitation returned to approximately average level for the area and litterfall was lower than the previous, very wet, season. This can be attributed to decreased leaf longevity during spells of below average precipitation.

Litterfall increases with age, but seem to stabilize and even decrease after canopy closure (Waring and Schlesinger, 1985; Dames *et al.* 1999b). Although it has been found that management practices such as pruning influence litterfall in *P. patula* plantations, the general pattern of an increase with age and stabilization after canopy closure stay the same (Dames *et al.* 1999b). Litterfall is dependent on age and growth rate, and in particular, foliage accumulation. Needle biomass accretion rates increase towards canopy closure, peaks, and then decline after canopy closure (Morris, 1992). Consistent with this pattern, the trees on the 4-year-old site produced less litter than the older trees. The average amount of annual litter produced during two full years of this study compares well with other studies on *P. patula*, such as that of Lundgren (1978) in Tanzania, Singh (1982) in India and Dames *et al.* (1999b) in the Drakensberg escarpment area. Litterfall in the 14-year-old site was somewhat higher than that found by Dames *et al.* (1999b). This can be attributed to the very high MAI_{20} of this site (28) as opposed the 4-year-old site (19) and the 22-year-old site (20). Canopy closure occurred on the 14-year-old site in the recent past.

Decomposition of leaf material is considered only in terms of mass loss and *P. patula* litter lost between 40 and 50% of initial mass in one year of field incubation. This is comparable to mass loss rates reported by Dames *et al.* (1999b) for the same species in the same area, but much higher than those reported Versveld and Donald (1991) for *P. radiata* in the Southern Cape area of South Africa. Versveld and Donald (1991) reported mass loss rates of roughly 50% mass loss of needles in litterbags over a three-year period. The difference can partly be ascribed to differences in methods. Versveld and Donald (1991) incubated litter on the forest floor surface, while in the current study litterbags were incubated in the fermentation layer. The higher rate of mass loss in *P. patula* plantations can also be attributed to generally wet, warm, summers in the

Drakensberg escarpment area as opposed to warm, dry summers in the Southern Cape (Versveld and Donald, 1991). The combination of high temperatures and high availability of moisture, as well as the periodic nature of moisture availability (wet/dry cycles) during summer usually translates to high rates of decomposition (Waring and Schlesinger, 1985). Dames *et al.* (1999b) used a similar method to the one employed in this study, and mass loss rates after one year were similar.

When a substrate with a high C:N ratio (cotton strips) was introduced into the *P. patula* ecosystem, it was found that additional N did increase the decomposition of this substrate. This could be due to a decrease in the C:N ratio of the substrate after addition of N to the forest floor and kickstart of the decomposition process. Absence of any increase in decomposition after N addition suggests that N deposition is unlikely to increase decomposition of *P. patula* litter in the short term. No evidence could be found from the litter decomposition bags, or the cotton strips, that the rates of decomposition are substantially different from each other underneath *P. patula* plantations of different ages. This was despite the greater exposure of soil under the 4-year-old site to sunlight by virtue of a more open canopy and thus more variable temperature and moisture conditions. Variable moisture and temperature conditions act as a primer to nitrification and decomposition in forest and agricultural soils (Waring and Schlesinger, 1985), and cause quicker turnover of organic matter. These results suggest that in these *P. patula* ecosystems decomposition is independent of stand age, except when both moisture and temperature are limiting factors.

Decomposition of plant material is highly dependant on litter quality, in particular the ratio of C:N (Waring and Schlesinger, 1985). Only a few cases are known where additions of N to the forest floor increased decomposition (Waring and Schlesinger, 1985); this is probably only valid

for substrates that have high C:N ratios and have the capacity to immobilize incoming N. Results presented in this study shows that additional N, although decreasing the C:N ratio of the F- and H-layers slightly, was not sufficient to increase the rate of mass loss significantly on any of the sites. This is probably due to the already low C:N ratios in these relatively N rich ecosystems.

Cotton TSL was highest during the warm, wet, summer period. This translates well to the seasonal pattern of actual decomposition that can be expected in these plantations and under these climatic conditions (Meentemeyer, 1978). However, there appears to exist a lag phase in decomposition rate of cotton strips that is independent of temperature and moisture availability. This can be seen in the high rates of decomposition after the rainfall has already dropped to below 30mm per month. This might reflect sustained high decomposer activity in soils, despite the decrease in rainfall.

Although the cotton strip technique has come in for criticism for not representing actual decomposition in the soil (Howard, 1988), it does provide researchers with an acceptable index of environmental influences on decomposition in various ecosystems (Smith *et al.* 1993). It has also shown good correlation with other methods of determining decomposition (Heal *et al.* 1974; 1981). The cotton strip assay allows for the substrate to be standardized and for comparisons to be drawn between different ecosystem types and different treatments. This method furthermore allows for determination and comparison of relative decomposition potential at different soil depths. It is thus a robust method to determine the decomposition potential of soils exposed to different treatments. This conclusion is borne out by the results obtained in this study. However, because of the differences in composition and substrate quality, results obtained cannot be directly compared to those of litterbags.

Net nitrification and mineralization have been found to increase under conditions of simulated N deposition. This trend is consistent across all three sites. Increases in net nitrification and mineralization rates following N input are typical of coniferous ecosystems experiencing N saturation (Aber *et al.* 1989; Gundersen *et al.* 1998). This is indicative of an acceleration of N cycling in the ecosystem that is usually the result of an increase in the microbial biomass in the soil.

The C:N ratios of the litter have been found to be good predictors of net N mineralization and net nitrification (Gundersen and Rasmussen, 1988; Taylor *et al.* 1989; Gundersen *et al.* 1998). C:N ratios of 23 - 25 in forests exposed to high N deposition rates were found to be a threshold for increased rates of nitrification in the forest floor and mineral soil and leaching of NO_3^-. Below this value, nitrification and leaching of NO_3^- increased significantly (Gundersen *et al.* 1998). It is clear that the C:N ratio of the forest floor in *P. patula* plantations is low, indicative of an N rich ecosystem. The rate of N cycling would be high and this can be seen from relatively high nitrification and mineralization rates in *P. patula* ecosystems found in this study.

Nitrification formed a greater proportion of net mineralization than is the case in northern hemisphere coniferous forests, where NH_4^+ is the main species of N available to the plant (Gundersen *et al.* 1998). This indicates fast conversion of NH_4^+ to NO_3^- and is consistent with what was found in other plantation ecosystems in the Southern Hemisphere (Adams and Attiwell, 1983). On the oldest site (22-years-old), after N fertilization, the formation of NO_3^- became the dominant mineralization process. These trends are consistent with a shift from a tight N cycle where most N is available as NH_4^+ to an open system with high availability and loss of NO_3^- (Aber *et al.* 1989).

Net nitrification in the laboratory has been found to be less reliable than *in situ* incubation of soil and organic matter (Gundersen *et al.* 1998). Sieving, drying and re-wetting of mineral soil can stimulate the processes of nitrification and mineralization (Alef, 1995). For the purposes of this study laboratory incubation was used to highlight possible changes in nitrification and mineralization under condition of simulated N deposition. In other studies of N fertilization effects on forest ecosystems a high degree of similarity was found between incubation of re-wetted soil in the laboratory and intact soil core incubations in the field (Smölander *et al.* 1995).

The application of water as part of the N application solution may lead to some responses that were noticed in the soil dynamics measured in this part of the field study. It is conceivable that litter decomposition may have been influenced by the increased availability of water; however, no significant differences were found between the decomposition rate of the control and those of the treatment sites at any age. The rate of decomposition of cotton strips in June 1996 may be explained as an artifact of water application. However, the precipitation in this area is mainly confined to late spring, summer and autumn. This coincided with the occasions when N, and subsequently, water was applied. Water is known to increase nitrification rate in soil (Powers, 1990), but no evidence exists that small amounts of water added to plantation forests increase or decrease litterfall in the short term.

Experimental evidence presented in this study such as the absence of increased litterfall and increased net nitrification and mineralization after exposure of *P. patula* ecosystems to simulated N deposition suggests that these ecosystems are not N limited. Low C:N ratios of forest floor material also supports the conclusion that these forests are N saturated rather than N limited. It is expected that the production of aboveground litter by *P. patula* plantations approaching N saturation will not be affected by increased N deposition in the short to medium

term. From growth data presented elsewhere (Chapter 3) these forests exhibit growth trends associated with N saturation in stages 1 and 2 (Aber *et al.* 1989), hence litter production will only be affected towards the end of stage 2, when forest decline starts. Litter decomposition will not be affected by additional N inputs, possibly due to the interactive effects of the already high N availability in the system and the inhibiting effect of N on decomposers, although litter with a high C:N ratio, such as new litter, may benefit from short term high inputs of N. Nitrification and mineralization will increase, reflecting an increase in N turnover, and contributing to high levels of NO_3^- leaching, and general decline in ecosystem health.

Chapter 6

The effect of simulated N deposition on the mycorrhizal population dynamics on the roots of *P. patula*

INTRODUCTION

Deposition of NO_3^- and NH_4^+ leads to several chemical and biological changes in forest ecosystems that may eventually adversely affect tree health. These changes can influence trees directly or indirectly through the mycorrhizas, a plant-fungal symbiotic system that is essential to many forest trees for uptake of nutrients and water. It is becoming clear that this plant-fungal interaction is being affected by chemical changes in soil due to N deposition (Termorshuizen *et al.* 1988; Jansen and Dighton 1990; Brunner and Scheidegger, 1994).

Nitrogen is limiting to growth of many forest ecosystems, but is also one of the most critical elements for growth (Jansen and Dighton 1990). Excess N in the ecosystem caused by deposition of NO_3^- and NH_4^+causes reductions in the growth of extramatrical hyphae, leading to reductions in the frequency and diversity of ectomycorrhizal roots and fungi (Jansen and Dighton, 1990). Special emphasis has been placed on the role of different species of N (NO_3^- vs NH_4^+) in the reported decline of mycorrhizas in areas exposed to elevated N deposition. Termorshuizen *et al.* (1988) found a decrease in carpophore production following N fertilization, which depended on the form of N applied. In *P. sylvestris* seedlings NO_3^- proved to be more damaging than NH_4^+ (Termorshuizen *et al.* 1988). Jansen and Dighton (1990) attributed this damaging effect of NO_3^- over NH_4^+ on mycorrhizal frequency to the inability of some mycorrhizal species to utilize NO_3^-. Other results showed NH_4^+ to be more detrimental to mycorrhizal frequency (Jentske *et al.* 1991) and root morphology (Brunner and Scheidegger,

1994) than NO_3^-. Although these conflicting results can be attributed to species and methodological differences, a general trend is that the higher the level of N applied to northern forests, the lower the number of carpophores and the mycorrhizal frequency.

Dames *et al.* (1999a) showed that pH optima for biomass accumulation exist in mycorrhizas growing on pine roots in the Drakensberg escarpment area. Apart from pH optima (Jansen and Dighton, 1990), fungi also show N optima for growth (Dames *et al.* 1999a) and low N inputs into N-poor soils are expected to increase growth for many species. However, at high N inputs, decreased growth is expected, except for nitrophyllous species (Jansen and Dighton, 1990). Pines, along with *Picea* and *Fagus* species are obligatory ectomycorrhizal. Meyer (1988) found that growth of obligatorily ectomycorrhizal trees declined more than facultatively ectomycorrhizal trees, which showed a lesser degree of damage.

Locally, no changes in the total numbers of mycorrhizal root tips on *P. patula* roots in stands that received NPK fertilizer were found, but the species composition of the ectomycorrhizal biomass changed significantly (Carlson, 1994). A similar trend was observed in 35-year-old *Picea sitchensis* in Britain supplied with N fertilizer, while new species also appeared (Alexander and Fairly, 1983). These trends may be due to differences in the efficiency of the mycorrhizal species in using NO_3^- and NH_4^+. Laboratory experiments showed that different mycorrhizal fungi have different preferences for different N species (Dames *et al.* 1999a).

In some instances, the effect of N on carpophore production and mycorrhizal species dynamics seem to be contradictory when analyzing results obtained in the field as opposed to those obtained in controlled seedling experiments. In the Netherlands, in an area with high inputs of anthropogenic N, medium-aged to mature stands of *Pseudotsuga menziesii* (Jansen and

Dighton, 1990) and *Pinus sylvestris* (Termorshuizen and Schaffers, 1987) showed lower fruiting body production and lower mycorrhizal frequency, while the same trend could not be shown for younger stands. These trends can possibly be linked to mycorrhizal succession. Succession of mycorrhizal species takes place throughout the lifetime of the ectomycorrhizal host. In young stands (pre-canopy stage) the number of species increases through each year (Fleming *et al.* 1984; Dighton *et al.* 1986; Last *et al.* 1987). In northern conifers the number of species is highest at canopy closure, when fungi from both pre- and post- canopy stages are present. Pre-canopy stage stands are characterized by pioneer fungi, which disappear at canopy closure when fungi characteristic of mature trees appear (Jansen and Dighton, 1990).

In contrast to the mostly negative effects of N on mycorrhizal population dynamics discussed above, other results have shown that N fertilization increased the longevity of mycorrhizas, while decreasing mortality (Alexander and Fairley, 1983). Both negative and positive effects of excess N on the microbial composition of trees can persist for up to 10 years after N application (Arnebrandt and Söderström, 1992). This result is especially relevant in areas that receive high levels of N deposition, where changes in mycorrhizal population composition changed the efficiency of uptake of water and nutrients such as P.

N deposition is expected to reduce the numbers of mycorrhizas, the functioning of mycorrhizas, the mycorrhizal biomass composition as well as the number of fine roots of affected trees. These changes in mycorrhizal dynamics might contribute to nutrient imbalances and growth decline (Jansen and Dighton, 1990). The aim of this study is to determine the effects of simulated N deposition on various aspects of population dynamics of ectomycorrhizal fungi and root growth in *P. patula* stands of different ages.

MATERIALS AND METHODS

The method of Carlson (1992) was used and modified to suit this experiment. In October 1996 a stainless steel tube with a sharpened edge was used to remove soil cores from the top 20 cm on every plot. The individual soil samples were numbered to ease identification and taken to the laboratory where the roots, stones and larger pieces of organic material were removed by hand. Root ingrowth bags were constructed from glass fibre mesh with a mesh size of 1mm. The root ingrowth bags measured 40cm in length and had a diameter of 4cm. The bags were inserted in the cylindrical holes left where soil was excavated. The diameter of the hole was 4 ± 0.2 cm, thus leaving as little space between the bag surface and the intact soil as possible. The cleaned soil was then returned to the corresponding hole and bag and gently firmed by hand. On every plot six ingrowth bags were inserted in the inner plots. The bags were positioned 1,5m from the trunk of three randomly selected trees, covering all four major directions.

The root ingrowth bags were removed from the plots in March 1997. A sharpened stainless steel tube, slightly bigger than the root bag, was used. Care was taken that all roots were severed before final removal of the bag. The intact cores were taken to the laboratory and the roots removed by hand. The soil was then used to determine soil volume in the bag. The roots were immediately covered in 30 % ethanol to prevent them from becoming brittle (Carlson, 1992). The roots were viewed under a stereomicroscope with a 20X and 30X magnification. Mycorrhizal root tips were classified according to the width and length of the tip, surface characteristics and colour of the mantle (Carlson, 1992). For determinations of physical characteristics and measurements of the mycorrhizal morphotypes at least 10 root tips were measured under a stereomicroscope.

The length of the roots was determined according to the method of Tennant (1975). The roots were placed in a petri dish on top of a 1 x 1 cm grid. The number of times the root crossed or touched a grid line was counted and was assigned a count of one (1). Where the curved portion of a root lay along a root, a count of two (2) was given. The length of the root was calculated as follows (Tennant, 1975):

Root length (cm) = Total number of grid intercepts x (11/14)

The mycorrhizal density (number of mycorrhizal root tips per cm root) for individual mycorrhizal morphotypes and for total number of mycorrhizas was calculated accordingly.

The same roots that have been used for mycorrhizal measurements were also used for root measurements. The root density and specific root length were calculated after the roots had been dried at 60°C for 3 days. Root density was calculated as the length of root per volume (cm/cm^3) of soil in the root ingrowth bags and the specific root weight is the root dry weight length of root (mg/cm). Soil analyses for use in the correlation matrix are described in Chapter 4.

Analysis of variance, followed by Duncan's test was used for significance testing across more than two means. In all cases, $p<0.05$ is used to denote significance at the 5% confidence level, which was taken as a statistically significant difference throughout. The Mann-Whitney test was used in cases where the number of values per sample was unequal. Summarized ANOVA tables can be found in Appendix I.

RESULTS

Four different types of mycorrhizas were found on the roots of *P. patula* (Table 6.1). A short description of each can be found in Table 6.1. Dames *et al.* (1999a) found that mycorrhizal type 2 corresponds to the carpophore of *Boletus pinacola* and type 3 to that of *Scleroderma citrinum*. Carpophores of both these fungi were plentiful on all the plots towards the end of the summer rainfall period every year although no quantitative estimates were performed. Carlson (1994) reported mycorrhizal root tips of similar description on the roots of *P. patula* in the Drakensberg escarpment, although no attempt was made to link carpophores to the mycorrhizas or to identify the fungi concerned.

Table 6.1 *Physical description of main morphotypes of mycorrhizas found on the roots of* P. patula *in this study. Carpophores associated with morphotypes 2 and 3 are from the work of Dames* et al. *(1999a).*

	Species 1	Species 2	Species 3	Species 4
Branching	Simple, dichotomous	Simple, dichotomous	Simple, dichotomous	Simple, dichotomous
Colour	Light brown with lighter tip	Dark brown with lighter tip	White	Dark brown with black tip
Length of tip (mm)	1.305 (± 0.274)	1.497 (±0.305)	1.652 (±0.425)	0.691 (±0.118)
Width of tip (mm)	0.380 (±0.162)	0.204 (±0.178)	0.345 (±0.215)	0.325 (±0.230)
Surface	Smooth, hyphae sparse	Rough appearance, hyphae sparse	Smooth, hyphae sparse	Smooth, dense hyphae emanating from surface
Associated carpophore	Unknown	*Boletus pinacola*	*Scleroderma citrinum*	Unknown

Specific root length of roots growing into root bags showed an increasing trend with age (Fig. 6.1). *P. patula* roots in the litter layer and upper soil layer are thus getting heavier as the trees age. This trend is reversed with the application of N. There were no significant differences between unfertilized and fertilized plots at any age/site in terms of root growth into root ingrowth bags in the period September 1996 to March 1997, although a decreasing trend in root density was observed in reaction to simulated N deposition (Fig. 6.2).

Mycorrhizas from *Boletus pinacola* and *Scleroderma citrinum* (species 2 and 3) dominated in terms of numbers, while morphotypes 1 and 4 were found only infrequently (Fig. 6.3). There were significantly more *Scleroderma citrinum* type mycorrhizas on the roots of fertilized samples from site the 22-year-old site relative to the unfertilized plots. None of the other comparisons were significantly different at the 5% level. All four species were represented at the three sites and there were no clear trends in population dynamics across the three ages. No new species appeared, although other studies found evidence of up to seven species on the roots of a 4-year-old *P. patula* stand in the same area (Carlson, 1994). It is clear that simulated N changed the relative abundance of the two dominant species, while species 1 and 4 were not influenced to the same extent. The roots isolated from the ingrowth bags of fertilized plots of the 22-year-old site possessed significantly more mycorrhizas than unfertilized plots of that site (Fig. 6.4). This was also significantly more than the unfertilized and fertilized plots for the 14-year-old site and the 4-year-old site. This increase was mainly due to a sharp increase in the numbers of *Scleroderma citrinum* in reaction to the simulated N treatment. The 22-year-old site showed a trend of lower ratio of type 2 to type 3 in response to simulated N levels (Fig. 6.5). This trend was significant at $p<0.05$. Neither of the other sites showed any significant trends.

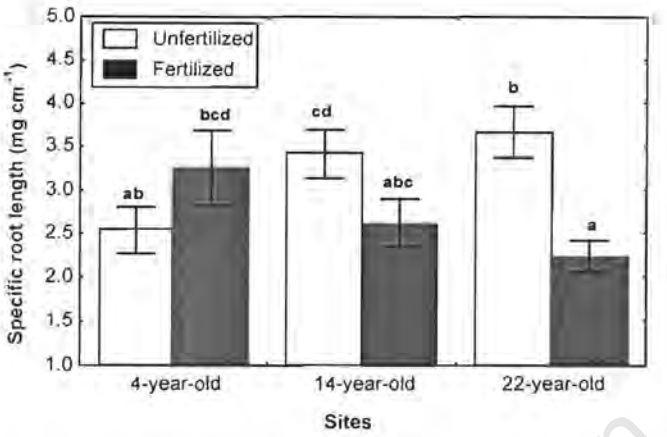

Fig. 6.1 *Specific root length of root samples from the root ingrowth bags taken from fertilized and unfertilized plots of the field sites in March 1997, after incubation for six months. Values followed by the same letter are not significantly different at* $p<0.05$ *by two-factor ANOVA followed by Duncan's multiple range post hoc test. Values are the means of at least eight samples per treatment and error bars represent one standard error from the mean.*

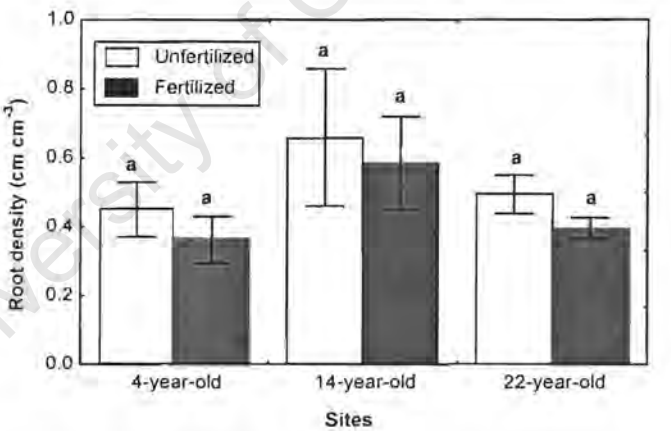

Fig. 6.2 *Root density in root ingrowth bags incubated in fertilized and unfertilized plots of the three sites investigated. The root bags were inserted in October 1996 and removed six months later. Values followed by the same letter are not significantly different at* $p<0.05$ *by two-factor ANOVA followed by Duncan's multiple range post hoc test. Values are the means of at least eight samples per treatment and error bars represent one standard error from the mean.*

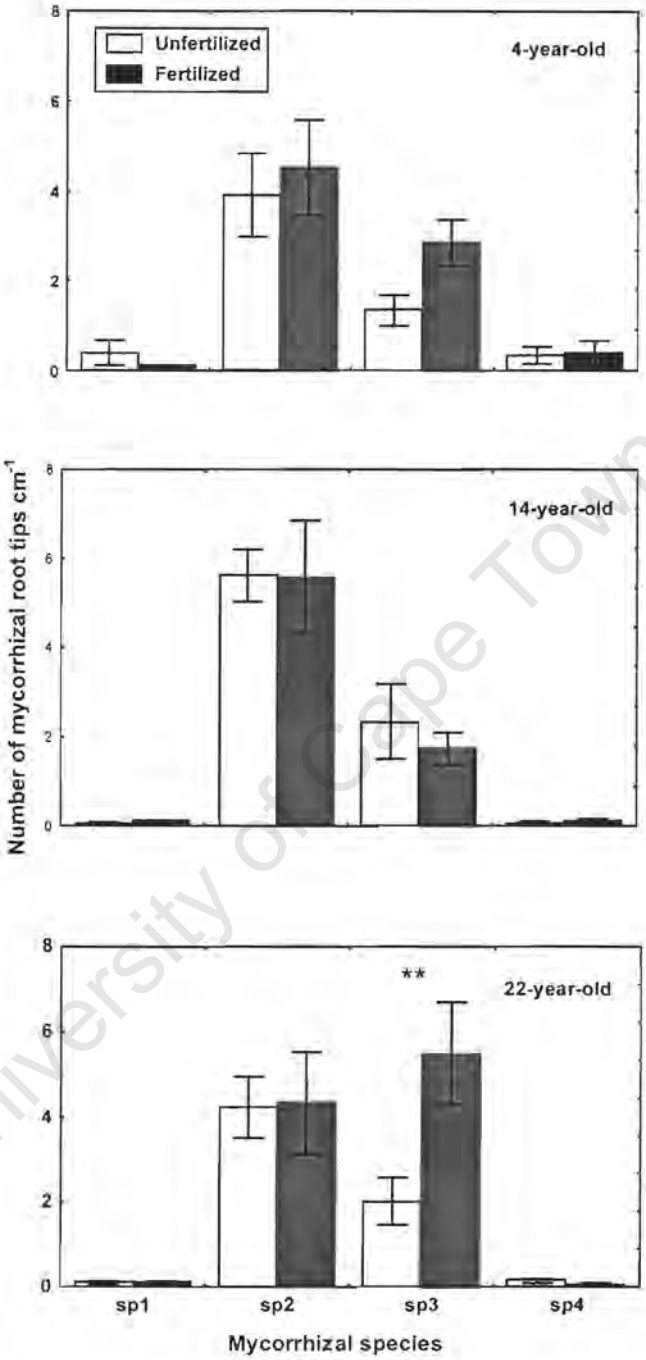

Fig. 6.3 *Number of mycorrhizal root tips of the four mycorrhizal species per length of root isolated from root ingrowth bags incubated in fertilized and unfertilized plots of the three sites investigated. The root bags were inserted in October 1996 and removed in March 1997. ** signifies a significant difference at* $p<0.1$ *with the Mann-Whitney U test. Values are the means of at least eight samples per treatment. Error bars represent one standard error from the mean. Sp1 = species 1; sp2 = species 2; sp3 = species 3; sp4 = species 4.*

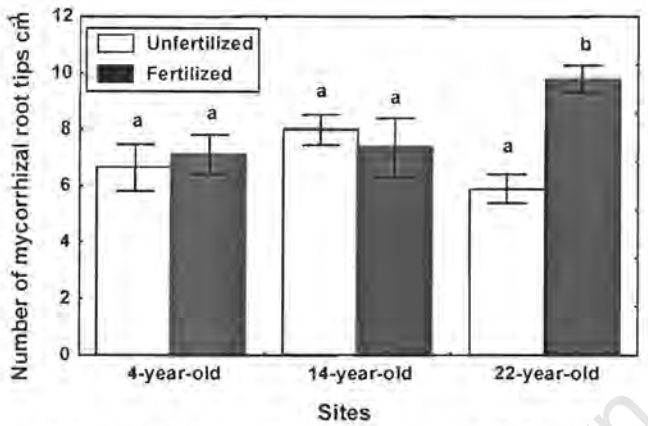

Fig. 6.4 *Total number of mycorrhizal root tips per length of root isolated from root ingrowth bags incubated in fertilized and unfertilized plots of the three sites investigated. Values followed by the same letter are not significantly different at p<0.05 by two-factor ANOVA followed by Duncan's multiple range post hoc test. Values are the means of at least eight samples per treatmen and error bars represent one standard error from the mean.*

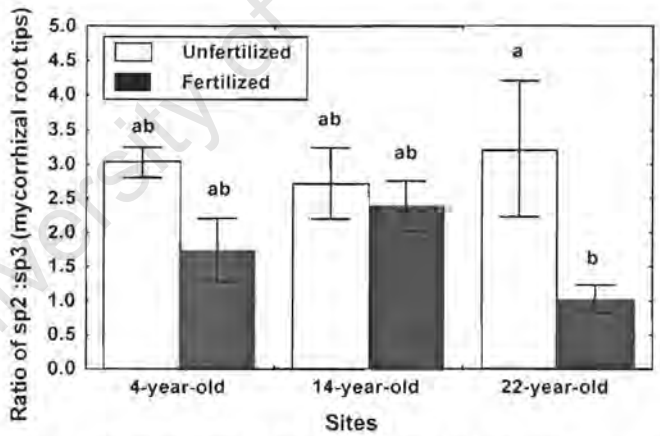

Fig. 6.5 *Ratio of mycorrhizal species 2 to species 3 on roots isolated from root ingrowth bags. These morphotypes have been found to be the most important in terms of biomass. Values followed by the same letter are not significantly different at p<0.05 by two-factor ANOVA followed by Duncan's multiple range post hoc test. Values are the means of at least eight samples per treatment and error bars represent one standard error from the mean. Sp2 = species 2; sp3 = species 3.*

Table 6.2 *Pearson correlation coefficients for mycorrhizal attributes and various soil parameters. Coefficients include both the unfertilized and fertilized treatments across the 4-, 14- and 22-year-old* P. patula *sites.*

	pH	Na	P	K	Ca	Mg	Mn	Fe	Org C	N
Root density	-0.11	0.05	0.01	-0.12	-0.05	-0.02	-0.01	0.06	0.07	0.02
Frequency of species 2	-0.02	0.10	0.11	0.16	0.07	0.15	-0.01	-0.28	0.29	0.17
Frequency of species 3	-0.12	-0.22	-0.37	-0.03	-0.31	-0.41	0.39	0.10	-0.41	-0.33
Total mycorrhizal	-0.05	-0.06	-0.20	0.12	-0.21	-0.19	0.33	-0.15	-0.12	-0.11

There was no correlation between mycorrhizal attributes and nutrients in the mineral soil across treatments and ages (Table 6.2). Within each site, correlations were found with individual elements, but these were not consistent across sites. Soil analysis revealed that the total P levels in the soil of the 22-year-old site were very much lower than on the 4- and 14-year-old site (Table 4.1, Chapter 4). There were, however, no strong correlations between the soil total P and any of the mycorrhizal attributes. Simulated N deposition over three years did not change the soil total P on the 22-year-old site significantly. Results from chapter 3 (Table 3.3), revealed a significant increase in the N:P ratios in the leaves of fertilized trees of the 22-year-old site. Similar trends were observed on the other sites.

DISCUSSION

More than two seasons of simulated N deposition treatment at 120 kg N ha^{-1} yr^{-1} of N did not have any significant effect on root density in the root ingrowth bags. Thus, increased N availability in this system did not lead to any significant adjustment in mycorrhizal biomass allocation, although a decreasing trend was observed on all the sites. This is supported by the lack of significant growth responses of the aboveground compartments (Chapter 3). This lack of change in the root biomass dynamics has been observed in other coniferous systems exposed to

simulated N deposition (Persson *et al.* 1998), indicating that root growth at a stand level may only respond to increased N availability in the medium to longer term. This is also consistent with the stages described by Aber *et al.* (1989), where root biomass only decreases towards the end of stage 2. This also confirms the conclusions of Gundersen *et al.* (1998), in a review of the hypotheses of Aber *et al.* (1989), that root biomass responses to elevated N in the rooting zone are only significant over the long term and are not easily prone to short term changes in soil N availability.

The root density inside the root bags is comparable to what was found in ingrowth cores in other coniferous forests (Persson *et al.* 1998). The lack of significant differences in root growth between the unfertilized plots of sites of different ages is surprising if the differences in growth rate are taken into account. Older trees would be expected to exhibit lower rates of growth since the biomass is already established and nutrient requirements is met mostly through remobilization from senescing foliage (Miller, 1981; Waring and Schlesinger, 1985). In this study, the rate of root growth is as high as that of the two younger sites. This may be related to the low levels of P in the soil of the 22-year-old site. Although foliage P levels in the 22-year-old site is as high as that of the other two sites (0.17 %; Chapter 3), proportionally more P is recycled from older leaves, while the soil P levels are significantly lower that the other sites. It has been shown that trees respond to nutrient deficiencies by increasing root biomass (Binkley, 1985). Phosphorus is also occasionally limiting to growth of *P. patula* in the Drakensberg escarpment area and responses to P fertilizer are frequently found (Schutz, 1975).

It has been shown that root vitality decreases when an ecosystem reaches N saturation (Gundersen *et al.* 1998). Although root vitality *per se* was not assessed during this study, the specific root length showed a decrease in response to simulated N deposition. This general trend

was evident especially in the older site. This indicates thinner roots and rootlets at high levels of N supply. Such a trend has been observed before in forest systems exposed to high levels of N deposition and is a first indication of root biomass decline (Persson, 1988; Majdi and Persson, 1995). Thinner roots reduce the absorbing surface of roots and contribute to the general decline of the fine root biomass vitality that is seen towards the end of stage 2 of forest decline (Aber *et al.* 1989).

The mycorrhizal densities of seven mycorrhizal root tips per cm of root was found in this study are almost twice that normally found on European conifers and also higher than what was found by Carlson (1994). This may be the result of the warmer climate and faster growth rate attained with conifers locally (Carlson, 1994). The disparity in the relative abundance between the current study and that of Carlson (1994) is possibly the result of differences in the timing of sampling, which is related to the seasonal differences in growth rate. As many as seven morphotypes have previously been found by Carlson (1992), while only four were found in a later study by the same author (Carlson, 1994). The two studies differed in seasonal timing of sampling, showing the effect on mycorrhizal population. Mycorrhizal densities will be higher in the actively growing season when nutrient uptake rates are highest. This is reflected in the current study where roots were sampled at the end of the growing season, while Carlson (1994) sampled in the winter.

Carlson (1994) has documented mycorrhizal response of a *P. patula* stand after NPK treatment. The work being presented in this study confirmed the conclusions of Carlson (1994) that a change in the mycorrhizal species composition resulted from the change in nutrient availability due to nutrient application to *P. patula* plantations. This can be seen as the first indication of a change in mycorrhizal dynamics, which, with continued N inputs will eventually

culminate in altered nutrient relations. The nature of the change in nutritional relations can, however, not be predicted, mainly due to the dearth of knowledge regarding the role the different mycorrhizal morphotypes play in nutrition.

The two dominant species of mycorrhizas on *P. patula* roots in South Africa, *Scleroderma citrinum* and *Boletus pinacola* were studied in detail by Dames (1999a). Both species showed definitive preferences for NO_3^- and NH_4^+ *in vitro*. There was no difference in the utilization of NO_3^- and NH_4^+ forms of N by *Scleroderma citrinum* while there was a definite preference for NH_4^+ by *Boletus pinacola*. Dames *et al.* (1999a) have also shown that *Boletus pinacola* had a very low pH optimum (2.00), while *Scleroderma citrinum* had a slightly higher optimum (3.00 - 5.00). In response to simulated N deposition treatment with NO_3^- and NH_4^+, the mycorrhizal density of *Scleroderma citrinum* increased while that of *Boletus pinacola* was reduced on the two youngest sites while it remained stable on the 22-year-old site. This change in the ratio of the two dominant species could thus be explained in terms of their preference for NO_3^- and NH_4^+. While *Scleroderma citrinum* utilized both inorganic forms of N, *Boletus pinacola* used only NH_4^+, conferring a competitive advantage on the former species, which is expressed in terms of an increase in numbers of mycorrhizal root tips. From the study of Dames *et al.* (1999a), whether *Scleroderma citrinum* can be regarded as a nitrophyllous mycorrhizal species is still unclear. Nitrophyllous species would be expected to remain stable during N saturation or even increase in numbers when exposed to N (Jansen and Dighton, 1990). Mycorrhizal species other than species 2 and 3 are only of minor importance in terms of numbers.

Neither root nor mycorrhizal attributes were well correlated to any soil nutrient parameters examined in this study. Of significance is the poor correlation between soil P availability and root and mycorrhizal attributes. In the light of the well-described relationship between P

availability and mycorrhial biomass it was expected that this correlation would be high. In addition, the P level in the upper mineral soil of the unfertilized plots of the 14-year-old site and the 4-year-old site is significantly higher than the 22-year-old site and remained unchanged after simulated N deposition (Table 4.4). Despite this, the number of mycorrhizal root tips was not different from the two younger sites. In combination with the similar levels of P in the foliage of the 22-year-old site, this suggests a decoupling of low soil P levels and high mycorrhizal biomass, possibly due to higher internal recycling and uptake of organic P from the organic soil.

Results from this study of mycorrhizal reaction to simulated N deposition to *P. patula* stands suggest that the likely medium to long term response to increased N availability will be changes in mycorrhizal population structure, with possible associated changes in nutrient relations. Seen in combination with the general trend of declining specific root length following simulated N deposition, this suggests that additional inputs of N will lead to a decline in root health and vitality associated with stage 2 of N saturation, as described by Aber *et al.* (1989).

Chapter7

^{15}N dynamics in vegetation and soil of three *P. patula* plantation ecosystems in relation to N cycling and simulated N deposition

INTRODUCTION

Large long-term inputs of NO_3^- and NH_4^+ to forest ecosystems lead to N saturation, which is characterized by increased N cycling which culminates in losses of NO_3^- to drainage waters (Aber *et al.* 1989). Fast turnover of N in an open N cycle is often associated with increased $\delta^{15}N$ values in ecosystem elements (Högberg, 1990; Högberg and Johannisson, 1993). Garten (1993) predicted that natural abundance of ^{15}N would progressively increase in ecosystems becoming N saturated, mainly due to enriching effect of N turnover in the ecosystem (all N turnover processes discriminate against the heavy isotope). Garten (1993) concluded that the $\delta^{15}N$ of ecosystem elements could therefore prove to be a useful indicator of N deficiency or N saturation.

$\delta^{15}N$ in forest ecosystems is a function of the ^{15}N natural abundance of the N compounds entering and leaving the system, the input-output balance, N transformations, N source partitioning and N compartmentalisation within the ecosystem (Handley and Raven, 1992). In forest ecosystems, these values lie between -10 and +15‰ (Mariotti *et al.* 1980; Hauck and Bremner, 1976). Most forest soils are enriched in ^{15}N relative to atmospheric N, partly due to nitrification followed by NO_3^- leaching, (Handley *et al.* 1996), volatilization of NH_3 (Johannisson and Högberg, 1994) and denitrification (Wellman *et al.* 1968; Delwiche and Steyn, 1970). A repeated pattern is that $\delta^{15}N$ values of undisturbed soils tend to increase with depth (Högberg *et al.* 1996; Nadelhoffer and Fry, 1988; Mariotti *et al.* 1980) which is attributed to age

related nitrification and downward movement of humic material (Mariotti *et al.* 1980; Nadelhoffer and Fry, 1994).

In most cases the foliage of forest species is slightly depleted in ^{15}N compared to the soil (Nadelhoffer and Fry, 1988). The difference between the $\delta^{15}N$ of the foliage and that of the soil layers is referred to as the enrichment factor (ε) and a positive relationship between ε and the extent of N saturation has been found in European sites (Emmett *et al.* 1998). A positive relationship has also been found between net nitrification and mineralization potential in soil and ε and foliar $\delta^{15}N$ values (Garten and van Miegroet, 1994). Differences between current and older needles have been observed and are possibly due to the onset of catabolic activity in the old needles and the subsequent translocation of N to the actively growing needles (Gebauer and Schulze, 1991). Older needles from a *Picea abies* plantation showing signs of growth decline and needle loss were more depleted in ^{15}N than older needles from a healthy site, probably due to an earlier onset of translocation in the stressed trees. In simulated N and fertilization experiments two stages of foliage $\delta^{15}N$ status are observed (Högberg *et al.* 1992; Nommik *et al.* 1994). Initially the $\delta^{15}N$ values resemble the signal of the added N, reflecting use of "new" N, followed by an increase in $\delta^{15}N$ as the rates of mineralization and nitrification increase.

Recent studies have shown that carpophores and the fungal sheath surrounding the root are highly enriched in ^{15}N (Handley *et al.* 1996), suggesting preferential storage of the heavy isotope of N. Handley *et al.* (1996) found large variation in fungal $\delta^{15}N$ that could be attributed to internal cycling of N, since the caps of fruiting bodies of mycorrhizal fungi were enriched relative to the stipes. This phenomenon was also found when the fungal sheaths of mycorrhizal roots were analyzed (Högberg *et al.* 1996).

In recent years the focus in N deposition research has shifted from merely monitoring deposition effects and investigating mechanisms of damage to researching and finding possible indicators of N saturation (Fenn *et al.* 1998; Gundersen *et al.* 1998). Recently it became clear that the C:N ratio of the organic soil and the upper mineral soil of forests in combination with other processes such as NO_3^- leaching and nitrification could serve as a tool to predict N saturation (Gundersen *et al.* 1998). The relationship between the C:N ratio of the F-layer, nitrification and the leaching of NO_3^- from the mineral soil has been investigated for coniferous ecosystems in the northern hemisphere (Emmett *et al.* 1998; Dise *et al.* 1999). A clear trend apparent in the sites that formed part of the NITREX consortium of ecosystem manipulation experiments is that the nitrification rate is very low and subsequently almost no leaching of NO_3^- takes place in sites with a C:N ratio exceeding 25-27 (Gundersen *et al.* 1998; Dise *et al.* 1999). At sites that exhibit a C:N ratio lower than 25, detectable leaching took place, while the nitrification rate also increased (Gundersen *et al.* 1998; Dise *et al.* 1999).

This study investigated the distribution of stable N isotopes in managed *P. patula* plantations to gain insight into N cycling dynamics in such plantation ecosystems in South Africa. The N isotope ratio after the addition of N to *P. patula* stands was also investigated to determine the fate of added N. The C:N ratio in the organic soil layers was investigated to examine the use of C:N ratios in assessing the N saturation of the three sites investigated in this study.

MATERIALS AND METHODS

Sampling

Samples were collected in April 1997, in the winter (dry season). Sampling was undertaken on three sites, namely *P. patula* sites that were 4-, 14- and 22-year-old in 1995. Samples were taken from the L_1-, L_2-, F and H- organic soil horizons (Dames, 1999a), the top 10 cm of the mineral soil (M10), the current years needle growth and the needles from the previous growing season. Roots were isolated from the F-layer of the three sites. Carpophores were sampled from the forest floor. These consisted mainly of *Scleroderma citrinum*. One mini-plot (1 x 1 m in size) was randomly selected for each site. One sample was taken from the freshly fallen litter at the top of the organic horizon by removing all the litter from that layer inside of the 1 x 1m square (L_1-layer). The same method was repeated with the other layers. Roots were isolated from the F- and H- layers by hand. All samples were taken to the laboratory, dried at 65°C for 96h, and two subsamples taken from each sample. The subsamples were ground in a rotator mill (Retch, Germany), and stored in airtight containers until analyzed.

Isotopic analyses

Simultaneous analyses of the N and C content and the N and C isotopic composition were undertaken on a system combining an elemental analyzer with a Finnigan Mat 242 gas isotope mass spectrometer. Results were expressed as δ values, which are relative to standard reference materials (Mariotti, 1984):

$$\delta^{15}N \text{ or } \delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000‰,$$

where:

R_{sample} = molar ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ of the sample

$R_{standard}$ = molar ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ of the standard

N isotopic compositions were expressed relative to an atmospheric air standard ($\delta^{15}N_{AIR}$ = 0) and carbon isotopic values were expressed against a Pee Dee Belemnite standard ($\delta^{13}C_{PDB}$ = 0). The $\delta^{15}N$ values of the N mixture applied to the fertilized plots were also determined. Five samples were taken and reduced to dryness in a freeze-dryer.

Analysis of variance, followed by Duncan's post hoc test, was used for significance testing across more than two means. Summarized ANOVA tables can be found in Appendix I.

RESULTS

$\delta^{15}N$ abundance of the soil

The natural ^{15}N enrichment of the organic soil horizons ranged from 1 to 4.5‰ (Fig. 7.1). All the $\delta^{15}N$ values for the different soil horizons had positive values, and increased with an increase in the depth of the organic horizon ($H>F>L_2>L_1$). There were, however, differences in the slope of the increase in $\delta^{15}N$ between the different sites. Differences between the L_1- and the H- layers were much larger on the 14- and 22-year-old sites than on the 4-year-old site. This can also be seen in the significantly higher H- layer $\delta^{15}N$ values for the 14- and 22-year-old sites. This trend is correlated with the age of the sites and the depth of the litter layer.

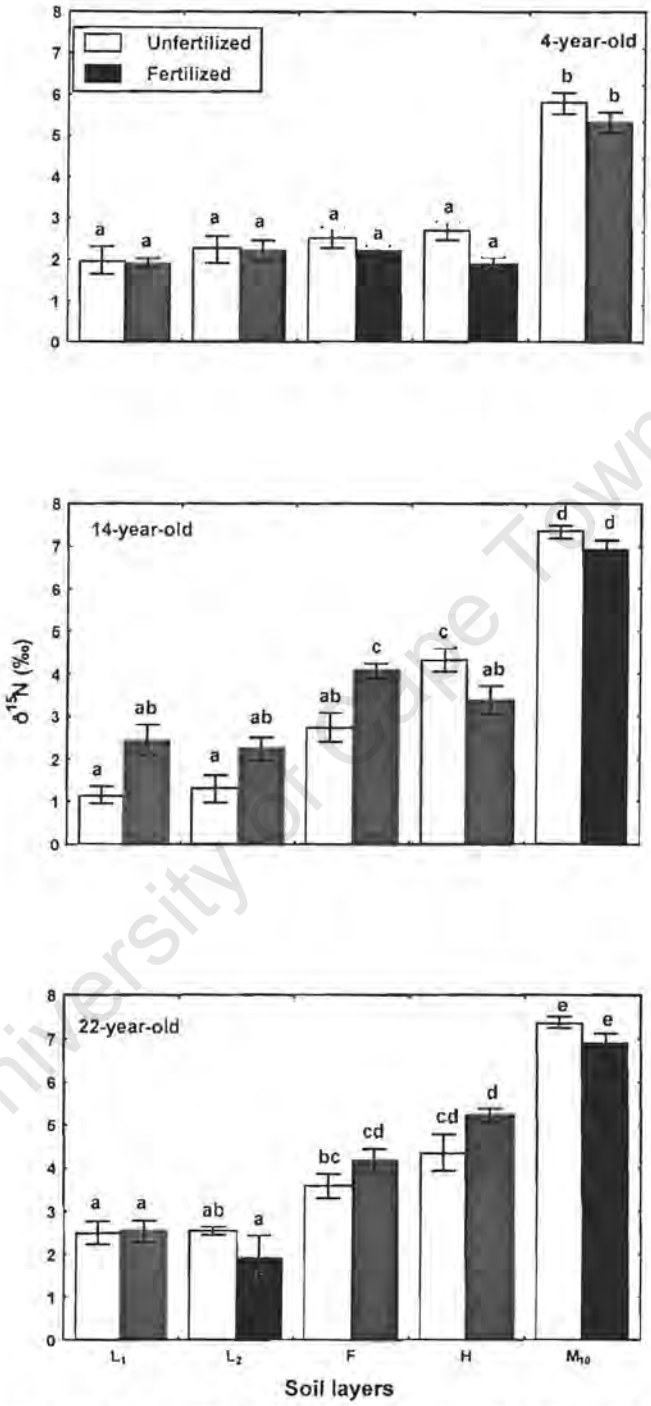

Fig. 7.1 *Mean $\delta^{15}N$ values of different organic and mineral soil horizons form fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *sites. Bars with the same letters are not significantly different at* $p<0.05$ *(three-factor ANOVA followed by Duncan's multiple range post hoc test). The post hoc test was carried out on each site individually, and the sites can thus not be directly compared. Values are the means of the four plots per treatment and error bars represent one SE from the mean.*

Application of N to these sites did not change the $\delta^{15}N$ values of the organic soil horizons to a large degree and no clear trend was observed, except for the 4-year-old site (Fig. 7.1). Here the fertilized values all tended to be lower than the unfertilized values, although not significantly so. The $\delta^{15}N$ of the applied N mixture was determined to be -0.55±0.15‰.

In all three sites the top 10cm of the mineral soil (M10) was significantly more enriched in $\delta^{15}N$ than any of the other horizons studied. The M_{10} horizon from the 14- and 22-year-old sites had higher $\delta^{15}N$ values than did the 4-year-old site (Fig. 7.1). In all cases application of N to the sites depleted the M_{10} horizon of fertilized in ^{15}N relative to the unfertilized plots.

$\delta^{15}N$ abundance of the plant components

The $\delta^{15}N$ values of roots isolated from the F- and H- layers increased with age, but were consistently lower than those of the F- and H- layers of the respective sites (Fig. 7.2). Carpophores sampled on the sites exhibited very high $\delta^{15}N$ values (Fig. 7.3), consistent with

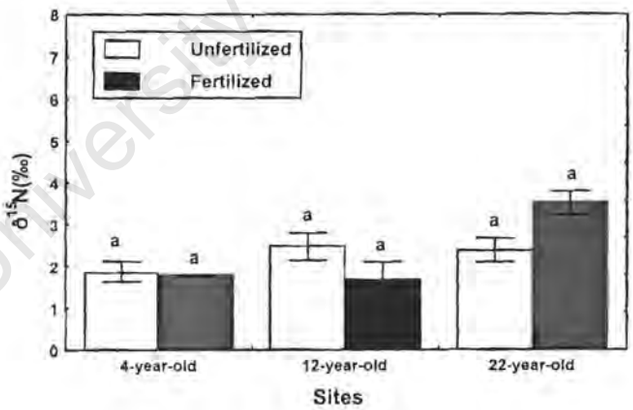

Fig. 7.2 *Mean $\delta^{15}N$ values of roots isolated form the F- and H- horizons of fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *sites. Bars with the same letters are not significantly different at $p<0.05$ (two-factor ANOVA followed by Duncan's multiple range post hoc test). Values are the means of the four plots per treatment and error bars represent one SE from the mean.*

other studies (Handley and Raven, 1992). Application of N to the sites lowered the $\delta^{15}N$ of carpophores on all sites.

The $\delta^{15}N$values of *P. patula* leaves ranged from 1.5 to 3.9‰. The leaves from the 4-year-old site showed significantly lower values than those of the 14- and 22-year-old sites (Fig. 7.4). In all cases the one-year-old needles exhibited lower $\delta^{15}N$ values than the current needles. There was a trend for the current-year needles of the fertilized plots of all three sites to have lower $\delta^{15}N$ values than the unfertilized needles. This trend was less obvious in the one-year-old needles.

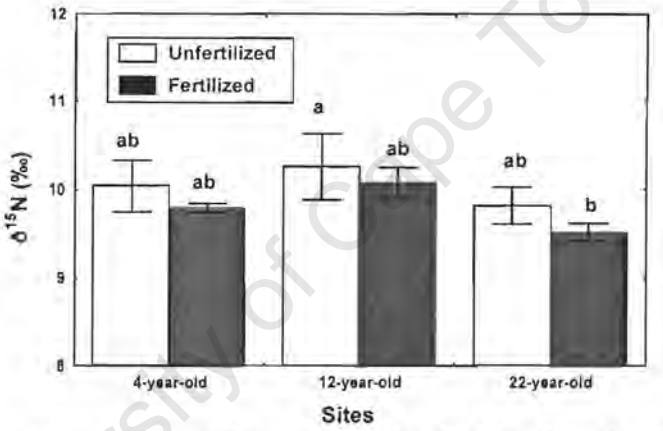

Fig. 7.3 *Mean $\delta^{15}N$ values of carpophores from the fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *sites. Bars with the same letters are not significantly different at* $p<0.05$ *(two-factor ANOVA followed by Duncan's multiple range post hoc test). Values are the means of the four plots per treatment and error bars represent one SE from the mean.*

The $\delta^{13}C$ of ecosystem components

The $\delta^{13}C$ of the ecosystem components show the typical values for C_3 plants. There were no significant differences in $\delta^{13}C$ between the organic horizons of the three sites (Fig. 7.5). No evidence could be found that chronic N addition changed the $\delta^{13}C$ of organic or mineral soil horizons. A notable departure from typical C_3 values was found in the top of the mineral soil (M_{10}) from all three sites. In the M_{10} horizon the $\delta^{13}C$ values ranged from -18 to -22‰ and

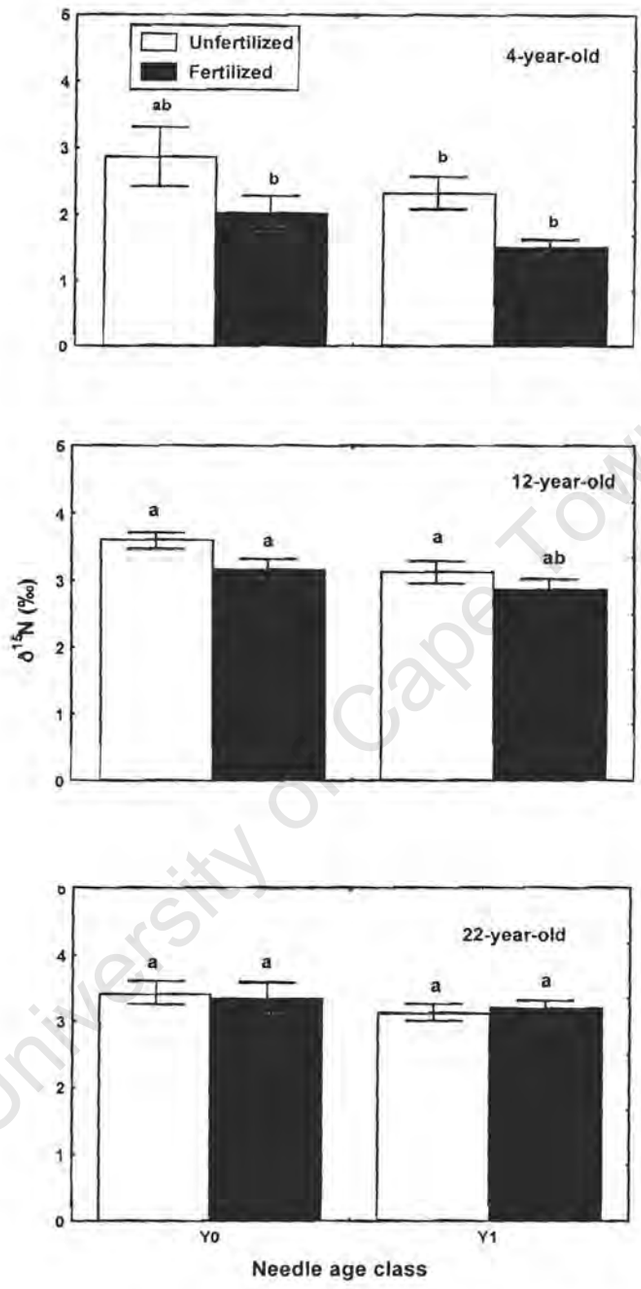

Fig. 7.4 *Mean $\delta^{15}N$ values of needles form different age classes collected from fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *sites. Bars with the same letters are not significantly different at* $p<0.05$ *(two-factor ANOVA followed by Duncan's multiple range post hoc test). Values are the means of the four plots per treatment and error bars represent one SE from the mean.*

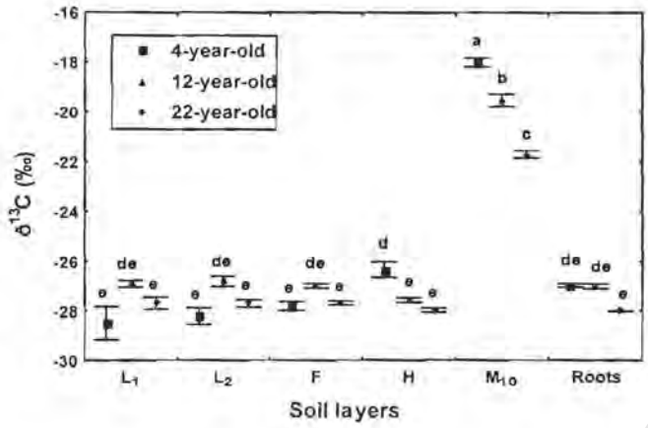

Fig. 7.5 *Mean $\delta^{13}C$ values of different organic and mineral soil horizons and roots isolated form the F- and H- horizons of fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *sites. Bars with the same letters are not significantly different at* $p<0.05$ *(ANOVA followed by Duncan's multiple range post hoc test). Values are the means of the four plots per treatment. Error bars represent one SE from the mean.*

decreased with age. $\delta^{13}C$ values from this layer were significantly higher than the other soil layers (Fig. 7.4).

C:N ratios of the soil horizons

The general trend in all the sites was a decrease in the C:N ratio with depth (Fig. 7.6). This was less clear on the 4-year-old site, which exhibited low C:N ratios even in the L_1- and L_2- layers, although the values for the F- and H- layers were similar to the 14- and 22-year-old sites. Contrary to what was found with the $\delta^{15}N$ values, the C:N ratios were influenced by the application of N (Fig. 7.7). Most of the values fell below the 1:1 line, suggesting lower C:N ratios in the organic and mineral soil horizons than the respective unfertilized components. This is consistent with immobilization of N in those horizons.

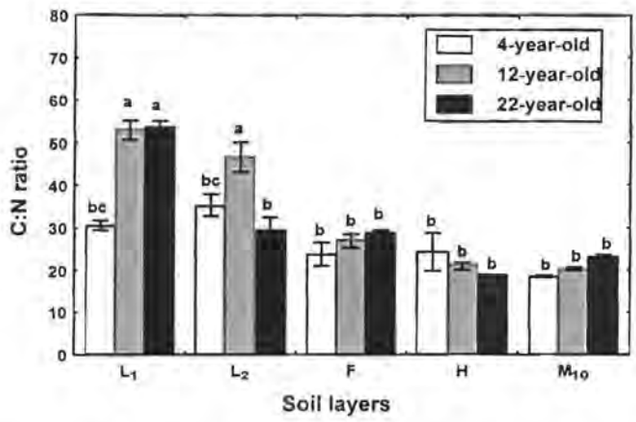

Fig. 7.6 *Mean C:N ratios of the organic and mineral soil horizons of the unfertilized plots of 4-, 14-, and 22-year-old* P. patula *sites. Bars with the same letters are not significantly different at* p<*0.05 (two-factor ANOVA followed by Duncan's multiple range post hoc test). Values are the means of the four plots per treatment and error bars represent one SE from the mean.*

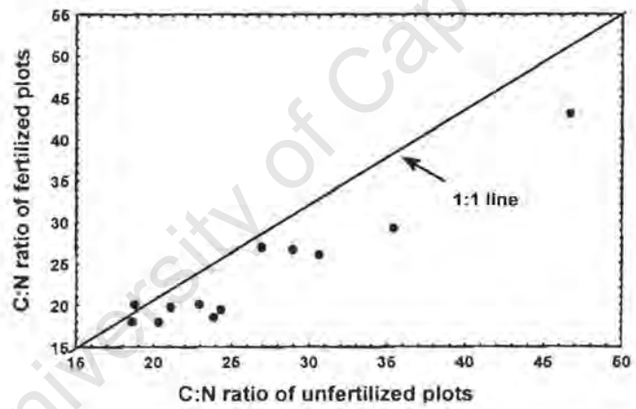

Fig. 7.7 *Relationship between C:N ratio of the fertilized and unfertilized plots of 4-, 14-, and 22-year-old* P. patula *sites. A 1:1 line was drawn in to illustrate the deviation of the C:N ratio of the fertilized plots from those of the unfertilized plots due to simulated N deposition.*

DISCUSSION

The $\delta^{15}N$ values in this *P. patula* plantation ecosystem were higher than what is normally expected of coniferous ecosystems (Emmett *et al.* 1998, Gebauer *et al.* 1994, Nadelhoffer and Fry, 1994). Several factors combine to create this effect. Firstly, the sites in the current study are second rotation coniferous plantations that have been left unplanted between rotations for up to two years. This management practice increases the rate of mineralization and nitrification and encourages N loss from the soil (Vitousek, 1981), leading to elevated $\delta^{15}N$ values in the soil and vegetation. The practices of ripping, pitting and ploughing, frequently carried out in forest compartments before planting of new seedlings would further disturb the site and add to mixing of soil layers thereby increasing the rate of nitrification. A similar conclusion has been reached to explain the exceptions to the normal range of $\delta^{15}N$ that have been found at Aber forest, Wales, where high $\delta^{15}N$ values were attributed to disturbance of the ecosystem through management practices (Emmett *et al.* 1998).

Secondly, prior to being converted to plantations, all three sites supported C_4 grasslands, similar to undisturbed grassland ecosystems in the Drakensberg escarpment (Scholes *et al.* 1996). Apart from anecdotal evidence, this can also be deduced from the carbon isotope signal of the sites. While the organic soil horizon reflects the C_3 photosynthetic pathway that is characteristic of most pines (Ehleringer *et al.* 1993; Warren and Adams, 2000), the C resident in the mineral soil has $\delta^{13}C$ values >-20‰. This is indicative of C originating from C_4 plants, which constitute a substantial portion of the grass species in the grassland ecosystem in the high altitude areas in the Drakensberg escarpment (Scholes *et al.* 1996). The N turnover rates in these grasslands are generally high, mainly due to the high levels of N in the foliage and the annual growth cycle combined with high litter production and decomposition, as well as annual burning

(Scholes *et al.* 1996). This would lead to a high turnover of N, and through high rates of nitrification and mineralization, increase the $\delta^{15}N$ values in the ecosystem. By the 1950's, plantations were established, replacing the grasslands, but with a resident soil N pool which was enriched in $\delta^{15}N$ due to the discriminatory effect of high rates of nitrification and mineralization against the heavy isotope (Vitousek *et al.* 1989).

Lastly, the N cycle in these ecosystems is generally fairly open, which supports high rates of nitrification and mineralization. It is estimated that the rate of anthropogenic N input in the Drakensberg escarpment area is between 15 and 20 $kg^{-1}.ha^{-1}yr^{-1}$ (Tyson *et al.* 1988; Olbrich, 1995). Schutz (1990) documented high levels of N in *P. patula* leaves in the area, an indication of high N loading of the ecosystem, while Nowicki (1997) reported high levels of NO_3^- in the streams draining the forestry areas, indicating leaching of N from the soil and an open N cycle. The net result of these processes is an increase in the $\delta^{15}N$ values of the ecosystem elements. Other indicators from the current study, such as low C:N ratios and low retention of added N in the system, also indicate possible long term exposure of this system to N deposition (Chapter 5; Chapter 6).

Soil $\delta^{15}N$ values generally increase with depth and this is usually attributed to downward transport of humus particles enriched in $\delta^{15}N$ as well as continuing nitrification at the different layers (Vitousek *et al.* 1989; Nadelhoffer and Fry, 1988). The *P. patula* ecosystem investigated in this study exhibited a steep gradient in ^{15}N downwards in the soil profile and this gradient increased with age of the site and with depth of the litter layer. Corresponding layers from the different sites tended to show an increase in $\delta^{15}N$ with age. Since nitrification and decomposition discriminate against the heavy isotope, this is indicative of the time of exposure to these processes. Furthermore, it was found that the carpophores from these sites were

enriched in ^{15}N (Fig. 7.5); normally, the mycorrhizal sheaths on the roots are also enriched in ^{15}N (Handley and Raven, 1992). After mortality of these fungal elements the decaying material becomes part of the necromass of the F- and H-layers.

After two seasons of adding of 120 kg N ha^{-1} yr^{-1} no clear trend in $\delta^{15}N$ of the fertilized plots relative to the unfertilized plots was evident. Soil total N usually consists of relatively inactive N, such as organically bound N, and does not immediately reflect the $\delta^{15}N$ value of the added N (Johannison and Högberg, 1994). A detectable and consistent treatment effect could establish only several years after the start of chronic N inputs, as the new N replaces the old residual N and starts to dominate the signal. This pre-treatment memory effect is especially evident in the mineral soil horizons (Handley and Scrimgeour, 1997). In contrast, the established plants and the understorey in plantations would provide clearer short-term indication of changes in the N pools than the soil (Johannison and Högberg, 1994). In a recent review, Handley and Scrimgeour (1997) maintained that plants would use the newest N available to them, which in the case of forest fertilization, would be fertilizer-N. Results from the current study are consistent with this conclusion.

Vegetation $\delta^{15}N$ values normally reflect the actively sampled soil N pool and therefore the recent history of the site. This can be seen in the $\delta^{15}N$ values of the fertilized plots. The isotopically light N that has been added to fertilized plots was reflected in the current needles of all three sites. This was not the case with the Y1 needles, possibly due to the effect of retranslocation from these needles. These findings suggest that some of the added N is taken up by the plant, and this is reflected in the N concentration of the current and one-year-old needles (Chapter 3).

Pinus patula needles on the three sites showed a decreasing trend in $\delta^{15}N$ with age of the needles involved. Gebauer and Schulze (1991) found a similar trend in *Picea abies* in the Fichtelgebirgte in NE Bavaria, Germany, which they attributed to the onset of catabolic activity in the older needles and subsequent export of N-containing compounds. Alternatively, it could be due to the live needles acting as a strong sink for assimilated N. This last scenario seems likely in the light of the work by Fife and Nambiar (1997) in which they showed that N is vigorously translocated within the plant (*P. radiata*) from the older needles to the youngest needles. The general trend of lower $\delta^{15}N$ values in the 4-year-old site than the other sites was also evident in the foliage. This further supports the conclusion that nitrification and ammonification are important determinants of $\delta^{15}N$ values in this ecosystem. The 4-year-old site has not had the exposure to these processes that the 14- and 22-year-old sites have had. Nitrogen produced during nitrification is lost from the ecosystem and will be depleted in ^{15}N, leading to gradual enrichment of plant available N. Generally, sites exposed to high levels of N deposition also have higher $\delta^{15}N$ values (Gebauer and Schulze, 1991).

The $\delta^{15}N$ values of carpophores from *P. patula* ecosystems were much higher than any other ecosystem elements. A similar trend was found for other coniferous ecosystems (Gebauer and Dietrich, 1993) and also in some common garden fungi (Handley *et al.* 1996). These studies suggest that the loading of carpophores with the heavy isotope of N could be due to internal recycling of N within the carpophore. However, the observed differences between cap and stipe reported in the aforementioned studies were too small to explain the reported difference in $\delta^{15}N$ between fruiting body and the organic soil, mineral soil, roots and leaves. In this study, the differences between the fruiting body of mycorrhizal species and the fermentation and humus

layers of the *P. patula* sites were 5 and 6‰, respectively. Simulated N lowered the $\delta^{15}N$ of carpophores, indicating use of the fertilizer N that was added to the fertilized plots.

Work by Dames (1996) showed that in *P. patula* ecosystems most of the fine feeder roots are found in the L_1 (40%) and the F-layers (31%). The uptake and storage of ^{15}N-enriched N by fruiting bodies of mycorrhizal fungi can, at least in part, explain the discrepancy between the live foliage and these organic soil zones where N is principally taken from. Roots from the fermentation layer showed consistently lower $\delta^{15}N$ values than the F- layer itself. That means that the plant is accessing N that is slightly depleted in ^{15}N. It is possible that the organic N in the decomposing plant litter is quite highly enriched in ^{15}N, since the measured $\delta^{15}N$ values reflect the total N-pool, which would also serve to explain the observed differences between fruiting bodies and L_1 and F layers. Several mycorrhizas possess the ability to take up organic C because they possess enzymes that hydrolyze organic N so that it can become available to plant roots (Näsholm *et al.* 1998). Another plausible way of explaining the difference between the live foliage and the N source is that the roots are taking up isotopically light N, which is the product of the processes of ammonification and nitrification (Nadelhoffer *et al.* 1996). Isotopic fractionation during the uptake of NH_4^+ (Montoya *et al.* 1991) and NO_3^- (Wada and Hattori, 1978) has been noticed in marine phytoplankton, but in N limited ecosystems this seems unlikely (Nadelhoffer and Fry, 1994).

Garten (1993) proposed using an enrichment factor, ε, the difference in $\delta^{15}N$ between soil and foliage, to indicate the degree of N saturation. In the NITREX project, a high correlation was found between ε, N deposition and other indicators of N loading into coniferous ecosystems (Gundersen *et al.* 1998). It is, however, clear that management practices can influence the usefulness of ε as an indicator of N saturation. If ε is the difference between the mean soil $\delta^{15}N$

(H- and M_{10}- layers) and mean foliage $\delta^{15}N$ (current + one-year-old needles), all three plots show ε values of 1-2, indicating a highly enriched ecosystem. This could have been due to high levels of N deposition or, as in the case of Aber forest, Wales, it could merely be the product of management practices that increased the rate of N cycling in the system (Emmett *et al.* 1998). The influence of anthropogenic N on the $\delta^{15}N$ values of the *P. patula* ecosystem, therefore, is not clear and the use of ε as an indicator of N saturation is therefore ambiguous. This suggests that ε, the enrichment factor will not be a good indicator of N saturation in this ecosystem.

The mean C:N ratio of the F- and H- layers combined in the unfertilized plots of the tree sites ranged from 23 to 24, reflecting N-rich sites (Emmett *et al.* 1998). A C:N ratio of 24 was found to be a critical point in the N cycle of forests exposed to N deposition (Gundersen *et al.* 1998). Both net nitrification and NO_3^- leaching have been shown to increase if the C:N ratio falls below this critical level, although a transitional zone has been found between 23 and 25 where nitrification and leaching cannot be predicted from the C:N ratio (Dise *et al.* 1999). This seemed to apply to *P. patula* stands in the Drakensberg escarpment, where the magnitude of NO_3^- leaching from untreated soils has not reached levels characteristic of a true N saturated ecosystem, although high levels of NO_3^- has been found in saturated soil extracts and stream water (Nowicki, 1997). The C:N ratio of the H-layer is generally lower than that of the F-layer because of the large proportion of humic particles in the H-layer, which contains large amounts of largely immobile N.

Changes in the C:N ratio are very slow in response to N deposition and it is expected to take decades for the C:N ratio to reach levels below 25 (Gundersen *et al.* 1998). The very low levels exhibited by the *P. patula* ecosystem are indicative of a system that has been exposed to moderate to high levels of N deposition for an extended period. Olbrich (1995) estimated inputs

into *P. patula* ecosystems in the Drakensberg escarpment area in excess of 20 kg N ha^{-1} yr^{-1} in the late 1980's and early 1990's. Nitrate deposition values in excess of 10 kg N ha^{-1} yr^{-1} have been measured in the early part of the last decade and very high emission figures for power stations, the major producers of NOx's, have been recorded as least as far back as the early 1980's (Tyson *et al.* 1988). Although pollution figures for the period 1960-1980 are not available, it can be assumed that the inputs to the system would have been relatively high, since a number of power stations in operation at that time.

These results suggest that *P. patula* ecosystems in the Drakensberg escarpment are enriched in the heavy isotope, probably because historically the areas were under grasslands with high N turnover rates as well as the management applied to the plantations. Nitrogen saturation is expected to increase the fraction of the heavy isotope further, since N turnover is accelerated and the N cycle become more open. The C:N ratio in the organic soil horizons of the sites investigated already show values that are associated with increased leaching of NO_3^- and an acceleration of N cycling (Gundersen *et al.* 1998). Simulated N deposition did not change the C:N ratio or the $\delta^{15}N$ values in the ecosystem appreciably, an indication of the conservative nature of the soil N pool. Increased deposition will, therefore, increase the rate of turnover of N in the ecosystem, which will change both the $\delta^{15}N$ values and the C:N ratios over the medium to longer term. It can be expected that the rate of decomposition will increase; however, inherently low P levels in the ecosystem could impede faster decomposition.

Chapter 8

Growth and nutrition of *P. patula* seedlings supplied with different rates of NO_3^- and NH_4^+ and different NO_3^-:NH_4^+ ratios

INTRODUCTION

Deposition of NH_4^+ and NO_3^- continue to increase globally, especially in the developing countries of East and South-East Asia, Latin America and Sub-Saharan Africa (Galloway *et al.* 1994; Galloway, 1998). Due to the increase in developing countries' reliance on industrial processes that utilize fossil fuels as a source of energy, NO_3^- dominates as the main anthropogenic N species. In contrast, NH_4^+ is the dominant ion in most parts of Western Europe, mainly due to emissions from livestock farming, with industrial processes producing progressively less air pollutants (Galloway *et al.* 1994). Most simulated N deposition studies have focused on NH_4^+ or an equal combination of NH_4^+ and NO_3^-, while little research has been done on N deposition profiles in developing countries. Most of the hypotheses developed for N saturation relate to Northern Hemisphere temperate forest ecosystems (Aber *et al.* 1989; Fenn *et al.* 1998) and these ideas remain untested for other ecosystems (Galloway, 1998).

Controlled, greenhouse-based seedling experiments have long been used in studies of the N nutrition of conifers to determine the underlying mechanisms involved. The use of conifer seedlings in conjunctions with field experiments has already provided insight into different aspects of N and growth dynamics in N saturated ecosystems (Wilson and Skeffington, 1994a; Köllig *et al.* 1997). Aber *et al.* (1989) predicted that N deposition would lead to decreases in C allocation to the roots, which then contributes to reduced uptake of nutrients, in turn leading to

nutrient deficiencies and a decline in total biomass. This trend was reflected in conifer seedling experiments where the root:shoot ratio of seedlings grown under controlled conditions tended to increase when nutrient limitations occur while excess nutrients lead to a decrease in root:shoot ratio (Canham *et al.* 1996). Nadelhoffer *et al.* (1985) found similar trends when net primary production of above and below ground biomass of coniferous forest trees were evaluated. Canham *et al.* 1996 hypothesized that the magnitude of the change in C allocation due to increased or decreased nutrient availability is species-specific. In general, the root:shoot ratio of plants decreases with increasing N availability, for both natural and manipulated systems over a wide variety of species (Reynolds and D'Antonio, 1996). The result of this trend is an inverse relationship between the N-content of the plant and the root:shoot ratio (Richards, 1965; Nadelhoffer *et al.* 1985). Root:shoot ratio increases as N or P become limiting to seedlings, while Mg limitations normally have the opposite effect (Ericsson, 1995).

Nitrogen:nutrient ratios are essential to sustained productivity of forest ecosystems (Linder, 1995; Fenn *et al.* 1998). High levels of N supply to conifers are associated with deficiencies in elements such as P and base cations (Hüttl and Wisniewski, 1987; Köllig *et al.* 1997) and this can also be induced in seedlings growing in greenhouses. Application of N to *Picea abies* greenhouse seedlings induced Mg deficiency and these trends were used to predict possible Mg deficiency in the field (Köllig *et al.* 1997). Nitrogen-induced Mg deficiencies are suspected to contribute to forest decline in Europe (Köllig *et al.* 1997). The work of Thomas and Miller (1992) showed significant decreases in foliar percentage of P after NH_4NO_3 application to young Sitka spruce plantations. In ecosystems with a naturally low P availability this could lead to P deficiencies, exacerbated by increased growth due to higher N availability. Both cation

and P deficiencies have been predicted to occur under conditions of high N supply (Aber *et al.* 1989).

Nitrogen taken up that cannot be utilized immediately by seedlings is stored in the leaf as amino acids (glutamine, asparagine, arginine) (Aber *et al.* 1989; Fenn *et al.* 1998). Several authors have reported elevated arginine levels in the leaves of trees growing in areas with high levels of N deposition (Oren *et al.* 1988; Edfast *et al.* 1990; Fenn *et al.* 1998).

Different chemical species influence soil and plant responses to N. Ammonium has greater potential to acidify the soil than NO_3^- and also competes with base cations for uptake (Wilson and Emmett, 1998). Uncertainty surrounds the preference of most northern conifers for NH_4^+ or NO_3^- sources of N. In general, most conifers prefer NH_4^+ over NO_3^- as their source of N (van den Driessche, 1971; Flaig and Mohr, 1992; Wallander *et al.* 1997). This could be related to the relatively higher availability of NH_4^+ as opposed to NO_3^- in the cold northern coniferous forests due to low nitrification rates (Waring and Schlesinger, 1985). However, the N preferences of temperate zone pines such as *P. elliottii, P. taeda, P. patula* and *P. radiata* are largely unknown.

In this section, a greenhouse-based seedling experiments is described that serves as a complementary study to the field experiments described in Chapters 3, 4, 5, 6 and 7. The greenhouse-based seedling experiments are used not as an alternative to field based experiments, but rather as a means to study in more detail some of the possible mechanisms involved in the response of the *P. patula* trees in the field to N fertilization. Interrelationships between different elements as well as growth patterns at different levels of N and different forms of N were studied. The aim was to identify possible mechanisms whereby growth and nutrition of *P.*

patula exposed to high levels of N could be affected and to relate this to trends observed in the field experiment.

MATERIALS AND METHODS

Plant and soil material

Shale-derived soil was obtained from Brooklands Plantation, Sabie, one of the sites where the field experiment was located (see chapter 2 for a full description). The soil is classified as a Nomanci (Soil Classification Working Group, 1991), and it has a high clay content (Bouyoucos, 1962; Table 8.1). The soil was put through a 5mm mesh to remove stones and course organic material. To conserve active mycorrhizal spores, the soil was not sterilized before the seedlings were planted, similar to the method of Crous *et al.* (1995). Therefore no inoculum was introduced, as was the case with other similar studies (Richards, 1965; Bledsoe and Zasoski, 1983). Three ℓ of air-dry soil were put into polythene planting bags, 15cm in diameter and 40cm high, which were open at the bottom. The bags were placed inside polythene planting pots (15cm in diameter and 30cm high) to provide stability. Seedlings were obtained from nursery stock at Ngodwana Nursery (Sappi Ltd), Ngodwana, Mpumalanga Province, South Africa. One seedling was planted per pot and the soil wetted to capacity. Seedlings were grown for six weeks before the start of the experiment. No fertilizer was given to any of the trees. The plants were grown in a greenhouse covered with plastic sheeting that let through infrared light (160 IR Sheet Polyethylene, Plastall Gundall Company). The pots were placed on a table, 0.8m high. Air movement and heat dissipation was achieved by keeping the doors open as well as regulating openings in the side of the greenhouse. The first treatments started in January 1997 and the experiment ended in December 1997.

Table 8.1 *Soil texture characteristics of the soil used in the seedling experiment. Values in parentheses represent one standard error from the average and n = 4.*

	Sand	Silt	Clay
Average	16 (± 0.64)	28.5 (± 0.20)	55.5 (± 0.67)

Experimental design

The effects of different rates of N and ratios of NO_3^- and NH_4^+ on the growth and nutrition of *P. patula* seedlings were tested (Table 8.2). A randomized design was used, with every treatment consisting of three replications of nine plants each, which were randomly located. The physical position of each of the individual replicates was changed once every three months to counteract the effect of the position and microclimate on the plants (Hattenschwiler and Körner, 1998). The total N applied to the 50 and 150 kg N ha^{-1} equivalent treatments were equal to field applications of 50 and 150 kg N ha^{-1} respectively, in different ratios, as indicated in Table 8.2. A combination of NH_4NO_3 and KNO_3 was used to get to the correct ratios. The aim of the experiment was to simulate soil conditions found in the field, and therefore, no attempt was made to balance any of the other nutrients.

Treatment

During summer and autumn (December to May of the experimental period) N solutions were applied three times a week to prevent the soil from drying out. Water-based N-solution (400ml) was applied to the soil surface of every pot. This provided enough water to wet the soil to full water capacity, while also allowing some drainage.

Growth measurements

The height and stem diameters of the seedlings were taken every four months and the crown diameter was determined at the end of the experimental period. The height of the tree was taken from the proximal point of the main stem, where the first roots appeared to the distal part (apical growing point). A caliper was used to measure the stem diameter at the point just below where the first of the lateral branches appeared. The diameter of two sides of the main stem, roughly perpendicularly from each other were measured and the average of the two measurements taken.

Table 8.2 *Compositions of the different treatments applied to the* P. patula *seedlings. The K treatment was equal to the concentration of K in the 150 kg N ha^{-1} equivalent 3:1 NO_3^-:NH_4^+ ratio treatment.*

	0 kg N ha^{-1}	K treatment	50 kg N ha^{-1}	150 kg N ha^{-1}
Water only	Y			
Water + K		Y		
NO_3^-:NH_4^+=1:1			Y	Y
NO_3^-:NH_4^+=3:1			Y	Y
NO_3^-:NH_4^+=6:1			Y	Y

The crown diameter of each seedling was measured on the leaves on the last internode on the main stem and the average of two measurements recorded. To account for visual symptoms of nutrient deficiencies, a Munsell Colour Chart (Munsell Colour Co., 1994) was used to evaluate the seedlings for signs of chlorosis by documenting the colour of the last internode on the main stem. The colour values according to the Munsell charts were transformed into numerical values according to the colour intensity to arrive at a single value relative to a reference colour (Melville and Atkinson, 1985). These values were then used in the statistical analyses.

Chemical analyses

On completion of the final growth measurements all seedlings were harvested prior to chemical analyses. Every seedling was separated into the root section and the shoot section. The youngest fully expanded leaves on the main stem were removed from the stem and kept separate. All plant parts were dried in an air circulation oven at 65°C until they achieved constant weight. The dried roots and shoots (including youngest fully expanded leaves) were weighed to three decimal points to determine root:shoot ratios. Only the youngest fully expanded leaves from each seedling were used for chemical analyses. The youngest fully expanded leaves were then bulked into four samples for every treatment and ground in a rotary mill. The samples were then acid digested and analyzed for base cations using an ICP Spetrometer. Total N concentrations were determined using indo-phenol blue colorimetric analysis after organic N transformation to NH_4^+ by kjeldahl digestion, while total P was determined using the Murphy and Riley (1962) method. Soil samples were taken at the end of the experimental period and bulked into four samples per treatment. Soil samples were analyzed for pH, electrical conductivity, total P (Murphy and Riley, 1962), total N (Murphy and Riley, 1962), Na, Ca , Mg, K, Al, Fe, Mn and organic C.

Data and Statistical analyses

Due to the nature of the experimental design, percentage differences from the control were used as the basis to determine significant differences using STATISTICA V5.5. Where the size of the sample population allowed, a two-factor ANOVA procedure, followed by Duncan's post hoc test were used to distinguish significantly different means (Zar, 1984; Bigg and Daniel, 1978). In all cases $p<0.05$ was used as the level of significance. Multiple linear regression analysis was used to determine the relationship between different variables, while Pearson correlation coefficients

were determined to test for correlations between variables. Summarized ANOVA tables can be found in Appendix I.

RESULTS

Growth measurements

The growth rate of *P. patula* seedlings, including control and treatments, was comparable to that found in other seedling studies with temperate conifers in South Africa (Donald and Young, 1982; Noble and Schumann, 1993; Crous *et al*, 1995). Application of N to *P. patula* seedlings led to a reduced height growth rate compared to the control (N0; Table 8.3; Fig. 8.1 and Fig. 8.2). The height increment over the 10 months of treatment showed a decline in growth correlated with an increase in N dose that was most pronounced in the 150 kg N ha^{-1} treatments (Fig. 8.1). Two-factor ANOVA, followed by Duncan's post hoc test to compare within ratios revealed that the final height of the 150 kg N ha^{-1} equivalent with NO_3^-:NH_4^+ ratios of 1:1 and 6:1 was significantly lower ($p<0.05$) than any other treatment. The 50 kg N ha^{-1} equivalent treatments did not, however, differ much from the control.

Although the same dose response trend described for the height growth parameters was evident in the final stem diameter and the stem diameter increment (Fig. 8.3 and 8.4), this was not as pronounced as the height growth parameters. As is the case with the height growth, the 150 kg N ha^{-1} equivalent treatment (NO_3^-:NH_4^+ ratio of 6:1) had the lowest final diameter growth at the end of the experiment and this was significantly different from the 50 kg N ha^{-1} equivalent treatment (NO_3^-:NH_4^+ ratio of 1:1) treatment (Fig. 8.3). Factorial analyses using ANOVA, followed by Duncan's post hoc test also did not show any significant differences, both in terms of ratios and levels of N applied. A similar trend to the height and stem diameter

increment was observed in the crown diameter where a trend of decreasing crown size resulted in significantly smaller crowns on the 150 kg N ha^{-1} equivalent treatments (NO_3^-:NH_4^+ ratio of 3:1 and 6:1) than the 50 kg N ha^{-1} equivalent treatment (NO_3^-:NH_4^+ ratio of 1:1) (Fig. 8.5).

Table 8.3. *Average values for various growth parameters of* P. patula *seedlings at the end of 12 months of treatment.* P. patula *seedlings were grown in pots at different levels of N and different ratios of NO_3^-:NH_4^+. Values in parentheses represent one standard error from the mean.*

	No Nitrogen	Potassium	50 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio
Final Height (mm)	352.00 (±14.26)	324.17 (±7.55)	337.41 (±11.61)	327.92 (±10.79)	319.71 (±15.56)	287.50 (±6.68)	318.40 (±10.83)	292.60 (±12.16)
Height Increment (mm)	174.52 (±9.71)	166.33 (±14.51)	158.71 (±8.09)	158.15 (±7.07)	146.70 (±10.61)	130.21 (±5.38)	135.77 (±6.99)	124.97 (±6.29)
Final Collar diameter (mm)	6.78 (±0.12)	6.52 (±0.15)	6.83 (±0.10)	6.73 (±0.13)	6.49 (±0.17)	6.61 (±0.12)	6.63 (±0.14)	6.34 (±0.13)
Collar Diameter Increment (mm)	111.82 (±3.48)	103.31 (±6.12)	112.78 (±8.94)	124.44 (±6.34)	99.65 (±11.11)	136.59 (±7.32)	95.20 (±7.71)	110.23 (±7.38)
Crown Diameter (mm)	276.25 (±12.74)	253.75 (±8.53)	286.14 (±9.59)	259.48 (±7.38)	257.08 (±9.51)	263.62 (±10.33)	237.29 (±8.31)	236.70 (±8.28)
Colour Values	4.00 (±0.12)	3.67 (±0.09)	4.17 (±0.09)	3.67 (±0.12)	3.77 (±0.15)	3.64 (±0.18)	3.64 (±0.18)	3.24 (±0.10)
Root:Shoot Ratio	0.58 (±0.04)	0.61 (±0.03)	0.59 (±0.04)	0.60 (±0.04)	0.61 (±0.03)	0.76 (±0.05)	0.74 (±0.05)	0.69 (±0.05)
Root weight (g)	5.61 (±0.24)	6.45 (±0.29)	7.16 (±0.57)	5.78 (±0.62)	5.89 (±0.50)	7.03 (±0.48)	7.15 (±0.44)	5.71 (±0.38)
Shoot weight (g)	10.43 (±0.55)	9.92 (±0.52)	12.14 (±0.29)	9.96 (±0.66)	9.49 (±0.59)	9.37 (±0.40)	9.92 (±0.45)	8.94 (±0.61)

Destructive sampling of the seedlings at the end of the experiment revealed that the root:shoot ratio of the seedlings increased with an increase in N applied (Fig. 8.6), but that absolute mean values of the root weight showed no clear trend (Fig. 8.7), resulting in the treatments having no significant differences between them. The shoot weight, however, showed a declining growth trend consistent with the trends observed in the height growth, diameter growth and the crown diameter growth (Fig. 8.8).

Seedling colour intensity, transformed to numerical values (Melville and Atkinson, 1985) also decreased as the level of N application increased, indicating chlorosis at the highest level of N applied (Fig. 8.9). At the end of the experiment no mortality due to treatment effects was found.

Plant-nutrient relations

Total N concentrations in the youngest fully expanded leaves of the seedlings showed an increase with increasing level of N applied, but no significant trend with an increase in the ratio of NO_3^-:NH_4^+ (Fig. 8.10). The ratio of total N:total P shows an increase with an increase of the level of N applied and this was positively correlated with the root:shoot ratio (Table 8.9).

Potassium concentrations in the foliage increased as the proportion of NO_3^- in the treatment increased (Tables 8.5 and 8.6). The base cation concentrations in the foliage of the seedlings at the end of the experimental period showed a high degree of variability between treatments, but there was no clear trend that could be related to the different ratios of NO_3^- :NH_4^+ or the levels of N applied.

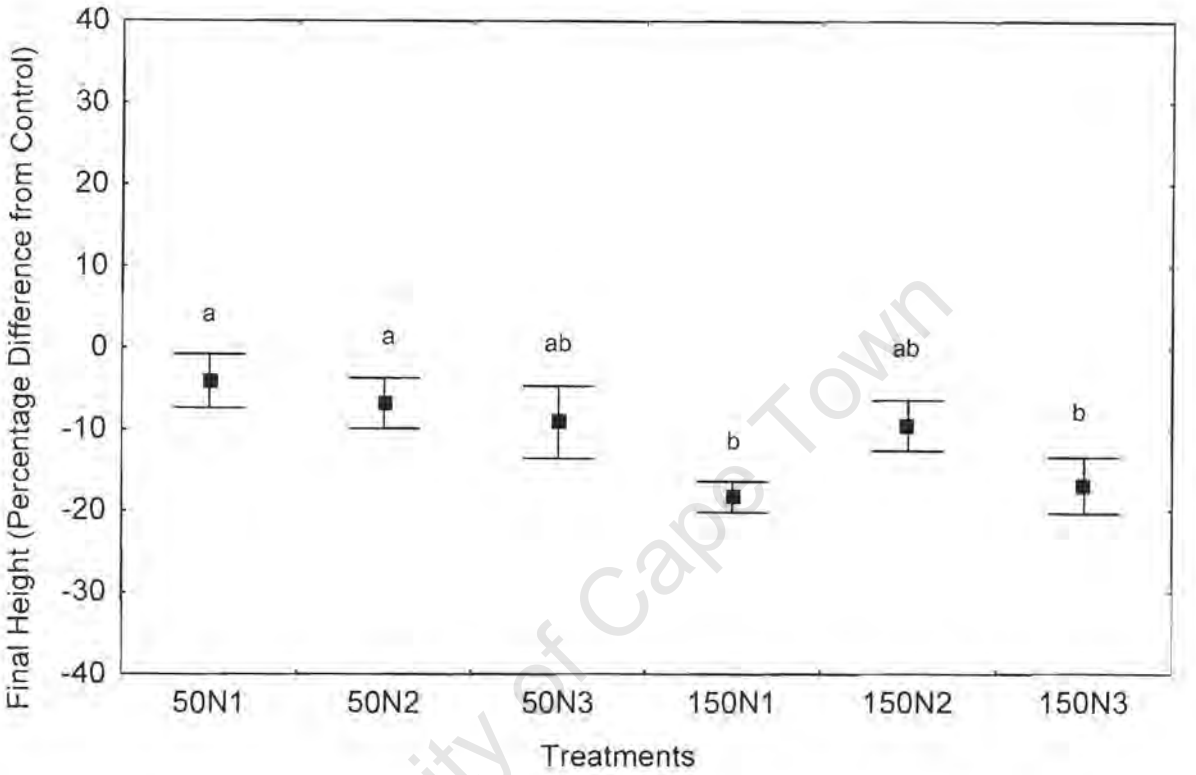

Fig. 8.1 *Percentage difference in final height of treatments from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of NO_3^-:NH_4^+. Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at $p<0.05$. The description of the treatments is as follows: 50N1 = 50 kg N ha^{-1} equivalent, 1:1 ratio; 50N2 = 50 kg N ha^{-1} equivalent, 3:1 ratio; 50N3 = 50 kg N ha^{-1} equivalent, 6:1 ratio; 150N1 = 150 kg N ha^{-1} equivalent, 1:1 ratio; 150N2 = 150 kg N ha^{-1} equivalent, 3:1 ratio; 150N3 = 150 kg N ha^{-1} equivalent, 6:1 ratio.*

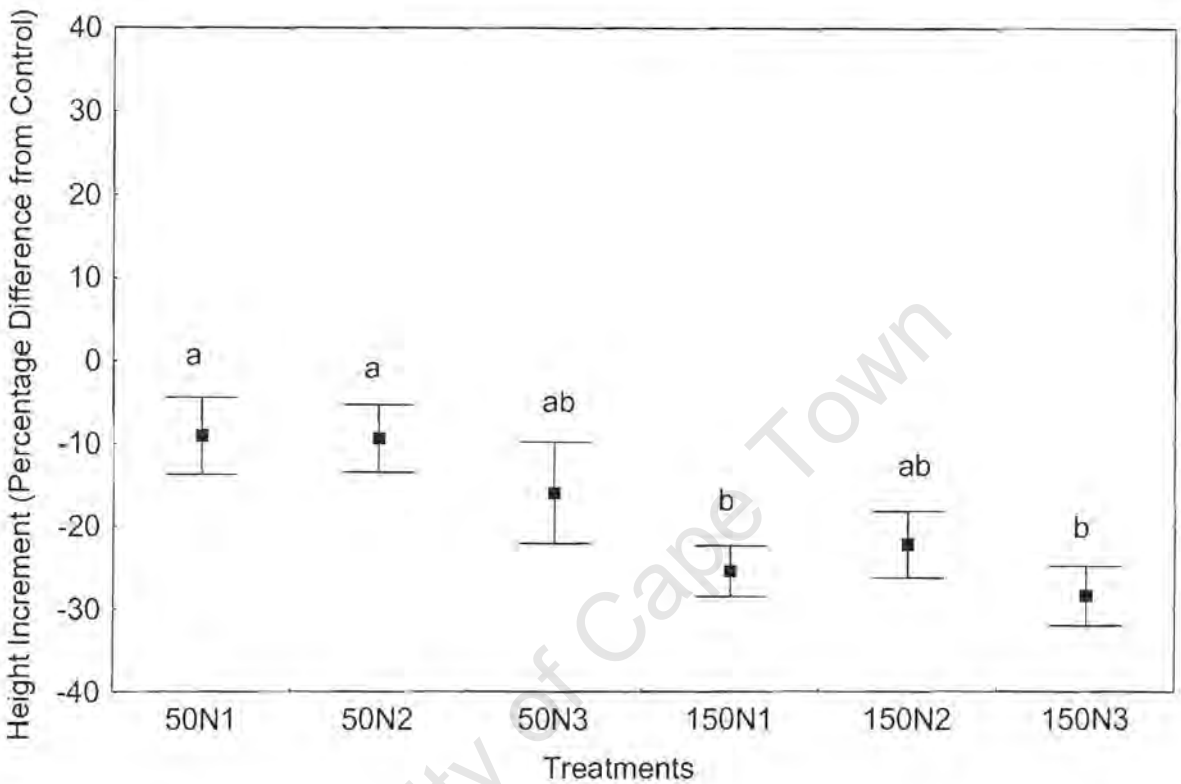

Fig. 8.2 *Percentage difference of height increment from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at* $p<0.05$. *The description of the treatments is as follows: 50N1 = 50 kg N* ha^{-1} *equivalent, 1:1 ratio; 50N2 = 50 kg N* ha^{-1} *equivalent, 3:1 ratio; 50N3 = 50 kg N* ha^{-1} *equivalent, 6:1 ratio; 150N1 = 150 kg N* ha^{-1} *equivalent, 1:1 ratio; 150N2 = 150 kg N* ha^{-1} *equivalent, 3:1 ratio; 150N3 = 150 kg N* ha^{-1} *equivalent, 6:1 ratio.*

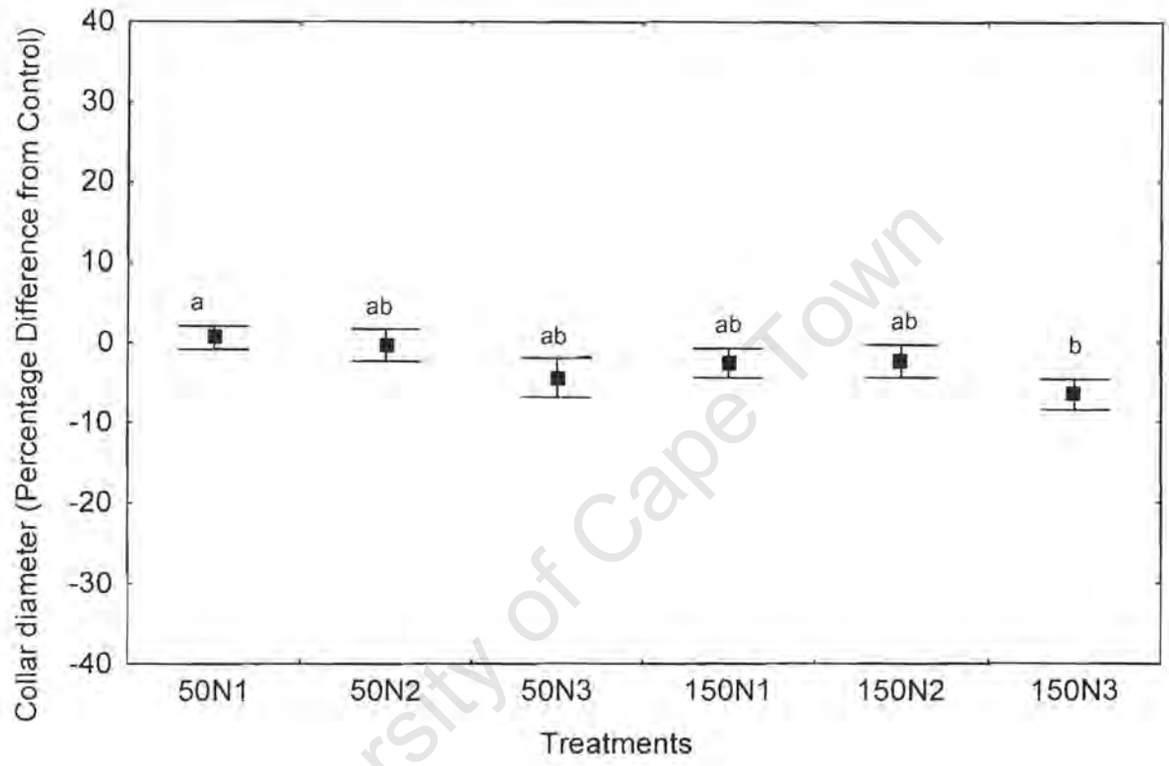

Fig. 8.3 *Percentage difference of final collar diameter from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-*:*NH_4^+*. Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at* $p<0.05$*. The description of the treatments is as follows: 50N1 = 50 kg N* ha^{-1} *equivalent, 1:1 ratio; 50N2 = 50 kg N* ha^{-1} *equivalent, 3:1 ratio; 50N3 = 50 kg N* ha^{-1} *equivalent, 6:1 ratio; 150N1 = 150 kg N* ha^{-1} *equivalent, 1:1 ratio; 150N2 = 150 kg N* ha^{-1} *equivalent, 3:1 ratio; 150N3 = 150 kg N* ha^{-1} *equivalent, 6:1 ratio.*

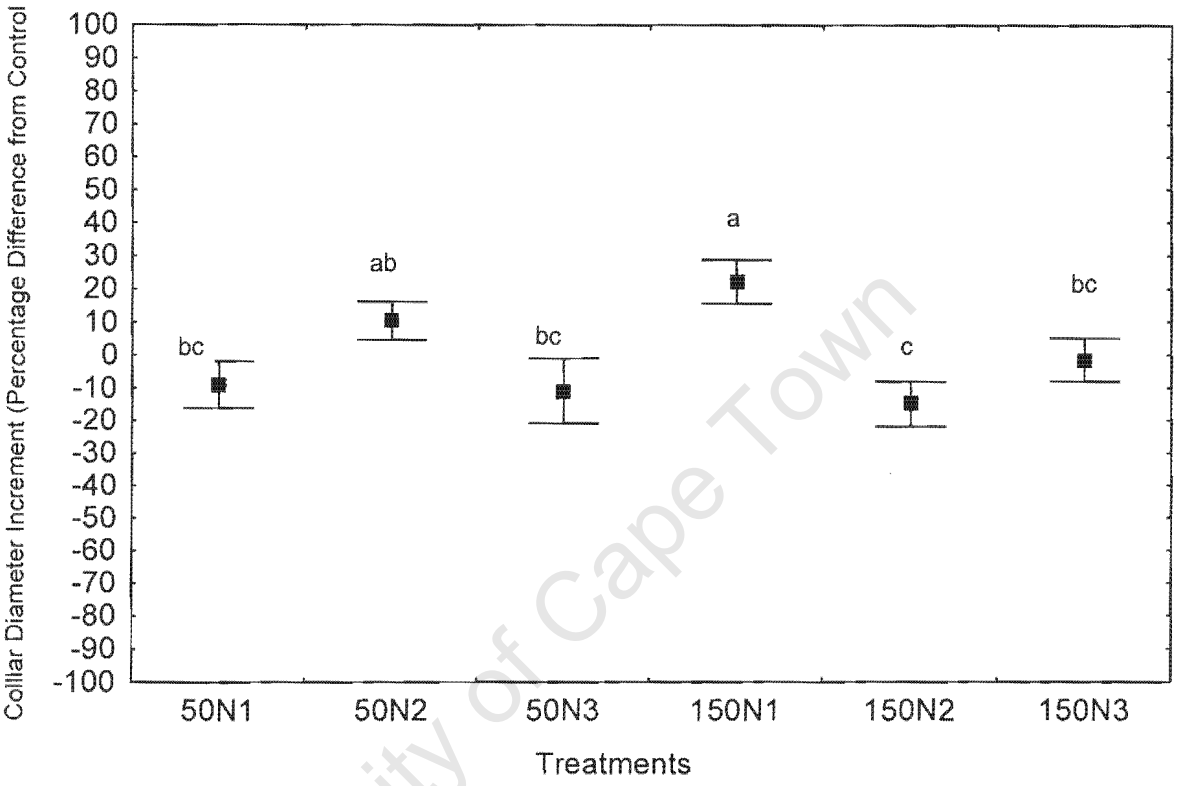

Fig. 8.4 *Percentage difference of stem diameter increment from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at* $p<0.05$. *The description of the treatments is as follows: 50N1 = 50 kg N* ha^{-1} *equivalent, 1:1 ratio; 50N2 = 50 kg N* ha^{-1} *equivalent, 3:1 ratio; 50N3 = 50 kg N* ha^{-1} *equivalent, 6:1 ratio; 150N1 = 150 kg N* ha^{-1} *equivalent, 1:1 ratio; 150N2 = 150 kg N* ha^{-1} *equivalent, 3:1 ratio; 150N3 = 150 kg N* ha^{-1} *equivalent, 6:1 ratio.*

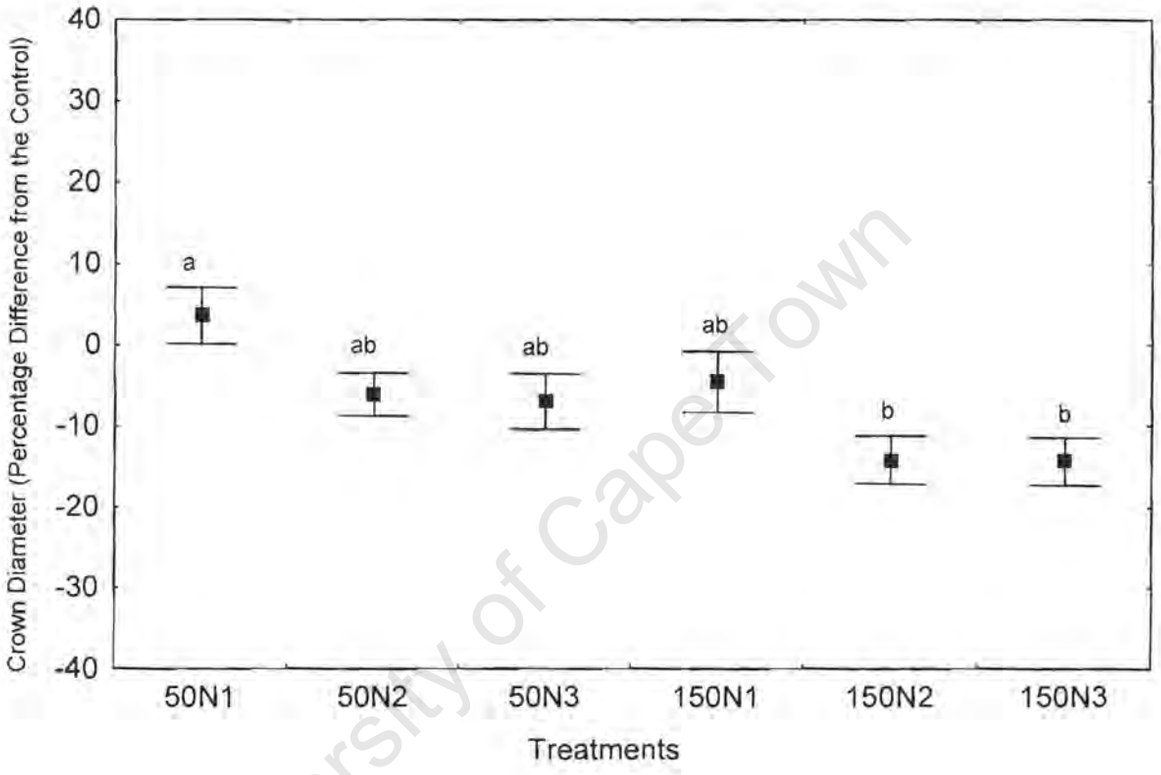

Fig. 8.5 *Percentage difference of crown diameter from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of NO_3^-:NH_4^+. Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at $p<0.05$. The description of the treatments is as follows: 50N1 = 50 kg N ha^{-1} equivalent, 1:1 ratio; 50N2 = 50 kg N ha^{-1} equivalent, 3:1 ratio; 50N3 = 50 kg N ha^{-1} equivalent, 6:1 ratio; 150N1 = 150 kg N ha^{-1} equivalent, 1:1 ratio; 150N2 = 150 kg N ha^{-1} equivalent, 3:1 ratio; 150N3 = 150 kg N ha^{-1} equivalent, 6:1 ratio.*

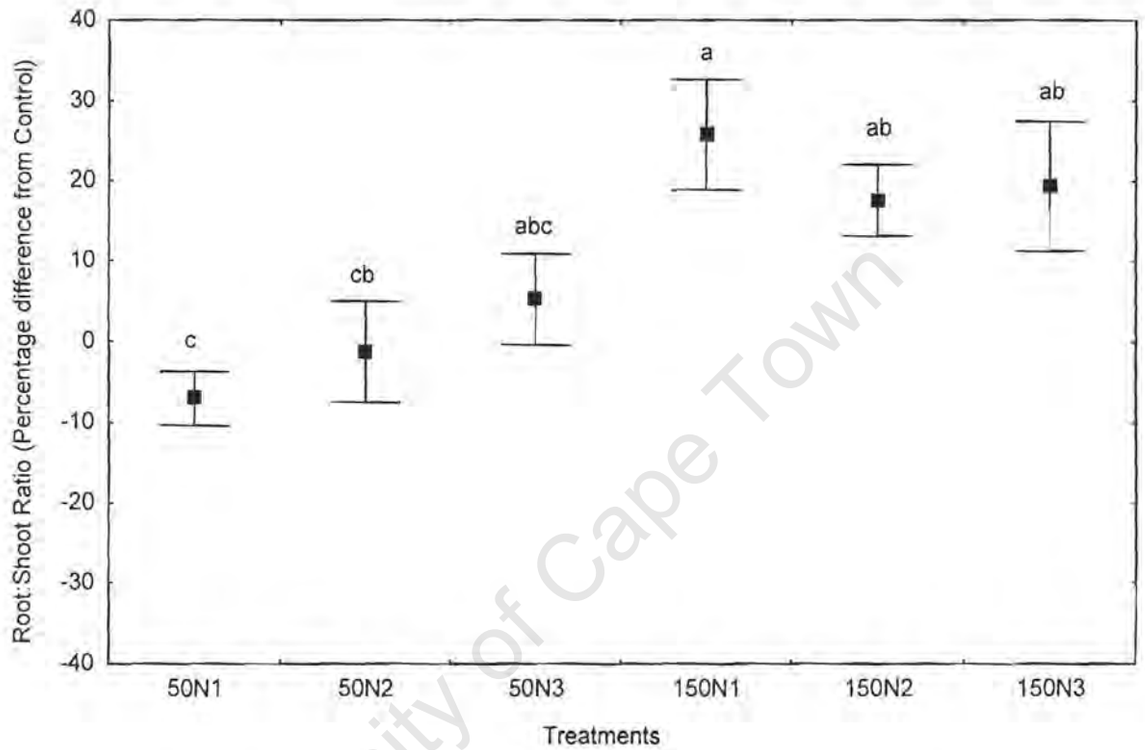

Fig. 8.6 *Percentage difference of root:shoot ratio from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at* $p<0.05$. *The description of the treatments is as follows: 50N1 = 50 kg N* ha^{-1} *equivalent, 1:1 ratio; 50N2 = 50 kg N* ha^{-1} *equivalent, 3:1 ratio; 50N3 = 50 kg N* ha^{-1} *equivalent, 6:1 ratio; 150N1 = 150 kg N* ha^{-1} *equivalent, 1:1 ratio; 150N2 = 150 kg N* ha^{-1} *equivalent, 3:1 ratio; 150N3 = 150 kg N* ha^{-1} *equivalent, 6:1 ratio.*

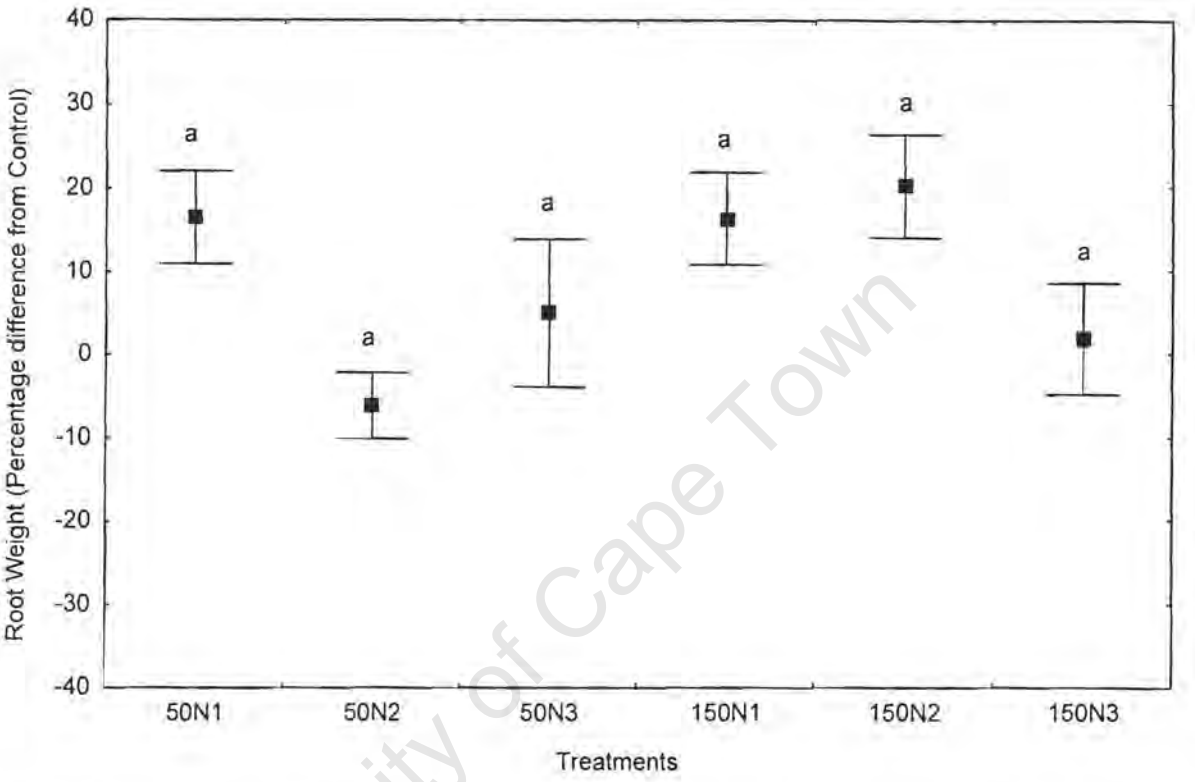

Fig. 8.7 *Percentage difference of root weight from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters is not significantly different at* $p<0.05$. *The description of the treatments are as follows: 50N1 = 50 kg N* ha^{-1} *equivalent, 1:1 ratio; 50N2 = 50 kg N* ha^{-1} *equivalent, 3:1 ratio; 50N3 = 50 kg N* ha^{-1} *equivalent, 6:1 ratio; 150N1 = 150 kg N* ha^{-1} *equivalent, 1:1 ratio; 150N2 = 150 kg N* ha^{-1} *equivalent, 3:1 ratio; 150N3 = 150 kg N* ha^{-1} *equivalent, 6:1 ratio.*

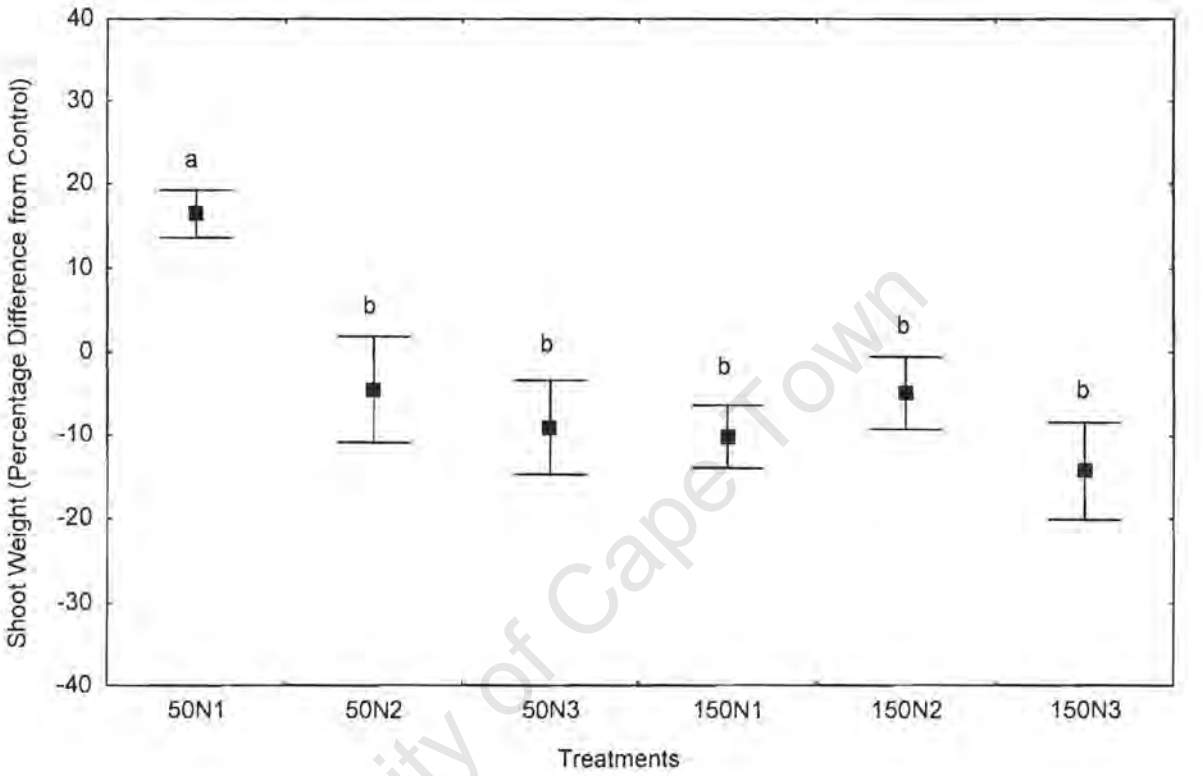

Fig. 8.8 *Percentage difference of shoot weight from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of NO_3^-:NH_4^+. Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters is not significantly different at $p<0.05$. The description of the treatments are as follows: 50N1 = 50 kg N ha^{-1} equivalent, 1:1 ratio; 50N2 = 50 kg N ha^{-1} equivalent, 3:1 ratio; 50N3 = 50 kg N ha^{-1} equivalent, 6:1 ratio; 150N1 = 150 kg N ha^{-1} equivalent, 1:1 ratio; 150N2 = 150 kg N ha^{-1} equivalent, 3:1 ratio; 150N3 = 150 kg N ha^{-1} equivalent, 6:1 ratio.*

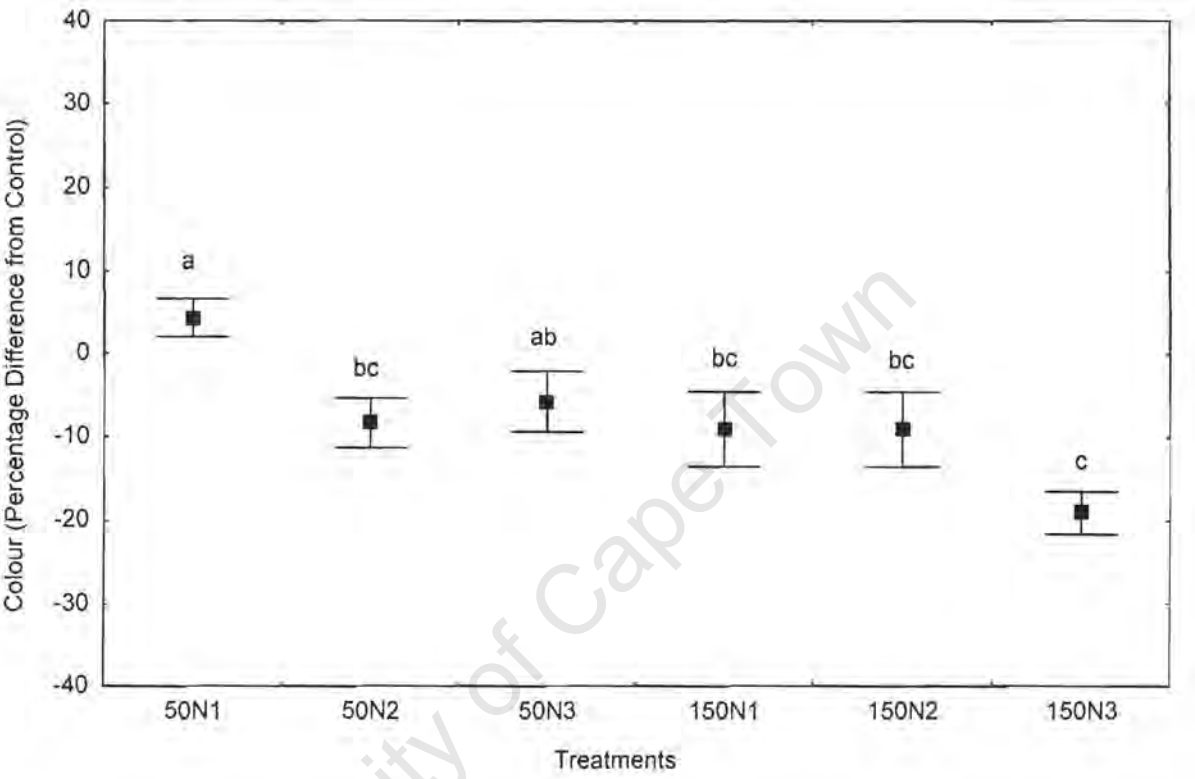

Fig. 8.9 *Percentage difference of colour values from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Error bars represent one standard error deviation from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at* $p<0.05$. *The description of the treatments is as follows: 50N1 = 50 kg N* ha^{-1} *equivalent, 1:1 ratio; 50N2 = 50 kg N* ha^{-1} *equivalent, 3:1 ratio; 50N3 = 50 kg N* ha^{-1} *equivalent, 6:1 ratio; 150N1 = 150 kg N* ha^{-1} *equivalent, 1:1 ratio; 150N2 = 150 kg N* ha^{-1} *equivalent, 3:1 ratio; 150N3 = 150 kg N* ha^{-1} *equivalent, 6:1 ratio.*

Table 8.4. *Average values for total N, total P and N:P ratio* P. patula *seedlings at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Values in parentheses represent one standard error from the mean.*

	No Nitrogen	Potassium	50 kg N. ha^{-1} equivalent; 1:1 NO_3^- :NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 3:1 NO_3^- :NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 6:1 NO_3^- :NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 1:1 NO_3^- :NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 3:1 NO_3^- :NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 6:1 NO_3^- :NH_4^+ ratio
Total N (%)	1.56 (± 0.05)	1.54 (±0.10)	1.70 (±0.21)	1.68 (±0.07)	1.55 (±0.08)	1.74 (±0.02)	2.14 (±0.12)	2.06 (±0.23)
Total P (%)	0.08 (±0.01)	0.07 (±0.02)	0.08 (±0.00)	0.07 (±0.00)	0.07 (±0.01)	0.05 (±0.01)	0.06 (±0.00)	0.07 (±0.02)
N:P Ratio	20.08 (±3.25)	23.21 (±6.06)	20.14 (±2.08)	25.40 (±1.93)	21.65 (±2.68)	31.93 (±3.34)	34.90 (±2.83)	29.94 (±12.85)

Soil analyses

Final analyses of the soil from the different treatments showed no differences between treatments in any parameter except EC, K concentration and total N concentration (Table 8.7 and Table 8.8). Electrical conductivity decreased significantly ($p<0.05$) with an increase in the K concentration in the treatment solution (K treatment). The K concentration in the soil was significantly higher in the treatments that contained the highest concentration of K in the treatment solution. No significant changes ($p<0.05$) in Zn, Mn and Fe contents were observed.

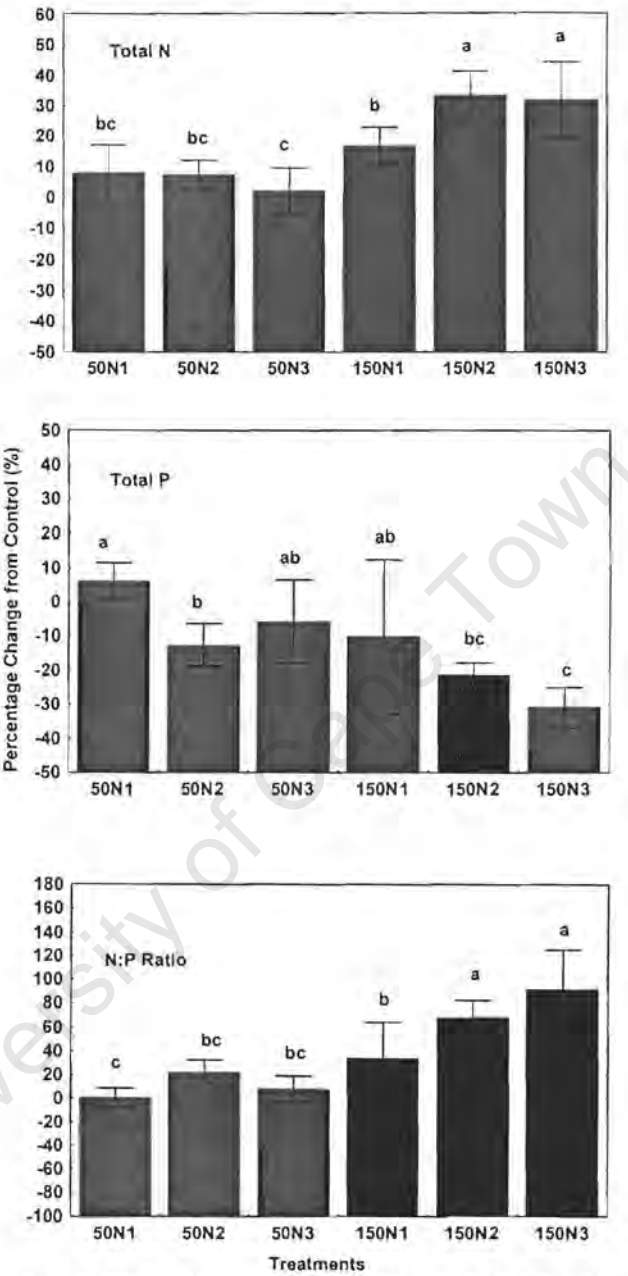

Fig. 8.10 *Percentage difference of total N, total P and N:P ratio from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Values in parentheses represent one standard error from the mean. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at* $p<0.05$. *The description of the treatments is as follows: 50N1 = 50 kg N* ha^{-1} *equivalent, 1:1 ratio; 50N2 = 50 kg N* ha^{-1} *equivalent, 3:1 ratio; 50N3 = 50 kg N* ha^{-1} *equivalent, 6:1 ratio; 150N1 = 150 kg N* ha^{-1} *equivalent, 1:1 ratio; 150N2 = 150 kg N* ha^{-1} *equivalent, 3:1 ratio; 150N3 = 150 kg N* ha^{-1} *equivalent, 6:1 ratio.*

Table 8.5 *Foliar nutrients in the youngest fully expanded leaves of the seedlings of* P. patula *at the end of 12 months of treatment. Seedlings were grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Values in parentheses represent one standard error from the mean.*

	No Nitrogen	Potassium	50 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio
K (%)	0.45 (±0.04)	1.22 (±0.29)	0.65 (±0.10)	1.29 (±0.19)	1.20 (±0.15)	0.64 (±0.20)	1.67 (±0.35)	1.68 (±0.34)
Ca (%)	0.40 (±0.08)	0.47 (±0.05)	0.4 (±0.13)	0.34 (±0.02)	0.43 (±0.04)	0.44 (±0.11)	0.43 (±0.11)	0.51 (±0.00)
Mg (%)	0.19 (±0.03)	0.20 (±0.01)	0.22 (±0.05)	0.19 (±0.02)	0.18 (±0.02)	0.18 (±0.06)	0.17 (±0.02)	0.17 (±0.02)
Mn (mg kg^{-1})	1,631.74 (±159.79)	1,663.70 (±28.75)	1,526.21 (±207.81)	1,637.85 (±229.69)	1,433.71 (±107.66)	1,345.41 (±107.79)	1,270.84 (±135.42)	1,470.65 (±46.22)
Fe (mg kg^{-1})	386.54 (±11.21)	459.45 (±28.19)	410.53 (±79.04)	458.89 (±185.86)	423.81 (±185.24)	292.47 (±73.48)	393.59 (±150.80)	737.86 (±9.60)
Cu (mg kg^{-1})	15.88 (±1.43)	16.39 (±4.64)	23.94 (±1.78)	16.29 (±1.84)	16.90 (±2.06)	24.42 (±4.30)	25.12 (±6.13)	25.34 (±11.38)
Al (mg kg^{-1})	899.74 (±125.73)	1,090.15 (±54.86)	1,071.31 (±95.85)	1,021.53 (±134.85)	963.13 (±89.76)	921.12 (±176.97)	971.82 (±165.52)	1,085.62 (±64.27)

Soil total N concentration increased significantly as the level of N in the treatment solution increased, while the total P levels did not change significantly. None of the other nutrient parameters, Mn, Al, Ca and Mg, were affected by the different treatments when compared to the control.

Correlations

Correlations between different growth and nutritional parameters (Table 8.9) show that most of the growth parameters were well correlated with each other.

Table 8.6 *Percentage difference of foliar nutrient parameters from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of NO_3^-:NH_4^+. Values in parentheses represent one standard error from the mean. In all cases n=4. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Values with the same letters are not significantly different at p<0.05.*

	50 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ratio	50 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ratio	50 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ratio	150 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ratio	150 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ratio	150 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ratio
K(%)	43.60c	187.06b	165.32b	40.88c	270.97a	271.56a
Ca(%)	5.25ab	-14.82b	7.10ab	9.28ab	6.87ab	26.31a
Mg(%)	17.53a	1.31a	-1.85a	-4.00a	-11.49a	-8.28a
Mn(%)	-6.47ab	0.37a	-12.14abc	-17.55bc	-22.12c	-9.87abc
Fe(%)	6.21b	-10.96b	9.64b	-24.34b	1.82b	90.89a
Cu(%)	50.74a	2.56a	6.42a	53.72a	58.17a	59.54a
Al(%)	19.07a	13.54a	7.05a	2.37a	8.01a	20.66a

The final height of the seedlings was highly correlated with the height increment (r=0.95; $p<0.05$) and it was also well correlated with the final diameter (r=0.74; $p<0.05$). It was similarly well correlated with the final crown diameter, final colour values of the youngest fully expanded leaves and negatively correlated with the N:P ratio in the youngest fully expanded leaves (r=-0.74; $p<0.05$) and the root:shoot ratio of the seedlings at the end of the experiment (r = -0.78; $p<0.05$).

Table 8.7 *Soil chemical parameters at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of* NO_3^-:NH_4^+. *Values in parentheses represent one standard error from the mean.*

	No Nitrogen	Potassium	50 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio
pH	3.98 (±0.02)	3.93 (±0.03)	3.93 (±0.02)	3.95 (±0.03)	3.97 (±0.03)	3.90 (±0.00)	3.93 (±0.03)	3.97 (±0.03)
EC mS	0.55 (±0.04)	0.75 (±0.04)	0.72 (±0.02)	0.97 (±0.14)	0.86 (±0.15)	1.27 (±0.15)	1.46 (±0.25)	1.43 (±0.22)
Acid Me%	6.04 (±0.05)	6.12 (±0.15)	6.38 (±0.07)	6.16 (±0.13)	5.99 (±0.09)	6.46 (±0.02)	5.97 (±0.01)	6.09 (±0.08)
Na mg kg^{-1}	72.25 (±4.87)	84.00 (±6.87)	86.25 (±5.25)	99.00 (±20.53)	94.00 (±3.61)	83.67 (±7.29)	100.33 (±4.16)	88.67 (±1.61)
K mg kg^{-1}	34.25 (±0.75)	119.00 (±12.17)	32.50 (±2.36)	95.50 (±18.59)	98.67 (±3.55)	29.67 (±2.75)	214.33 (±11.09)	265.33 (±3.25)
Mg Me%	0.17 (±0.01)	0.16 (±0.01)	0.17 (±0.01)	0.21 (±0.04)	0.17 (±0.01)	0.19 (±0.02)	0.20 (±0.01)	0.18 (±0.00)
Ca Me%	1.03 (±0.04)	0.92 (±0.06)	0.89 (±0.03)	1.13 (±0.16)	1.07 (±0.10)	0.93 (±0.03)	1.07 (±0.05)	0.92 (±0.04)
Cu mg kg^{-1}	4.03 (±0.10)	4.19 (±0.10)	4.17 (±0.09)	3.96 (±0.22)	4.07 (±0.16)	4.07 (±0.15)	4.01 (±0.06)	4.00 (±0.07)
Zn mg kg^{-1}	0.93 (±0.06)	1.07 (±0.07)	1.20 (±0.14)	1.07 (±0.04)	1.23 (±0.19)	1.07 (±0.11)	1.03 (±0.05)	1.17 (±0.10)
Mn mg kg^{-1}	16.26 (±0.45)	16.27 (±0.27)	19.99 (±0.98)	16.54 (±1.13)	17.06 (±0.80)	16.18 (±0.45)	16.58 (±0.65)	19.70 (±0.30)
Fe mg kg^{-1}	287.75 (±16.07)	147.34 (±62.98)	219.50 (±6.74)	233.25 (±21.85)	250.67 (±16.93)	260.33 (±22.74)	236.00 (±21.38)	236.00 (±15.87)
Org. C %	7.65 (±0.19)	7.61 (±0.06)	6.96 (±0.33)	6.83 (±0.13)	6.79 (±0.10)	7.75 (±0.18)	7.44 (±0.05)	7.06 (±0.02)
Total N mg g^{-1}	9.31 (±0.12)	9.24 (±0.30)	8.69 (±0.069)	8.94 (±0.44)	9.43 (±0.23)	9.42 (±0.42)	9.61 (±0.09)	9.12 (±0.48)
Total P μg g^{-1}	9.36 (±0.07)	9.51 (±0.24)	9.38 (±0.08)	9.24 (±0.05)	9.23 (±0.12)	9.23 (±0.05)	9.01 (±0.06)	9.26 (±0.06)

Table 8.8 *Percentage difference of soil chemical parameters from the control at the end of 12 months of treatment of* P. patula *seedlings grown in pots at different levels of N and different ratios of NO_3^-:NH_4^+. Values in parentheses represent one standard error from the mean. In all cases n=4. A two-factor ANOVA was to test for differences between treatments, followed by Duncan's post hoc test to determine which values differed from each other. Within each parameter, values with the same letters are not significantly different at p<0.05.*

	50 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	50 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 1:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 3:1 NO_3^-:NH_4^+ ratio	150 kg N. ha^{-1} equivalent; 6:1 NO_3^-:NH_4^+ ratio
pH (%)	-1.26a (±0.63)	-0.63a (±0.73)	-0.63a (±0.73)	-0.63a (±0.73)	-1.26a (±0.63)	-1.26a (±0.63)
EC (%)	30.61b (±3.04	74.06ab (±24.46)	55.38b (±26.45)	127.99ab (±26.66)	162.09a (±45.63)	157.45a (±40.13)
Acid (%)	5.59a (±1.14	1.90a (±2.10)	1.08a (±2.26)	1.86a (±0.58)	4.56a (±2.37)	-1.04a (±0.24)
Na (%)	19.38a (±7.27	15.23a (±8.71)	22.49a (±8.63)	26.99a (±8.18)	21.11a (±9.80)	32.18a (±8.18)
K (%)	-5.11de (±6.90	178.83c (±54.29)	182.48c (±10.14)	266.42b (±18.98)	-13.87c (±6.58)	515.33a (±28.44)
Mg (%)	-4.44a (±5.10	25.37a (±22.47)	-4.89a (±6.62)	17.91a (±6.62)	10.45a (±8.62)	11.94a (±6.15)
Ca (%)	-13.87a (±2.65	10.22a (±15.50)	-2.68a (±10.49)	0.48a (±3.47)	-7.54a (±3.25)	0.97a (±4.57)
Cu (%)	3.54c (±2.23	-1.55c (±5.35)	0.37c (±3.31)	-0.99c (±1.41)	2.61c (±3.52)	1.37c (±2.22)
Zn (%)	28.15a (±15.19	14.75a (±4.49)	24.93a (±18.10)	11.26a (±11.32)	18.77a (±10.49)	9.12a (±4.60)
Mn (%)	22.98a (±6.02	1.740bc (±6.96)	4.08bc (±4.12)	16.56ab (±5.34)	-1.28c (±2.41)	2.11bc (±3.27)
Fe (%)	-23.72a (±2.34	-18.94a (±7.59)	-15.12a (±5.30)	-2.87a (±9.38)	-13.47a (±7.56)	-38.31a (±21.22)
Org. C (%)	-8.93b (±4.35	-10.73b (±1.76)	-8.44b (±3.00)	-5.10ab (±1.51)	1.28a (±1.95)	-2.58ab (±0.51)
Total N (%)	-5.95b (±2.96	-7.22b (±4.80)	-5.13b (±3.14)	4.24a (±1.03)	3.05a (±1.40)	4.39a (±0.67)
Total P (%)	5.41a (±3.49	8.11a (±14.80)	-13.51a (±2.21)	-2.70a (±2.21)	-2.70a (±5.84)	-1.35a (±4.05)

Table 8.9 *Relationships between growth and nutritional parameters in* P. patula *seedlings, based on the measured nutrient concentrations at the end of 12 months of treatment at different levels of N and different ratios of* NO_3^-:NH_4^+. *Results are shown for Pearson correlation analyses at* $p<0.05$ *and linear regression analyses and the probability values are also indicated. In all cased n=8.*

Variables	Pearson correlation coefficient		Linear regression	
	r	p	r^2	p
Final height v. height increment	0.95	0.05	0.91	0.01
Final height v. final stem diameter	0.74	0.05	0.55	0.04
Final height v. final crown diameter	0.60	0.05	0.35	0.12
Final height v. colour	0.80	0.05	0.64	0.02
Final height v. N:P ratio	-0.74	0.05	0.52	0.04
Final height v. N dose	0.82	0.05	0.67	0.01
Final height v. root:shoot ratio	-0.78	0.05	0.60	0.02
Final colour v. N:P ratio	-0.62	0.05	0.38	0.10
Final colour v. final stem diameter	0.85	0.05	0.72	0.01
Height increment v. root:shoot ratio	-0.78	0.05	0.67	0.01
Height increment v. stem diameter increment	-0.08	0.05	0.02	0.77
N:P ratio v. root:shoot ratio	0.84	0.05	0.70	0.05
N:P ratio v. height increment	-0.72	0.05	0.55	0.04
N dose v. electrical conductivity	-0.92	0.05	-0.84	0.01
N dose v. leaf total N	0.83	0.05	0.69	0.01
N dose v. N:P ratio	0.73	0.05	0.54	0.04

The N:P ratio of the youngest fully expanded leaves was significantly correlated with the final colour values of the seedling foliage and highly correlated with the root:shoot ratio ($r = 0.70$; $p<0.05$). The NO_3^- dose was also correlated with the soil EC at the end of the experiment ($r=0.92$; $p<0.05$). None of the other foliage base cation parameters were well correlated with the growth parameters or the total N and total P values (data not shown).

DISCUSSION

In this experiment, *P. patula* seedlings performed best in the absence of any supplemented NO_3^- or NH_4^+ in the irrigation water, i.e. the control treatment. Both height increment and final height of the seedlings indicate that seedlings from the control (no N) treatment performed best, while growth rate declined progressively as the N dose increased. This trend in aboveground C allocation is confirmed with the crown diameter results, taken at the end of the experiments, which also showed a clear decrease in growth of the crown with an increase in the N dose and NO_3^- concentration in the irrigation water. This trend was also evident in the final diameter that was measured 10 months after the treatment started, although the trend was not as clear as that of the height growth. These results are in contrast to most other studies on N application to coniferous seedlings, where growth tends to increase in reaction to an increase of N availability (Richards, 1965; van den Driesche, 1971; Crous *et al.* 1995), which may be related to nutrient deficiencies.

The shoot biomass decreased progressively towards the 150 kg N ha^{-1} equivalent treatments. The decrease in growth was most pronounced in the 150 kg N ha^{-1} equivalent 6:1 NO_3^-:NH_4^+ ratio treatment, consisting of the highest N concentration, with six times more

NO_3^- than NH_4^+. This, combined with the root biomass trends resulted in an increase in the root:shoot ratio that is related to the concentration of N in the irrigation water.An increase in the availability of N and P, the nutrients most often in short supply, leads to an increase in the root:shoot ratio of trees, while a decrease in N and P or a deficiency of other essential elements would lead to a decrease in the root:shoot ratio (Ingestad, 1991).

Fine root biomass was negatively correlated with stand N status in coniferous forests in Europe (Gundersen *et al.* 1998). In this study application of N to *P. patula* seedlings increased the root:shoot ratio of the seedlings, although this was significant only in the 150 kg N ha^{-1} equivalent treatments. There is also a strong correlation between the N:P ratio and the increase in the root:shoot ratio at high levels of N application. This suggests that P could be a limiting factor to the growth of *P. patula* seedlings in this experiment and that the soils have inherently high N availabilities. This is further supported by the increase in root:shoot ratios, a trend which is frequently observed under conditions of P limitation (Ericsson, 1995).

However, mere disruption of the N:P ratio due to a unilateral increase in the N levels in coniferous trees has also been linked to growth decreases (Mohren *et al.* 1986). In the field, decreased root biomass in N saturated sites could exacerbate nutrient imbalances and nutrient deficiencies (Aber *et al.* 1989).

The growth patterns exhibited by *P. patula* seedlings in this study are consistent with a N-induced nutrient deficiency that is exacerbated at high N levels (Aber *et al.* 1989; Köllig *et al.* 1997). No additional nutrients were added to the soil during the treatment period, leading to possible nutrient deficiency after all needs for N were fulfilled. This has been shown in a number of studies which have reported deficiencies in essential elements after high N levels were applied to conifer seedlings. Köllig *et al.* (1997) found that high levels of N applied to *Picea*

abies led to a reduction in shoot growth at high N levels while Mg deficiencies were induced in the high N treatments. Even if Mg is not limiting, a mere disruption of optimal N:Mg ratios can contribute to growth declines (Oren *et al.* 1988). In this study, no significant trends in base cation nutrition were observed that could be attributed to the addition of N to the seedlings. Trends in foliage total N exhibited a significant increase in N with the dose of N applied, while total P levels did not show consistent trends. However, the results of these changes in nutrition show a significant increase in the N:P ratio, although this was only significant in the 150 kg N ha^{-1} equivalent treatments. Poor growth of *Pinus radiata* seedlings in a field trial following application of N and P were attributed to an imbalance in the N:P ratio, while a combination of the two fertilizers provided a better balance (Nielsen *et al.* 1984).

Phosphorus is frequently in short supply in forest ecosystems (Waring and Schlesinger, 1985). This is also the case in South African forestry soils, especially in the Southern Cape (Payn and Clough, 1988), but also in the Escarpment forestry areas where application of P fertilizers to the soil increases growth of *P. patula* up to $1m^3$ per year (Schutz, 1975). It is clear that the N:P ratio of seedlings in all the treatments as well as the control was well in excess of the optimum for plant growth, taken to be between 12 and 16 (Koerselman and Meuleman, 1996). Despite having a mean N:P ratio of more than 20, the seedlings of the control did not show any signs of chlorosis at the end of the experiment. The growth rate of the seedlings over the course of the experiment was comparable to other experiments on temperate conifers in South Africa (Donald and Young, 1982; Noble and Schumann, 1993; Crous *et al.* 1995). Most of the growth parameters were correlated with the N:P ratio, emphasizing the crucial role the N:P ratio plays in the growth of the seedlings. Poor growth of *P. radiata* seedlings after application of N was related to an imbalance in the N:P ratio, which can be remedied by application of both N and P

(Nielsen *et al.* 1984). Aber *et al.* (1989) hypothesized that plant P levels will decrease in N saturated sites. For example, N-induced P deficiency has been shown in mature Douglas-fir exposed to high levels of N deposition (Mohren *et al.* 1986). High foliar N levels have been negatively correlated with low P levels (Poth *et al.* 1991), while N saturation can be induced in trees exposed to high levels of N deposition by other elements such as P or base cations being in short supply. Nitrogen saturation may be reversed by application of limiting nutrients to forest stands (Mohren *et al.* 1986; Stevens *et al.* 1993). High N:P ratios have been shown in the field and have been shown to be affecting forest productivity (Mohren *et al.* 1986; Ericsson *et al.* 1993).

The controlling influence of the N:P ratio on forest productivity is further illustrated by the results obtained in *P. elliottii* in the Southern Cape region of South Africa by Wienand and Stock (1995). In contrast to the situation of control of nutrient turnover by P and N induced P deficiency, the reverse have been found, where application of P to these P deficient soils has led to a reduction in productivity and nutrient turnover (Wienand and Stock, 1995). This resulted in mid-rotation deficiency of N, exacerbated by the application of P.

Available P in the soil of *P. patula* stands examined in this study was low and gets progressively lower with an increase in age of the trees on the stand (Chapter 4). Although none of the mature trees in the field show any signs of P deficiency and suboptimal N:P ratios were only found in 1996, these optimal P concentrations are only maintained by internal retranslocation of P (Chapter 3). The additional factor of reduced P availability in some clay-rich soils will have a synergistic effect on the N:P ratio. The main effect of the change in the N:P ratio in the seedlings was an increase in the root:shoot ratio. Both the trends in N:P ratio and the root:shoot ratio were correlated with foliage colour at the end of the experiment, suggesting

common causal factors involved. This implies that the photosynthetic capacity of the seedlings was probably inhibited by N-induced P deficiency. This has severe consequences for industrial pine plantations in South Africa that are exposed to N deposition, not only because P is generally very low, but also because it has already been shown that these forests are exhibiting symptoms of N saturation (Chapter 3) and this N saturation will be exacerbated by P limitations to growth.

No mortality was found in response to treatment effects, even at the high level of 150 kg N ha^{-1} equivalent. At this level of N application, however, all the seedlings exhibited signs of chlorosis. Similar results have been found with *Picea abies* in Germany, which was found to be due to Mg limitation (Köllig *et al.* 1997). Nitrogen pollution in Europe has lead to nutrient imbalances in forest trees in the field such that the growth of the trees is eventually negatively affected. Magnesium has been identified in the field as well as in seedling experiments as the element most likely to be deficient in high N sites (Köllig *et al.* 1997). In this experiment base cation deficiency could not be induced by varying the dose of N applied or by varying the ratio of NO_3^- to NH_4^+.

Colour analysis can be a useful indicator of nutrient deficiencies (Crous *et al.* 1995) and has been positively linked to nutrient deficiencies in conifer seedlings (Melville and Atkinson, 1985). In the current study, the measurement of colour variation among the treatments was highly correlated to most growth parameters, an indication of the relationship between growth and photosynthetic activity in that the colour of the upper crown leaves was well correlated with growth parameters and was an accurate reflection of overall seedling health.

Application of N did not change the cation nutrition of the seedlings significantly, except for the K concentration, which increased significantly ($p<0.05$), and concomitantly with an increase in the NO_3^- concentration in the irrigation water. Although absolute changes in the

base cation concentrations did not take place, high N levels in the 150 kg N ha^{-1} equivalent treatments increased the ratio of N to base cations. Base cation deficiencies have been shown to contribute to decreases in growth in conifer seedlings (Oren *et al.* 1988). This is consistent with trends found in the field experiment, where applications of N did not change the cation composition of the trees significantly. Phosphorus, rather than cations, is in short supply in South African forestry soils (Payn and Clough, 1988). However, this situation could change with continuing high inputs of N, with base cations also becoming limiting.

Soil nutrient concentrations were largely unaffected by N applications, the only exception being K concentration. The high K and NO_3^- concentrations in the soil of treated pots contributed to the trend of increasing electrical conductivity (EC, in mS) seen in the treatments. Soil pH remained unaffected by high N application, despite suspected high nitrification rates in the 50 kg N ha^{-1} equivalent with NO_3^-:NH_4^+ of 1:1 and the 50 kg N ha^{-1} equivalent with NO_3^- :NH_4^+ of 6:1. Nitrate increases soil pH, while NH_4^+ decreased the soil pH and also led to base cation leaching (Wilson and Skeffington, 1994a). In this study the application of N over a period of 10 months did not affect the pH of the soil significantly.

While the reasons underlying the poor growth of *P. patula* at high NO_3^- concentrations can be related to a N:P ratio imbalance, the primary cause could be a change in the mycorrhizal biomass and/or the mycorrhizal composition in the 150 kg N ha^{-1} equivalent treatments as opposed to the control and 50 kg N ha^{-1} equivalent treatments. Holopainen and Heihonen-Tanski (1992) showed that the number of mycorrhizal rootlets on *Pinus sylvestris* seedlings decreased at high NO_3^- concentrations, while application of NH_4^+ did not significantly affect the mycorrhizal biomass. This would have significant effects on the P and possibly the N

nutrition of seedlings. Mycorrhizas are essential for optimal P nutrition of conifer seedlings, while it has been shown to be beneficial for N nutrition (Dighton and Skeffington, 1987; Dames *et al*, 1999a). In this seedling experiment deceases in mycorrhizal biomass were not found for *P. patula*, however, compositional changes in mycorrhizal biomass have been shown in the field experimental component of this study (Chapter 6).

This experiment highlights the nutritional consequences of excess N on *P. patula* seedlings and the possible effects that excess N will have on growth and nutrition of *P. patula* trees in the field. These results show that high additions of N to *P. patula* trees growing on shale soils will disrupt N and P nutrition to the extent that losses in productivity will result, as suggested by Aber *et al.* (1989). Chronic inputs of N into *P. patula* forests, many of which are situated on highly dystrophic shale soils, will serve to exacerbate these P deficiencies, while possibly also contributing to base cation leaching. While it has been shown that NH_4^+ can enhance the uptake of phosphate (Campion, 1998), excess NO_3^- in the soil will at best not influence P uptake, and may reduce P availability (Carriera *et al.* 1997). A continuing trend of the current high NO_3^- over NH_4^+ deposition will lead to P deficiencies in *P. patula* forests. Other trends such as the removal of biomass in the form of logs and reduced decomposition rates at high altitudes, which are also exposed to the highest N deposition rates, will further decrease the P stocks available in pine plantations. Although none of the mature trees in the field showed any signs of P deficiency and suboptimal N:P ratios were only found in 1996, these optimal P concentrations are only maintained by internal retranslocation of P (Chapter 3). Results from the seedling experiments, although not directly applicable to the field, identified the N:P ratio as a potentially crucial controlling factor of conifer growth in the Drakensberg escarpment forestry

area. The continuing inputs of N into conifer ecosystem will disrupt this ratio, merely by increasing the concentration of N relative to P.

Chapter 9

Implications of N deposition for forest sustainability in the Drakensberg escarpment *P. patula* forests

Nitrogen deposition globally and locally

The environmental consequences of high levels of anthropogenic N on sustainability and productivity in forest ecosystems include NO_3^- leaching, nutrient disorders, decline in growth and ultimately, forest dieback. Various stages of decline have become apparent in some northern hemisphere ecosystems, such as *Picea abies* forests in Germany (Oren *et al.* 1988), *P. sylvestris* forests in the Netherlands (Mohren *et al.* 1986), dry conifer forests of western North America (Fenn *et al.* 1998) and vast tracts of mixed conifer and deciduous forests North America (Aber *et al.* 1991). A common denominator in these forest ecosystems is an open N cycle, with N in excess of plant and microbial demand, a process described by Aber *et al.* (1989) as N saturation. In some of these ecosystems N deposition is coupled with high levels of S pollution, which have the effect of acidifying the soil, while the excess N leads to further acidification through an increase in nitrification.

Rapid industrial development and concomitant high levels of air pollution is leading to increased levels of anthropogenic N and S to be deposited in natural and commercial forest ecosystems in South East Asia, Africa and South America (Fowler *et al.* 1999). In South Africa the major source of N is from industrial activity in a relatively small area, surrounded by agricultural croplands and rangelands, grasslands, commercial forests and small areas of natural forests (Held *et al.* 1996). High levels of anthropogenic S are also produced in the same area and add to the cocktail of pollutants that get trapped in a very stable layer over the Southern African

continent during winter (Scholes and Scholes, 1999). A substantial portion of N deposition is also derived from biogenic and pyrogenic emissions from surrounding grassland and savanna ecosystems (Scholes *et al.* 1996, Scholes and Scholes, 1999).

The concept of N saturation in context

Fenn *et al.* (1998) and Gundersen *et al.* (1998) reviewed the N saturation hypothesis put forth by Aber *et al.* (1989) and showed that N deposition in excess of plant and microbial demand can impact on the growth and nutrition of forests, with further consequences for sustainability of the forest ecosystems involved. In the early stages, production will benefit from additional N (Aber *et al.* 1989; See Fig. 1.1). This type of response is consistent with the trend found by Spiecker *et al.* (1996) in an analysis of the growth trends in European forests. Many forest ecosystems benefited from moderate addition of N deposition, and grew faster; other forests showed no response, while some showed a negative response. Some of the latter forests occur in regions experiencing high N inputs. The responses exhibited by these forests approximate the hypotheses of Aber *et al.* (1989) regarding the sequential stages of N deposition. According to these hypotheses the beneficial effect of low doses of N to N-limited ecosystems in stage 1 will eventually be replaced by a shift in carbon allocation away from the roots, to be followed later by a decrease in aboveground biomass in stage 3, mainly due to nutrient imbalances. In addition, the N cycle becomes more open, with NO_3^- being lost from the ecosystem, maily due to increased nitrification and a reduced ability to hold N. In a new addition to the original hypothesis, Aber *et al.* (1998) suggested that the increase in nitrification and reduction in N retention may be the consequence of a loss in mycorrhizal biomass as N saturation progress.

The basic tenets of the N saturation hypotheses put forth by Aber *et al.* (1989) has been tested in several ecosystem types exposed to elevated N and the basic concepts describing N saturation has remained remarkably robust (Fenn *et al.* 1998; Gundersen *et al.* 1998). It has been found that the ultimate outcome of N deposition in different forest ecosystems is dependent on the biological and physical characteristics of the ecosystem exposed to high N levels. Different species composition and environmental conditions combine to result in different temporal and spatial expressions of N saturation symptoms. Most experimental evidence of N saturation has been gathered in the Northern Hemisphere. The environmental conditions in the Northern Hemisphere forests exposed to N saturation may be sufficiently different from *P. patula* plantation ecosystems in South Africa to suggest that direct extrapolations are equivocal. The review of Fenn *et al.* (1998) also suggests that many of the severe symptoms of N deposition, such as forest dieback, may never manifest in drier forests such as those found in the Western United States. This is the result of the differences in climate, such as higher ambient temperatures and highly seasonal rainfall trends, resulting in longer growing seasons and higher growth rates in these ecosystems. These ecosystems approximate conditions similar to the *P. patula* ecosystems in South Africa and it is clear that at least some of the more severe symptoms of N saturation, such as forest dieback, may not manifest in South African plantation ecosystems, although other symptoms may be present.

In addition to high levels of N introduced aerially into the ecosystem, the *P. patula* forests of the Escarpment also seem to be predisposed to N saturation, having relatively low levels of P, especially in the older stands (Chapter 4) and a relatively large overall N pool (Nowicki, 1997; Chapter 4 of this thesis). Shale and quartzite soils, which are the growing medium for a significant portion of the *P. patula* forests in the Drakensberg escarpment, are

relatively sensitive to acidification by S and N (Olbrich, 1995). Forest growth has also been shown to increase soil acidification by immobilization of base cations in standing biomass and export of base cations in harvests (Scholes and Nowicki, 1996; Scholes and Scholes, 1999). Loss of base cations is exacerbated by the accumulation of litter at high elevations and immobilization of base cations, especially Ca, and to a lesser extent, Mg (Morris, 1995; Dames *et al.* 1999b). While neither P, Ca nor Mg have been shown to be acutely deficient to growth of *P. patula*, the compounded effect of these routes to immobilization is expected to manifest itself in near the future.

The effect of simulated N deposition on *P. patula* forests in Mpumalanga

The objective of this thesis was to study changes that occur in ecological functioning of a *P. patula* plantation ecosystem during and after N availability in the ecosystem had been increased by applying NO_3^- and NH_4^+ to experimental plots.

The growth of *P. patula* in from Mpumalanga has not benefited from the addition of N, except for the very young and vigorously growing trees, which have the highest growth rate. This suggests that future inputs of N will benefit the younger, fast growing stands most. However, in all sites, the level of N in the leaves increased over the course of three years of N application, leading to increased N:P and N:cation ratios. With the demonstrated immobilization of Ca and Mg in the litter accumulating in the higher sites (Morris, 1995), export of base cations in harvested wood and the relatively low levels of plant available P, this suggests that growth may be inhibited by supra-optimal N:base cation and N:P ratios as more N is added to the ecosystem. Thus, while additional N will increase growth rates in reactive sites, biomass accumulation will eventually stabilize and ultimately decline as nutrient deficiencies take hold,

as predicted by Aber *et al.* (1989). These trends will take hold in the older sites before the younger sites, especially with the demonstrated low plant available P levels in the soil of those sites. Although the young trees benefited from increased N availability, the C allocation pattern seen in the young trees seems to suggest that additional N may not only disrupt the N:P balance, but may not benefit tree growth as a whole. This unusual allocation pattern may be the direct result of the change in N:P ratio when NO_3^- and NH_4^+ was applied, although adequate proof for this is lacking. A graphical summary of the effects of applied N on *P. patula* of different ages can be found in Fig. 9.1.

The trends in the nutrient vector analysis are well correlated to changes in nutrition and growth in the next growing season (Timmer and Stone, 1978; Proe *et al.* 1999). The trends observed in this study (Chapter 3) indicate possible N induced skewing of the N:P ratio of these trees at the expense of sustained productivity in the long term. The importance of the relationship between N and P to control growth of *P. patula* is supported by the results obtained in the seedling experiment, using shale-derived soil from the Mpumalanga forestry area as growing medium. The more N added to the seedlings, the lower the growth rate, measured by height and stem diameter, as well as by biomass parameters. This growth trend has a direct positive relationship with the increase in the N:P ratio, suggesting that the increase in N:P ratio with sustained high levels of N input will lead to a reduced growth rate. This is consistent with the hypotheses set forth by Aber *et al.* (1989), and suggests that these forests have already progressed beyond the first stage of N saturation.

In addition to soil-mediated changes in tree nutrition, N deposition may also influence nutrition through induced changes in mycorrhizal population structure. Simulated N deposition did not change the size of the mycorrhizal population, but the composition of the population was

altered (Chapter 6). This may be related to the relative preferences of different morphotypes for NO_3^- and NH_4^+. The relative contributions of the affected morphotypes to nutrition of the tree are not known, but continued N inputs may change the nutritional relationships in the tree. The effect of N deposition on mycorrhizal-plant interactions in different forest ecosystems has been identified as one of research needs for the continuation of N saturation research in dry conifer forests (Fenn *et al.* 1998).

The high levels of N in the upper mineral soil and foliage of standing trees in *P. patula* plantations of the escarpment area found in this study is suggestive of a relatively large pool of N in the pedosphere, a conclusion that is confirmed by the high levels of NO_3^- leaching in this area that was found in a previous study (Nowicki, 1997). This could be indicative of a combination of a high rate of nitrification in these forest soils, high rate of N inputs and low retention of N. These conclusions are also reflected by the low C:N ratios in the litter layer of all three sites, a characteristic recognized as one of the factors that predispose forests to N saturation (Fenn *et al.* 1998). In this study the litter C:N values were in the region of 25, a value suggested by Gundersen *et al.* (1998) as the breakthrough point for NO_3^- leaching. This is borne out by the presence of NO_3^- leaching in all three sites in the unfertilized controls, and the rapid response of NO_3^- leaching to application of NO_3^- and NH_4^+ (simulated N deposition). Seen in conjunction with the high levels of NO_3^- in streams draining the escarpment forestry area (Nowicki, 1997), this suggests that a significant portion of the pine plantations is N saturated or approaching N saturation. Application of N to the three sites increased the rate of nitrification, and the increase in microbial activity can also be seen in the increased decomposition of a low N substrate (cotton strips).

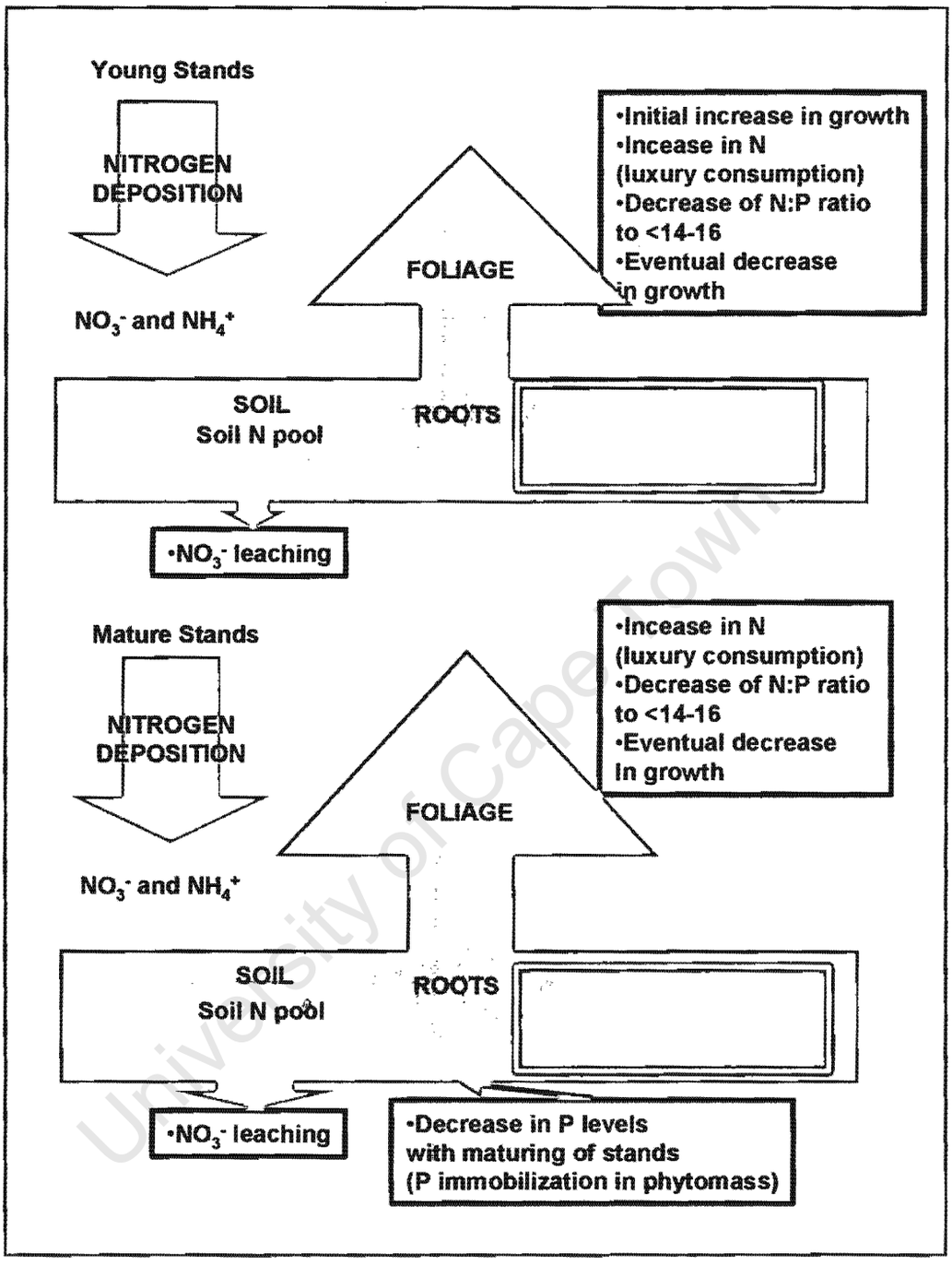

Fig. 9.1 *Summary diagram showing the effects of N deposition on* P. patula *ecosystems of different ages. The first section shows the effect of N deposition on young stands and the second section those on mature stands. The components of the ecosystem and effects of stand development are annotated in black, while the effects of N deposition on the ecosystem is annotated in blue.*

It can be concluded that these forests are approaching the breakthrough point suggested by Gunderson *et al.* (1998). Any further increases in the rate of nitrification could lead to mobilization of this pool of N and further contribute to the volume of NO_3^- leaching into the streams. Longer-term inputs to these ecosystems will also improve litter quality, leading to potential increases in decomposition rates and litter turnover, releasing more N and opening up the N cycle even more.

The pH of forestry soils in the Drakensberg escarpment forestry is generally low. Application of N did not change the pH of the topsoil significantly, in contrast to several other studies that showed a reduction in pH related to increased nitrification rate and stripping of cations due to the leaching of NO_3^- from the mineral soil. This suggests that there is enough buffering capacity currently to prevent acidification of the soil, and only in areas with highly elevated N rates in combination with low base cation levels is this likely to change. However, an increase in the level of nitrification may speed up the rate of acidification in these soils, mainly due the extra proton produced.

Although the transient nature of N saturation in simulated N saturation experiments in general is recognised (Cannell and Thornley, 2000), the control plots on the *P. patula* sites showed enough evidence to suggest that even in the natural state, these ecosystems are approaching N saturation.

Indicators of N saturation in forest ecosystems

With N saturation becoming a reality in many forest ecosystems, a concerted effort is needed to develop indicators of N saturation that can be used to show the N saturation status of forest ecosystems without time-consuming and intricate experimentation. Results from this thesis

show that foliar nutrient vector analysis may prove a good indicator for monitoring N dynamics in forest ecosystems prone to N saturation. The trends obtained using vector analysis are supported by other indices of growth and nutrition of *P. patula*, and supports the work done by Kiefer and Fenn (1997) in the dry conifer forests of California. The basic indices of growth and nutrition can also be used to indicate N saturation, as shown by the good collaboration with an integrative measure such as vector analysis. However, the combination of the different techniques may provide insight that may not be gained if the individual techniques are used.

In addition, the C:N ratio of the litter layer in this study shows trends that are very similar to those found in other forests exhibiting various stages of N saturation (Gundersen *et al.* 1998). Based upon the results obtained by Gundersen *et al.* (1998), the C:N ratios in the *P. patula* ecosystems investigated reflect a high level of N loading, and together with previous work showing high levels of NO_3^- in the streams draining the area (Nowicki, 1997), this is indicative of N saturation. Monitoring of NO_3^- levels in streams draining afforested areas will be an essential first warning of N saturation, while monitoring of the rate of nitrification will be an good indicator of impending 'breakthrough' of NO_3^- leaching.

Examination of the trends between the ^{15}N signatures of the soil and leaves (enrichment factor, ε) of the control plots revealed an inconsistency that can be attributed to the influence of forest site preparation and other forms of disturbance. This is in contrast to the results obtained by Gundersen *et al.* (1998), who found the enrichment factor to be a good indicator of N saturation. The use of ε as an indicator N saturation in *P. patula* ecosystems is thus not recommended, but further study is needed to evaluate its suitability in a larger sample set.

The amino acid arginine accumulates in conifers exposed to high N deposition and is currently being developed as an indicator of high N status in Europe (Näsholm *et al.* 1997) and

North America (Fenn *et al.* 1998), but needs experimental study to evaluate the use of this indicator in South Africa.

Causes of N saturation in South African *P. patula* ecosystems

The cycling of N in the Drakensberg escarpment has been the subject of other studies such as that of Dames (1996) and Nowicki (1997), the latter investigating the differences in soil chemistry between pine plantations and adjoining grasslands. Historically, the vegetation of the escarpment area, from the southern Drakensberg in KwaZulu-Natal to Mpumalanga and some parts of the Nothern province consisted of acid grasslands, with rapid turnover of N and other nutrients (Scholes and Scholes, 1999). These grasslands were replaced by plantation forests, which slowed down the annual rate of cycling of N, but increased the amount of N participating in cycling, thus increasing the potential of opening up the N cycle when disturbed. Any further increase in N from anthropogenic sources would increase the disturbance in N cycling and increase the risk of a breakthrough in NO_3^- leaching.

Nowicki (1997) investigated the dynamics of acid anions in the afforested areas of South Africa and measured elevated leaching of NO_3^- in the commercial forests of Mpumalanga. It has been suggested that the elevated level of NO_3^- in the streamwater of the escarpment in Mpumalanga is due to this combination of more the conversion of grasslands to tree crops and the influence of management of the tree crops on N cycling, such as suggested by Vitousek (1981). However, the work of Nowicki (1997) showed that the level of NO_3^- is highest in the Mpumalanga Drakensberg escarpment, which begs the question why these high levels have not been found in other forestry regions in the escarpment. One possible explanation is that the geology in the Mpumalanga Drakensberg escapment is different, but in addition, the area also

receives considerable annual inputs of N in the form acid precipitation. This N would immeadiately become part of the N cycle, and the fact that most of this anthropogenic N is in the form of highly mobile NO_3^-, would be very prone to leaching. By virtue of the close proximity of the Mpumalanga Drakensberg escarpment and the higher biomass of trees compared to grassland, the forests receive more N from anthropogenic sources (Held *et al.* 1996). This, seen with the relatively high levels of NO_3^- in the streamwater in the area suggests more than a mere casual relationship with the symptoms of N saturation reported.

Potential for N saturation in other forestry regions in South Africa

The forestry areas to the west of the Drakensberg escarpment forestry area in Mpumalanga are exposed to high levels of N deposition, mainly due to their proximity to coal-fired power stations. These forests are growing on soils that are relatively less sensitive to acidification, such as granites and basalts and are relatively more buffered to acidification than those in the escarpment (Olbrich, 1995). Thus, while soil-based effects of N deposition may not manifest in the short to medium term, purely as a consequence of the higher exposure to N (and S) deposition, physical and chemical injuries are expected to develop relatively soon.

The high-lying region of KwaZulu-Natal is one of the areas where pollutants are released from the air mass circulating over the Southern African subcontinent (Scholes and Scholes, 1999). The commercial forests are exposed to anthropogenic N deposition, although relatively lower levels than those measured in the commercial forests of Mpumalanga. The KwaZulu-Natal forests consists of *P. patula* plantations as well as various cold-tolerant *Eucalyptus* species and are established on shale-, mud- and sandstone-derived soils. The *P. patula* stands may react in much the same way as those stands in Mpumalanga, although it is expected that N

breakthrough may take longer because of the lower levels of N entering these ecosystems, or may not develop at all due to the lower N inputs. Internationally relatively little is know about the effects of excess N on growth and nutrition of angiosperms like *Eucalyptus* species. However, it is known that *Eucalyptus* spp. are more efficient in obtaining nutrients than conifers (Attiwill and Adams, 1996) and owing to their faster growth rate, probably more nutrient use efficient. By immobilizing nutrients in this way, *Eucalyptus* plantations are thus expected to use the additional N to greater effect than pines, possibly delaying the onset of N saturation.

Nitrogen saturation and the critical load concept

A critical load is a quantitative estimate of exposure to one or more pollutants below which significant harmful effects on sensitive elements of the environment do not occur (Hornung, 1994; Sverdrup and de Vries, 1994). The basis of this approach is to define the pollutant threshold above which harmful effects on a sensitive receptor are caused. This approach will aid in the development and implementation of policy to keep deposition levels below the critical load suggested for the most sensitive ecosystem in a particular region.

Critical loads for N as a nutrient are determined using a mass-balance approach. In most European forest ecosystems this value range from 10-30 kg N ha^{-1} yr^{-1} (Hornung, 1994). While values for the determination of critical loads are currently lacking for South African plantation forests, the results obtained in this study suggest that it has been exceeded substantially by N additions of 120 kg N ha^{-1} yr^{-1}. All three sites showed supra-optimal N:nutrient ratios, low C:N ratios, high levels of NO_3^- leaching and an increase in nitrification. In addition, the lack of growth response to added N seem to suggest significant negative changes in the ecosystem that can be contributed to changes in N cycling due to anthropogenic N cycling, possibly coupled

with transformations of the N cycle due to the conversion of natural grasslands to commercial forests. These factors may work in combination to predispose *P. patula* ecosystems in the Mpumalanga escarpment to N saturation and lead to relatively low critical loads.

A major factor that will influence the value of a critical load for a particular ecosystem is the incidence of nutrient limitations in the ecosystems (Harrison *et al.* 1995). In the presence of low plant available P levels in the oldest sites in *P. patula* stands, it could be argued that the critical load for N as a nutrient for this site should be significantly lower than determined using the basic mass balance approach. In cases where the mass balance approach has been modified by including nutrient limitations in the calculation, values of between 3 and 19 kg N ha^{-1} yr^{-1} have been obtained for European ecosystems, significantly lower than that suggested by the mass balance approach (Reynolds *et al.* 1998). It could thus be argued that the critical load for N as a nutrient have already been exceeded for *P. patula* stands growing in the escarpment area, especially in older stands. The value of critical loads for N as a nutrient has been demonstrated in the rapid recovery of ecosystems after the deposition of N has been reduced to pre-industrial levels (Boxman *et al.* 1995; Boxman *et al.* 1998a).

Future directions for research

This study has demonstrated the phenomenon of N saturation in *P. patula* plantations of the Drakensberg escarpment. Previous research showed elevated NO_3^- levels in forests streams in Mpumalanga, but not in other afforested areas on the eastern seaboard of South Africa (Nowicki, 1997). Collectively, these studies highlight the need to extend ecosystem monitoring to a larger area in the escarpment, covering the whole area affected by air pollution. However, due to the extremely high costs of ecosystem manipulation experiments such as this one, the use of

indicators such as foliage total N, N:P ratios, foliage vector analyses, NO_3^- leaching (streamwater monitoring) and C:N ratios of the organic soil layer need to form part of a structured research and monitoring programme. The development of an N saturation monitoring programme will be assisted by current initiatives to establish sustainable forestry programmes by the major forestry companies,and this will provide an institutional framework for an N saturation monitoring programme. More rapid indicators need to be developed for use in a permanent monitoring programme that need to involve the major role player such as the forestry land owners, the government and research organizations.

More research is needed to develop the suitability of foliage vector analysis, organic soil C:N ratios and arginine accumulation in foliage as indicators of N saturation in other pine spp. and *Eucalyptus* plantation ecosystems. In addition, the effect of high N availability on mycorrhizas commonly found on pine root in South Africa need to be investigated further in the light of the variable NO_3^- and NH_4^+ preferences found by Dames *et al.* (1999a) and changes in mycorrhizal population found after simulated N deposition in this study. The use of the enrichment factor ε, has only been studied in three sites of variable ages in this study. It is suggested that the trends in $\delta^{15}N$ of soils and trees exposed to N deposition need to be studied over a larger sample size.

The effects of N on whole plant ecophysiology such as water use and hydraulic properties of stems have not received attention in this study. It has been showed that increasing the levels of N may influence plant water relationships in declining stands negatively (Oren *et al.* 1988). Conversely, an increase in the growth rate of younger sites and sites on the periphery of the affected areas may increase the water use of trees, since larger trees transpire more due a larger transpiring surface. Seen against the background of the scarcity of water in South Africa studies

need to be conducted to investigate these aspects. Differences in water use due to changes in nutrient availability in the ecosystem may induce changes in the hydraulic and anatomical properties of the stem of trees exposed (Zimmerman, 1978). A synergistic effect between N, P and available water on xylem anatomical characteristics has been shown for *Eucalyptus globulus* fertilized with N and P fertilizer (Raymond and Muneri, 2000). With the current emphasis on producing wood with specific quality characteristics, it is imperative that the effects of high N levels and skewed N:P ratio on wood fibers be investigated. The synergy between elevated CO_2, elevated temperature and elevated N availability has been shown in European countries (Spieker *et al.* 1996), and while global change scenarios for forestry in South Africa have been developed (Kunz *et al.* 1995), this did not include changes in forest nutrient cycling induced by atmospheric deposition.

Conclusions

In conclusion, the results presented in this thesis show that chronic high N deposition in the Drakensberg escarpment will lead to progressive N saturation of the pine plantations. The unfertilized plots used as controls for the field experiment show symptoms of N saturation that may be the consequence of high N deposition measured in the area, combined with the alteration of the N cycle with the transformation of natural grasslands to commercial plantations. As further evidence of N saturation these sites exhibited a rapid reaction of soil water NO_3^- levels to added N. This will be exacerbated by an increase in the nitrification rate, with adverse effects for stream water quality. Seen in conjunction with previous studies that documented relatively high levels of NO_3^- in the streams draining the escarpment (Nowicki, 1997) this suggests that some commercial plantation areas may already be N saturated, or approaching N saturation.

Nitrogen saturation is a considerable threat to productivity of pine forests in the Drakensberg escarpment, principally because of the reduction in optimal N:nutrient ratios through luxury uptake of N, and, eventually reducing growth. The optimal nutrient balance required for growth may be further disturbed by changes in mycorrhizal population composition, which were demonstrated in this study. The younger sites have the largest capacity to accommodate and use increased N in the ecosystem, mainly due to faster biomass accumulation in the young trees. In contrast, older forest has higher scavenging capabilities and therefore higher deposition of N to these sites. In addition, older sites also have low P levels, which predispose these sites to N saturation. It is recommended that indicators for N saturation, such as annual vector analyses determination, C:N ratios, growth dynamics and determinations of N inputs to different ages of pine plantations be incorporated into site sustainability programs for these forests.

References

Aber JD (1992) Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. *Tree* **7**: 220-224.

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. *Bioscience* **39**: 378-386.

Aber JD, Melillo JM, Nadelhoffer KJ, Pastor J, Boone RD (1991) Factors controlling nitrogen cycling and nitrogen saturation in northern temperate forest ecosystems. *Ecological Applications* **1**: 303-315.

Aber JD, Magill AH, Boone RD, Melillo JM, Steudler PA, Bowden RD (1993) Plant and soil responses to chronic nitrogen additions at the Harvard forest, Massachusetts. *Ecological Applications* **3**: 156-166.

Aber JD, Magill AH, McNulty SG, Boone RD, Nadelhoffer KJ, Downs M, Hallett R (1995) Forest biogeochemistry and primary production altered by nitrogen saturation. *Water Air and Soil Pollution* **85** : 1665-1670.

Aber J, McDowell W, Nadelhoffer K, Magill K, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernadez I (1998) Nitrogen saturation in temperate forest ecosystems. *Bioscience* **48**: 921-934.

Adams MA, Attiwill PM (1982) Nitrate reductase activity and growth response of forest species to ammonium and nitrate sources of nitrogen. *Plant and Soil* **66**: 373-381.

Adams MA, Attiwill PM (1983) Patterns of nitrogen mineralization in 23-year-old pine forest following nitrogen fertilization. *Forest Ecology and Management* **7**: 241-248.

Adams MB, Campbell RB, Allen HL, Davey CB (1987) Root and foliar nutrient concentrations in loblolly pine: effects of season, site, and fertilization. *Forest Science* **33**: 984-996.

Ågren GI, Bosatta E (1988) Nitrogen saturation of terrestrial ecosystems. *Environmental Pollution* **54**: 185-197.

Alef K (1995) Nitrogen mineralization in soils. *In*: Alef K, Nannipieri P (Eds.), *Methods in Applied Soil Biology and Biochemistry*. Academic Press. 608 p.

Alef K, Nannipieri P (1995) Methods in Applied Soil Biology and Biochemistry. Academic Press. 608 p.

Alexander IJ (1983) The significance of ectomycorrhizas in the nitrogen cycle. *In*: Lee JA, McNeil S, Rorison IH (Eds.) *Nitrogen as an ecological factor.* Blackwell, Oxford.

Alexander IJ, Fairley (1983) Effects of N fertilization on populations of fine roots and mycorrhizas in spruce humus. *Plant and Soil* **71**: 49-53.

Alexander IJ, Fairley RI (1986) Growth and nitrogen uptake rates of ectomycorrhizal spruce seedlings. *In* Gianinazzi V, Gianinazzi S (Eds) *Physiological and genetic aspects of mycorrhizae.* Proceedings of the 1st European Symposium on Mycorrhizae, Dijon, France.

Arnebrand K, Söderström B (1992) Effects of different fertilizer treatment on mycorrhizal colonization potential in two Scots pine forests in Sweden. *Forest Ecology and Management* (in press).

Arnold B (1992) Soil acidification as caused by the nitrogen uptake pattern of Scots pine *(Pinus sylvestris).* *Plant and Soil* **142**: 41-51.

Attiwill PM, Adams MA (1993). Nutrient cycling in forests. Tansley Review No. 50, *New Phytologist* **124**: 561-582.

Attiwill PM, Adams MA (1996) Nutrition of Eucalypts. CSIRO Publishing.

Berendse F (1987) The effect of lignin and nitrogen on the decomposition of litter in nutrient-poor ecosystems: a theoretical approach. *Canadian Journal of Botany* **65**: 1116-1120.

Berg B, Ekbohm G (1983) Nitrogen immobilization in decomposing needle litter at variable carbon: nitrogen ratios. *Ecology* **64**: 63-67.

Bielek P (1998) Nitrate in nature. *Environmental Pollution* Special Issue: Nitrogen, the Confer-N-s, First International Nitrogen Conference, Noorwijkerhout, The Netherlands, 23-27 March, 1998. pp. 527-530.

Bigg WL, Daniel TW (1978) Effects of nitrate, ammonium and pH on the growth of conifer seedlings and their production of nitrate reductase. *Plant and Soil* **50**: 371-385.

Binkley D (1985) Forest Nutrition Management. Wiley-Interscience.

Binkley D, Högberg P (1997) Does atmospheric deposition of nitrogen threaten Swedish forests? *Forest Ecology and Management* **92**: 119-152.

Bledsoe CS, Zasoski RJ (1983) Effects of ammonium and nitrate on growth and nitrogen uptake by mycorrhizal Douglas-fir seedlings. *Plant and Soil* **71**: 445-454.

Bouyoucos GJ (1962) Hydrometer method improved for making particles and size analysis of soils. *Agronomy Journal* **54**: 464-465.

Boxman AW, Van Dam D, Van Dijk HFG, Hogervorst RF, Koopmans CJ (1995) Ecosystem responses to reduced nitrogen and sulphur inputs into two coniferous stands in the Netherlands. *Forest Ecology and Management* **71**: 7-29.

Boxman AW, van der Ven PJM, Roelofs JGM (1998a) Ecosystem recovery after a decrease in nitrogen input to a Scots pine stand at Ysselsteyn, the Netherlands. *Forest Ecology and Management* **101**: 155-163.

Boxman AW, Blanck K, Brandrud TE, Emmett BA, Gundersen P, Hogervorst RF, Kjonaas OJ, Persson HA, Timmerman V (1998b) Vegetation and soil biota response to experimentally changed nitrogen inputs in coniferous forest ecosystems of the NITREX project. *Forest Ecology and Management* **101**: 65-79.

Brandrud TE (1998) Ectomycorrhizal fungi in the NITREX site at Gardsjon, Sweden: below- and above-ground responses to experimentally changed N inputs (1990-1995). *Forest Ecology And Management* **101**: 207-214.

Brunner I, Scheidegger C (1994) Effects of high nitrogen concentrations on ectomycorrhizal structure and growth of seedlings of *Picea abies* (L.) Karst. *New Phytologist* **129**: 83-95.

Bytnerowicz A, Fenn ME (1996) Nitrogen deposition in California forests: a review. *Environmental Pollution* **92**: 127-146

Campion J (1998) Nitrogen nutrition in *Eucalyptus grandis* and *Pinus patula* plantations. Hons-BSc project, Department of Botany, University of the Witwatersrand, Johannesburg, South Africa.

Canham CD, Berkowitz AR, Kelly VR, Lovett GM, Ollinger SV, Schnurr J (1996) Biomass allocation and multiple resource limitation in tree seedlings. *Candian Journal of Forest Research* **26**: 1521-1530.

Cannell MGR, Thornley JHM (2000) Nitrogen states in plants ecosystems: a viewpoint. *Annals of Botany* **86**: 1161-1167.

Carlson CA (1992) Effects of acidic precipitation on the calcium and magnesium uptake by *Pinus patula*. MSc-thesis. University of the Witwatersrand, Johannesburg, South Africa.

Carlson CA (1994) The influence of fertilization on ectomycorrhizal colonisation of *Pinus patula* roots. *Suid Afrikaanse Bosboutydskrif* **171**: 1-6.

Carriera JA, Harrison AF, Sheppard LJ, Woods C (1997) Reduced soil P availability in a Sitka spruce (*Picea sitchensis* (Bong.)Carr) plantation induced by applied acid-mist: significance in forest decline. *Forest Ecology and Management* **92**: 153-166.

Cronan CS, Grigal DF (1995) Use of Calcium/Aluminum ratios as indicators of stress in forest ecosystems. *Journal of Environmental Quality* **24**: 209-226.

Crous JW, Ellis F, Theron JM (1995) Die invloed van bemesting op die groei van jong *Pinus radiata* in potte met twee tipiese bosbougronde van die Wes-Kaap. *South African Forestry Journal* **172**: 7-12.

Dames JF (1996) Litter accumualtion in a *Pinus patula* plantations and the role of ectomycorrhizal fungi in a forest ecosystem. PhD dissertation, Department of Botany, University of the Witwatersrand, Johannesburg, South Africa.

Dames JF, Straker CJ, Scholes MC (1999a) Ecological and anatomical characterization of some *Pinus patula* ectomycorrhizas from Mpumalanga, South Africa. *Mycorrhiza* **9**: 9-14.

Dames JF, Scoles MC, Straker CJ (1999b) Litter production and accumulation in *Pinus patula* plantations in Mpumalanga province, South Afirca. *Plant and Soil* **203**: 183-190.

De Clerq WP, Wessels WPJ, Moolman JH (1993) An automated sample retrieval system for soil water samplers. Proceedings from the South African Association of Soil Scientists Congress, January 1993.

Delwiche CC, Steyn PL (1970) Nitrogen isotope fractionation in soil and microbial reactions. *Environmental Science and Technology* **4**: 929-935.

Dighton J, Poskitt JM, Howard DM (1986) Changes in the occurance of basidiomycete fruit bodies during forest stand development: with specific reference to mycorrhizal species. *Transcripts of the Bryological and Mycological Society* **87**: 163-171.

Dighton J, Skeffington RA (1987) Effects of artificial acid precipitation on the mycorrhizas of Scots pine seedlings. *New Phytologist* **107**: 191-202.

Dise NB, Wright RF (1995) Nitrogen leaching from European forests in relation to nitrogen deposition. *Forest Ecoclogy and Management* **71**: 153-161.

Dise NB, Matzner E, Forsius M (1999) Evaluation of organic horizon C:N ratio as an indicator of nitrate leaching in conifer forests across Europe. *Environmental Pollution* Special Issue: Nitrogen, the Confer-N-s, First International Nitrogen Conference, Noorwijkerhout, The Netherlands, 23-27 March, 1998. pp. 453-456.

Donald DGM, Young I (1982) The growth of pine seedlings in South African nurseries. *South African Forestry Journal* **123**: 36-48.

Durham P (1965) Nursery practice. *Tanzania Forestry Department Technical Note* (Silviculture) 80.

Edfast A-B, Nasholm T, Ericsson A (1990) Free amino acid concentrations in the needles of Norway spruce and Scots pine trees on different sites in areas with two levels of nitrogen deposition. *Canadian Journal of Forestry Research* **20**: 1132-1136.

Ehleringer JR, Hall AE, Farquhar GD (1993) Stable Isotopes and Plant Carbon-Water Relations. Physiological Ecology Series of Monographs, Texts, and Treatises. Academic Press Inc.

Emmett BA, Brittain SA, Hughes S, Gorres J, Kennedy V, Norris D, Rafarel R, Reynolds B, Stevens PA (1995b) Nitrogen additions ($NaNO_3$ and NH_4NO_3) at Aber forest, Wales: I. Response of throughfall and soil water chemistry. *Forest Ecology and Management* **71**: 45-59.

Emmett BA, Brittain SA, Hughes S, Kennedy V (1995a) Nitrogen additions ($NaNO_3$ and NH_4NO_3) at Aber forest, Wales: II. Response of trees and nitrogen transformations. *Forest Ecology and Management* **71**: 61-73.

Emmett BA, Boxman D, Bredemeier M, Moldan F, Gundersen P, Kjonaas OJ, Schleppi P, Tietema A, Wright RF (1998) Predicting the Effects of Atmospheric Nitrogen Deposition in Conifer Stands: Evidence from the NITREX Ecosystem-Scale Experiments. *Ecosystems* **1**: 352-360.

Ericsson T, Rytter L, Linder S (1992) Nutritional dynamics and requirements of short rotations forests. *In* Mitchell CP, Ford-Robertson JB, Hinckley T (Eds) *Ecophysiology of Short Rotation Forest Crops*. Elsevier, London).

Ericsson AL, Norden G, Nashom T, Walheim M (1993) Mineral nutrient imbalances and arginine concentrations in needles of *Picea abies* (L.) Karst from two areas with different levels of airborne deposition. *Trees* **8**: 67-74.

Ericsson T (1995) Growth and shoot root ratio of seedling in relation to nutrient availability. *Plant and Soil* **168/169**: 205-214.

Evans J (1978) A further report on second rotation productivity in the Usutu forest, Swaziland. Results of the 1977 assessment. *Commonwealth Forestry Review* **57**: 253-261.

Fenn ME, Poth MA, Johnson DW (1996) Evidence for nitrogen saturation in the San Bernardino Mountains in southern California. *Forest Ecology and Management* **82**: 211-230.

Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan DF, Stottlemyer R (1998) Nitrogen excess in North American ecosystems: A review of predisposing factors, geographical extent, ecosystems responses and management strategies. *Ecological Applications* **8**: 706-733.

Fife DN, Nambiar EKS (1997) Changes in the canopy and growth of *Pinus radiata* in response to nitrogen supply. *Forest Ecology and Management* **93**: 137-152.

Flaig H, Mohr H (1992) Assimilation of nitrate and ammonium by the Scots pine (*Pinus sylvestris*) seedling under conditions of high nitrogen supply. *Physiologia Plantarum* **84** : 568-576.

Fleming LV, Deacon JW, Last FT, Donaldson S (1984) Influence of propagation soil on the mycorrhizal succession of birch seedlings transplanted to a field site. *Transactions of the British Mycological Society* **82**: 707-711.

Flückiger W, Braun S (1998) Nitrogen deposition in Swiss forests and its possible relevance for leaf nutrient status, parasite attacks and soil acidification. *Environmental Pollution*, Special edition. Nitrogen, the Confer-N-s, First International Nitrogen Conference, Noorwijkerhout, The Netherlands, 23-27 March, 1998. pp. 69-76.

Fög K (1988) The effect of added nitrogen on the rate of decomposition of organic matter. *Biological Review* **63**: 433-462.

Forest Owners Association, South Africa (1997) Forestry facts and figures. Department of Water Affairs and Forestry.

Fowler D, Cape NJ, Coyle M, Flechard C, Kuylenstierna J, Hicks K, Derwent D, Johnson C, Stevenson D (1999) The global exposure of ferest to air pollution. *Water, Air and Soil Pollution* **116**: 5-32.

French DD (1988) Seasonal patterns in cotton strip decomposition in soils. *In* Harrison AF, Latter PM, Walton DWH (*Eds*), *Cotton strip assay: an Index of Decomposition* pp. 34-42. ITE Symposium 24, Institute of Terrestrial Ecology, Grange-over-Sands.

Galloway JN (1998) The global nitrogen cycle: changes and consequences. *Environmental Pollution Special Issue:* Nitrogen, the Confer-N-s, First International Nitrogen Conference, Noorwijkerhout, The Netherlands, 23-27 March, 1998. pp. 15-26.

Galloway JN, Likens GE, Hawley ME (1984) Acid precipitation: natural versus anthropogenic components. *Science* **226**: 829-831.

Galloway JN, Levy H II, Kasibhatla PS (1994) Year 2020: Consequences of population growth and development on deposition of oxidized nitrogen. *Ambio* **23**: 120-123.

Galloway JN, Schlesinger WH, Levy II H, Michaels A, Schnoor JL (1995) Nitrogen fixation: anthropogenic enhancement - environmental response. *Global Biogeochemical Cycles* **9**: 235-252.

Galloway JN, Melillo KM (Eds). 1998. Asian Change in the Context of Global Change. Cambridge University Press, UK. 360 p.

Garten CT, Jr. (1993). Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker Branch Watershed. *Ecology* **74**: 2098-2113.

Garten CT, van Miegroet H (1994) Relationship between nitrogen dynamics and natural ^{15}N abundance in plant foliage from Great Smoky Mountains National Park. *Canadian Journal of Forest Research* **24**: 1636-1645.

Gebauer G, Dietrich P (1993) Nitrogen isotope ratios in different compartments of a mixed stand of spruce, larch and beech trees and of understory vegetation including fungi. *Isopenpraxis Environmental Health Studies* **29**: 35-44.

Gebauer G, Schulze E-D (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirgte, NE Bavaria. *Oecologia* **87**: 198-207.

Gebauer G, Giesemann A, Schulze E-D, Jager H-J (1994) Isotope ratios and concentrations of sulphur and nitrogen in needles and soils of *Picea abies* stands as influenced by atmospheric deposition of sulphur and nitrogen compounds. *Plant and Soil* **164**: 267-281.

Gower ST, Son Y (1992) Differences in leaf litterfall nitrogen dynamics for five forest plantations. *Soil Science Society of America Journal* **56**: 1959-1966.

Gundersen P (1991) Nitrogen deposition and the forest cycle: the role of denitrification. *Forest Ecology and Management* **44**: 15-28.

Gundersen P, Rasmussen L (1988) Nitrification, acidification and ammonium release in forest soils. *In*: Nilsson J, Grennfelt P (Eds), *Critical load for sulphur and nitrogen*. Report from Skokloster Workshop 19-24 March, 1988. Pp. 225-268.

Gundersen P, Emmett BA, Kjonaas OJ, Koopmans CJ, Tietema A (1998) Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest Ecology and Management* **101**: 37-55.

Handley LL, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology: A review. *Plant Cell and Environment* **15**: 965-985.

Handley LL, Scrimgeour CM (1997) Terrestrial plant ecology and ^{15}N natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish Old Field. *Advances in Ecological Research* **27**: 134-213.

Handley LL, Brendel O, Scrimgeour CM, Schmidt S, Raven JA, Turmbull MH, Steward GR (1996) The ^{15}N natural abundance pattens of field collected fungi from three kinds of ecosystems. *Rapid Communications in Mass Spectrometry* **10**: 974-978.

Harrison AF, Latter PM, Walton DWH (1988) Cotton Strip Assay: An Index of Decomposition. ITE Symposium 24, Institute of Terrestrial Ecology, Grange-over-Sands, UK.

Harrison AF, Stevens PA, Dighton J, Quarmby C, Dickenson AL, Jones HE, Howard DM (1995) The critical load of nitrogen for Sitka spruce forests on stagnopodsols in Wales: Role of nutrient limitations. *Forest Ecology and Management* **76**: 139-148.

Hattenschwiler S, Körner C (1998) Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. *Oecologia* **113**: 104-114.

Hauck RD, Bremner JM (1976) Use of tracers for soil and fertilizer nitrogen research. *Advances in Agronomy* **28**: 219-266.

Heal OW, Howson G, French DD, Jeffers JNR (1974) Decomposition of cotton strips in Tundra. *In* Holding AJ, Heal OW, Maclean SF, Flanagan PW (Eds*) Soil organisms and Decomposition in Tundra*. pp.341-362. Tundra Biome Steering Committee, Stockholm, Sweden.

Heal OW, Flanagan PW, French DD, Maclean SF (1981) Decomposition and Accumulation of Organic Material in Tundra. *In* Bliss LC, Heal OW, Moore JJ (Eds), *Tundra Ecosystems*. pp. 279-309, Cambridge University Press, Cambridge.

Heinsdorf D (1993) The role of nitrogen deposition in declining Scots pine (*Pinus sylvestris*) in the lowland of East Germany. *Water, Air and Soil Pollution* **69**: 21-35.

Held G, Gore BJ, Surridge AD, Tosen GR, Turner CR, Walmsley RD (1996) *Air Pollution and its Impacts on the South African Highveld*. Environmental Scientific Association, Johannesburg, South Africa. 144pp.

Högberg P (1990) Forests losing large quantities of nitrogen have elevated $^{15}N:^{14}N$ ratios. *Oecologia* **84**: 229-231.

Högberg P, Johannisen C (1993) ^{15}N abundance of forests are correlated with losses of nitrogen. *Plant and Soil* **157**: 147-150.

Högberg P, Tamm C-O, Högberg M (1992) Variations in ^{15}N abundance in a forest fertilization trial: Critical loads of N, N saturation, contamination and effect of revitalization fertilization. *Plant and Soil* **142**: 211-219.

Högberg P, Högbom L, Schinkel H, Högberg M, Johannisson C, Wallmark H (1996) ^{15}N natural abundance of surface soils, roots and mycorrhizas in profiles of European soils. *Oecologia* **108**: 207 - 214.

Holopainen T, Heinonen-Tanski H (1992) Effects of nitrogen sources on the growth of Scots pine seedlings and the ultrastructure and development of their mycorrhizae. *Canadian Journal of Forestry Research* **23**: 362-372.

Hornung M (1994) Impacts of nitrogen deposition in terrestrial ecosystems. Report of The United Kingdom Review Group on Impacts of Atmospheric Nitrogen. Department of the Environment, UK.

Howard PJA (1988) A critical assay of the cotton strip assay method. *In* Harrison AF, Latter PM, Walton DWH (*Eds*), *Cotton Strip Assay: an Index of Decomposition* pp. 34-42. ITE Symposium 24, Institute of Terrestrial Ecology, Grange-over-Sands.

Huttl RF, Wisniewski J (1987) Fertilization as a tool to mitigate forest decline associated with nutrient deficiencies. *Water, Air and Soil Pollution* **33**: 265-276.

Ingestad T (1991) Nutrition and growth of forests. *Tappi Jounal* **74**: 55-62.

Jansen AE, Dighton J (1990) Effect of Air Pollutants on ectomycorrhizas: A Review. Air Pollution Research Report 30. Commission of the European Communities.

Jeffries RL, Maron JL (1997) The embarrassment of riches: atmospheric deposition of nitrogen and community and ecosystem processes. *Trees* **12**: 74-78.

Jentschke G, Godbold DL, Huttermann A (1991) Culture of mycorrhizal tree seedlings under controlled conditions: effects of nitrogen and aluminium. *Physiologia Plantarum* **81**: 408-416.

Johannisson C, Högberg P (1994) ^{15}N abundance of plants and soils along an experimentally induced forest nitrogen supply. *Oecologia* **97**: 322-325.

Johnson DW (1992) Nitrogen retention in forest soils. *Journal of Environmental Quality* **21**: 1-12.

Johnson DW, Lindberg SE (1992) Atmospheric deposition and forests nutrient cycling. Ecological Studies 91. Springer-Verlag, New York, USA.

Katzensteiner K, Glatzel G, Kazda M (1992) Nitrogen induced nutritional imbalances - a contributing factor to Norway spruce decline in the Bohemian Forests (Austria). *Forest Ecology and Management* **51**: 29-42.

Kandeler E (1996) Nitrification. *In* Schinner F, Olinger R, Margesin R, Kandeler E, (Eds.), *Methods in Soil Biology*. Springer Verlag, Berlin.

Kelly BI (1986) Visual response of *Pinus patula, Pinus elliottii* and *Pinus taeda* to sulphur dioxide fumigations. Impact group, Specialist Environmental Investigations Section, Eskom, Johannesburg, South Africa. Report Number TRR/N86/101.

Kiefer JW, Fenn ME (1997) Using vector analysis to assess nitrogen status of ponderosa and Jeffrey pine along deposition gradients in forests of southern California. *Forest Ecology and Management* **94**: 47-59

Koerselman W, Meuleman AFM (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology* **33**: 1441-1450.

Köllig C, Pauli B, Häberle K-H, Rehfuss KE (1997) Magnesium deficiency in young Norway spruce (*Picea abies* [L.] Karst.) trees induced by NH_4NO_3 application. *Plant and Soil* **195**: 283-291.

Kotze H (1995) Merchantable volume and taper equations for *Pinus patula.* MSc-thesis, Faculty of Forestry, University of Stelllenbosch, Stellenbosch, South Africa.

Kunz RP, Schulze RE, Scholes RJ (1995) An approach to modelling spatial changes of plant carbon: nitrogen ratios in southern Africa in relation to anticipated global climate change. *Journal of Biogeography* **22**: 401-408.

Lajtha K (1988) The use of ion-exchange resin bags for measuring nutrient availability in an arid ecosystem. *Plant and Soil* **105**: 105-111.

Landsberg JJ, Gower ST (1997) Applications of Physiological Ecology to Forest Management. Academic Press, San Diego.

Last FT, Dighton J, Mason PA (1987) Succession of sheathing mycorrhizal fungi. *Tree* **2**: 157-161.

Lewis AOM (1986) Plants and Nitrogen. Studies in Biology no. 166. Edward Arnold, London.

Louw JH (1995) Site classification and evaluation for commercial forestry in the Crocodile River Catchment, Eastern Transvaal. MSc-thesis, University of Stellenbosch, Stellenbosch.

Louw JH (1997) A site growth study of *Eucalyptus grandis* in the Mpumalanga Escarpment area. *Southern African Forestry Journal* **180**: 1-14.

Louw JH (1999) A review of site-growth studies in South Africa. *Southern African Forestry Journal* **185**: 57-65.

Lundgren B (1978) Soil conditions and nutrient cycling under natural and plantation forests in the Tanzanian highlands. Forest Ecology and Forest Soils. University of Agricultural Science, Uppsala, Sweden. Report No 31.

Majdi H, Persson H (1995) Effect of ammonium sulphate application on the chemistry of bulk soil, rhizosphere, fine roots and fine-root distribution in a *Picea abies* (L.) Karst. Stand. *Plant and Soil* **168**: 151-161.

Malkönen E, Derome J, Kukkola M (1992) Effects of nitrogen inputs on forest ecosystems estimation based on long-term fertilization experiments. *In* Kauppi P, Antilla K, Kenttamies (Eds), *Acidification in Finland*. Pp. 325-347. Springer-Verlag, Belin.

Mariotti A (1984) Natural ^{15}N abundance measurements and atmospheric nitrogen standard calibration. *Nature* **311**: 251-252.

Mariotti A, Pierre D, les Bains T, Vedy JC, Bruckert S, Guillemot J (1980) The abundance of natural nitrogen-15 in the organic matter of soils along a altitudinal gradient (Chalais, Haute Savoie, France). *Catena* 7: 293-300.

Max TA, Burkhart HE (1976) Segmented polynomial regression applied to taper equations. *Forest Science* **22**: 283-289.

McNulty SG, Aber JD, McLellan TM, Katt SM (1990) Nitrogen cycling in high elevation forests of the northeastern US in relation to nitrogen deposition. *Ambio* **19**: 38-40.

McNulty SG, Aber JD, Newman SD (1996) Nitrogen saturation in a high elevation spruce-fir stand. *Forest Ecology and Management* **84**: 109-121.

Meentemeyer V (1978) Macroclimate and lignin quality of litter decomposition rates. *Ecology* **59**: 465-472.

Melville MD, Atkinson GA (1985) Soil colour: its measurement and its designation in models of uniform colour space. *Journal of Soil Science* **26**: 495-512.

Meyer J, Schneider BU, Werk K, Oren R, Schulze E-D (1988) Performance of two *Picea abies* (L.) Karst. Stands at different stages of decline. V. Root tip and ectomycorrhizal development and their relations to aboveground soil and nutrients. *Oecologia* **77**: 7-13.

Miller HG (1981) Forest fertilisation: some guiding concepts. *Forestry* **54**: 157-167.

Mohren GMJ, Van den Burg J, Burger FW (1986) Phosphorus deficiency induced by nitrogen input in Douglas-fir in the Netherlands. *Plant and Soil* **95**: 191-200.

Morris AR (1986) Soil fertility and long term productivity of *Pinus patula* plantations in Swaziland. PhD dissetation, University of Reading, Reading.

Morris AR (1992) Dry matter and nutrients in the biomass of an age series of *Pinus patula* plantations in the Usutu forest, Swaziland. *South African Forestry Journal* **163**: 5-11.

Morris AR (1993) Forest floor accumulation under *Pinus patula* in the Usutu Forest, Swaziland. *Commonwealth Forestry Review* **72**: 114-117.

Morris AR (1995) Forest floor accumulation, nutrition and productivity of *Pinus patula* in the Usutu Forest, Swaziland. *Plant and Soil* **168-169**: 178-191.

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytical Chemica Acta* **27**: 31-36.

Nadelhoffer KJ, Fry B (1988) Controls on nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Science Society of America Journal* **52**: 1633-1640.

Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest systems. *In* Lajtha K, Michener RH (Eds) *Stable Isotopes and Environmental Science*. pp. 22-44. Blackwell Scientific Publishers, London.

Nadelhoffer KJ, Aber JD, Melillo JM (1983) Leaf-litter production and soil organic matter dynamics along a nitrogen-availability gradient in southern Wisconsin (USA). *Canadian Journal of Forest Research* **13**: 12-21.

Nadelhoffer KJ, Aber JD, Melillo JM (1985) Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. *Ecology* **66**: 1377-1390.

Nadelhoffer KJ, Downs M, Fry B, Aber DJ, Magill AH, Mellilo JM (1995) The fate of ^{15}N-labelled additions to a northern hardwood forest in Maine, USA. *Oecologia* **103**: 292-301.

Nadelhoffer K, Shaver G, Fry B, Giblin A, Johnson L, McKane R (1996) ^{15}N natural abundance and N use by tundra plants. *Oecologia* **107**: 386-394.

Näsholm T, Nordin A, Edfast A-B, Högberg P (1997) Identification of coniferous forests with incipient nitrogen saturation through analysis of arginine and nitrogen-15 abundance of trees. *Journal of Environmental Quality* **26**: 302-309

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal forest plants take up organic nitrogen. *Nature* **392**: 914-916.

Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. *In*: Page AL, Miller RH, Keeney DR (*Eds*), *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*, (Second edition.). Pp. 539-579. American Society of Agronomy and Soil Science Society of America: Madison, Wisconsin.

Nielsen WA, Davis GR, McDavitt JG, Pataczek W (1984) Growth and nutrient uptake of *Pinus radiata* seedlings over the first 3 years following treatment with nitrogen and phosphorus fertilizers. *Australian Forestry Research* **14**: 30-36.

Nihlgård B (1985) The ammonium hypothesis - An additional explanation to the forest dieback in Europe. *Ambio* **14**: 2-8.

Nilsson SI (1986) Critical Loads for Nitrogen and Sulphur, Nordic Council of Ministers, Stockholm, Sweden.

Noble AD, Schumann AW (1993) The amelioration of *Pinus patula* mortality on former agricultural sites through fertilisation: A bioassay and greenhouse study. *South African Forestry Journal* **164**: 35-41.

Nohrstedt H-O (1992) Soil chemistry in a *Pinus sylvestris* stand after repeated treatment with two types of ammonium nitrate fertilizer. *Scandinavian Journal of Forest Research* **7**: 143-153.

Nommik H, Pluth DJ, Larsson K, Mahendrappa MK (1994) Isotopic fractionation accompanying fertilizer nitrogen transformations in soil and trees of a Scots pine ecosystems. *Plant and Soil* **158**: 169-182.

Novozamski I, van Eck R, van Schouwenburg JC, Walinga I (1974) Total nitrogen determination in plant material by means of the indolphenol blue method. *Netherlands Journal of Agricultural Science* **22**: 3-5.

Nowicki TN (1997) The effect of exotic tree plantations on the chemistry of acid- sensitive soils and associated surface waters in upland catchments of South Africa. PhD Dissertation, University of Cape Town.

Olbrich KA (1991) Variation in branch, shoot and needle characteristics of 8-year-old *Pinus patula*. Division of Forest Science and Technology Report FOR-DEA 00023, CSIR, Pretoria.

Olbrich KA (1993a) The chemistry and frequency of mist events on the eastern Transvaal escarpment. Report to the Department of Water Affairs and Forestry, Division of Forest Science and Technology Report FOR-DEA 657, CSIR, Pretoria.

Olbrich KA (1993b) Final report: the patterns of shoot growth, needle growth and foliar symptom development in *Pinus patula* in a fertilised and unfertilised stand. Division of Forest Science and Technology Report, FOR-DEA 655, CSIR, Pretoria.

Olbrich KA (1995) Air pollution impacts on South African commercial forests: A research synthesis: 1990-1995 and strategy for the future. Division of Forest Science and Technology Report, FOR DEA-874, CSIR, Pretoria.

Olbrich KA, Du Toit B (1993) Assessing the risks posed by air pollution to forestry in the Eastern Transvaal, South Africa. Division of Forest Science and Technology Report, FOR-C 214, CSIR, Pretoria.

Oren R, Schulze E-D, Werk KS, Meyer J, Schneider BU, Heilmeier H (1988) Performance of two *Picea abies* (L.) Karst. stands at different stages of decline. I. Carbon relations and stand growth. *Oecologia* **75:** 25-37.

Payn TW, Clough ME (1988) Differential fertilization of pine plantations on acid forest soils. *South African Forestry Journal* **147**: 16-25.

Payn TW, Schutz CJ, Clough ME (1989) Determination of the most stable period for sampling *P. patula* foliage in the summer rainfall region of South Africa. *Communications in Soil Science and Plant Analysis* **20**: 403-420.

Persson H (1988) Root growth and root damage in Swedish coniferous stands. *In*: Jansen AE, Dighton J, Bresser AHM (Eds.), *Ectomycorrhiza and Acid Rain*. Proceeding of Workshop/Expert Meeting, Berg en Dal 1987: 53-59.

Persson H, Ahlström K, Clemensson-Lindell A (1998) Nitrogen addition and removal at Gårdsjön – effects of fine roots and fine root chemistry. *Forest Ecology and Management* **101** : 199-205.

Poth MA, Peterson DL, Fenn ME (1991) The effect of air pollution on bigcone Douglas-fir in southern California. A progress report submitted to the USDA, Southern California, Edison, California, 8 August 1991.

Powers RF (1990) Nitrogen mineralization along an altitudinal gradient: Interactions of soil temperature, moisture, and substrate quality. *Forest Ecology and Management* **30**: 19-29.

Prescott CE (1995) Does nitrogen availability control rates of litter decomposition in forests? *Plant and Soil* **168-169**: 83-88.

Proe MF, Craig J, Dutch J, Griffiths J (1999) Use of vector analysis to determine the effects of harvest residues on early growth of second-rotation sitka spruce. *Forest Ecology and Management* **122**: 87-105.

Radin JW (1981) Water relations of cotton plants under nitrogen deficiency. IV. Leaf senescence during drought and its relation to stomatal closure. *Physiologia Plantarum* **51**: 145-149.

Radin JW, Parker LL (1979) Water relations of cotton plants under nitrogen deficiency. I. Dependance upon leaf structure. *Plant Physiology* **64**: 495-498.

Raymond CA, Muneri A (2000) Effects of fertilizer on wood properties of *Eucalyptus globulus*. *Canadian Journal of Forestry Research* **30**:136-144.

Reuss JO, Cosby BJ, Wright RF (1987) Chemical processes governing soil water acidification. *Nature* **329**: 27-32.

Reynolds HL, D'Antonio C (1996) The ecological significance of plasticity in root weight ratio in response to nitrogen: opinion. *Plant and Soil* **185**: 75-97.

Reynolds B, Wilson EJ, Emmett BA (1998) Evaluating critical loads of nutrient nitrogen and acidity for terrestrial systems using ecosystem-scale experiments (NITREX). *Forest Ecology and Management* **101**: 81-94.

Richards BN (1965) Mycorrhiza development of loblolly pine seedlings in relation to soil reaction and the supply of nitrate. *Plant and Soil* **22**: 187-199.

Sadanandan Nambiar EK (1999) Pursuit of sustainable forestry. *Southern African Forestry Journal* **184**: 45-62.

Schaberg PG, Perkins TD, McNulty SG (1997) Effects of chronic low level N additions on foliar elemental concentrations, morphology, and gas exchange of mature montane red spruce. *Canadian Journal of Forest Research* **27**: 1622-1629.

Scholes ME, Nowicki TE (1996) The effect of pines on soil properties and processes. *In*: Richardson DM (Ed.), *Ecology and Biogeography of* Pinus. Cambridge University Press, Cambridge, UK.

Scholes RJ, Scholes MC (1997) Applications of biogeochemical modelling in South Africa. *Progress in Physical Geography* **21**: 102-112.

Scholes RJ, Scholes MC (1999) Nutrient imbalances following conversion of grasslands to plantation forests in South Africa. *In* Smaling EMA, Oenema O, Fresco LO (Eds), *Nutrient Disequilibria in Agroecosystems: Concepts and Case Studies*. CABI Publishing.

Scholes RJ, Ward DE, Justice CO (1996), Emissions of trace gases and aerosol particles due to vegetation burning in southern hemisphere Africa. *Journal of Geophysical Research* **101**: 23677-23682.

Schonau APG (1983) Fertilization in South African forestry. *South African Journal of Forestry Research* **125**: 1-19.

Schulze E-D (1989) Air pollution and forest decline in a spruce (*Picea abies*) forest. *Science* **244**: 776-783.

Schutz CJ (1975) A review of fertilizer research on some of the more important conifers and eucalypts planted in subtropical countries, with special reference to South Africa. *Department of Forestry Bulletin* No. 53.

Schutz CJ (1990) Site relationships for *Pinus patula* in the Eastern Transvaal Escarpment area. PhD Dissertation, University of Natal, Pietermaritzburg.

Schutz CJ (1992) Monitoring long term productivity in South African forestry. *South African Forestry Journal* **120**: 3-6.

Singh B (1982) Nutrient content of standing crop and biological cycling in *Pinus patula* ecosystem. *Forest Ecology and Management* **4**: 317-332.

Skeffington RA (1990) Accelerated nitrogen inputs - A new problem or new perspective? *Plant and Soil* **128**: 1-11.

Skeffington RA, Wilson EJ (1988) Excess nitrogen deposition: Issues for consideration. *Environmental Pollution* **54**: 159-184.

Smethurst PJ, Nambiar EKS (1995) Changes in soil carbon and nitrogen during the establishment of a second crop of *Pinus radiata*. *Forest Ecology and Management* **73**: 145-155.

Smit CJ, Hauptfleisch V (1970) Bepaling van grond-pH in 0.01M $CaCl_2$. *NIVV Prosedures en Tegnieke*, Number 62.

Smith VR, Steenkamp M, French DD (1993) Soil decomposition potential in relation to environmental factors on Marion Island (Sub-Antarctic). *Soil Biology and Biochemistry* **25**: 1619-1633.

Soil Classification Working Group (1991) Soil classification: a taxonomic system for South Africa. Memoirs on the Agricultural Natural Resources of South Africa No. 15, Department of Agricultural Development, Pretoria. 257pp.

Spiecker H, Mielikainen K, Kohl K, Skovsgaard J (1996) Growth Trends in European Forests. European Forest Institute Research Report No.5. Springer-Verlag, Berlin, Heidelberg, 372 pp.

Stevens PA, Harrison AF, Jones HE, Williams TG, Hughes S (1993). Nitrate leaching from a Sitka spruce plantation and the effect of fertilization with phosphorus and potassium. *Forest Ecology and Management* **58**: 233-247.

Stevens PA, Norris DA, Sparks TH, Hodgson AL (1994) The impacts of atmospheric N inputs on through fall, soil and streamwater interactions for different aged forest and moorland catchments in Wales. *Water, Air and Soil Pollution* **73**: 297-317.

Stock WD (1983) An evaluation of some manual colorimetric methods for the determination of inorganic nitrogen in soil extracts. *Communications in Soil Science and Plant Analysis* **14**: 925-936.

Sullivan TJ (1993) Whole-ecosystem nitrogen effects research in Europe. *Environmental Science and Technology* **27**: 1482-1486.

Sverdrup H, de Vries W (1994) Calculating critical loads for acidity with the simple mass balance approach. *Water, Air and Soil Pollution* **72**: 143-162.

Tamm CO (1985) Nitrogen in terrestrial ecosystems. *Ecological Studies* **81**.

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. *Ecology* **70**: 97-104.

Tennant D (1975) A test of the modified line intercept method for estimating root length. *Journal of Ecology* **63**: 995-1001.

Termorshuizen AJ, Schaffers AP (1987) Occurrence of carpophores of ectomycorrhizal fungi in selected stands of *Pinus sylvestris* in the Netherlands in relation to stand vitality and air pollution. *Plant and Soil* **104**: 209-217.

Termorshuizen AJ, Schaffers AP, Ket PC, ter Stege EA (1988) The significance of nitrogen pollution on the mycorrhizas of *Pinus sylvestris*. *In* Jansen AE, Dighton J, Bresser AHM (Eds.), *Ectomycorrhiza and Acid Rain*, Proceeding of Workshop/Expert Meeting, Berg en Dal 1987: 53-59.

The Non-Affiliated Soil Analysis Work Committee (1990) Handbook of Standard Soil Testing Methods for Advisory Purposes.

Thomas RC, Miller HG (1992) Impact of foliar and soil applications of nitrogen on the nutritional status of a young Sitka spruce plantation. *Forest Ecology and Management* **51**: 217-225.

Timmer VR, Armstrong G (1987) Diagnosing nutritional stutus of containerised tree seedlings: Comparative plant analyses. *Soil Science Society of America Journal* **51**: 1082-1087.

Timmer VR, Morrow LD (1993) Predicting fertilizer growth response aand nutrient status of Jack pine by foliar diagnosis. *In* Stone EL (Ed.), *Forest Soils and Treatment Impacts.* Proceedings of the Sixth North American Forest Soils Conference, Knoxville, Tennessee.

Timmer VR, Stone EL (1978) Comparative foliar analysis of yound balsam fir fertilized with nitrogen, phosphorus, potassium and lime. *Soil Science Society of America Journal* **42**: 125-130.

Tyson PD, Kruger PJ, Louw CW (1988) Atmospheric pollution and its implications on the Eastern Transvaal Highveld. South African National Scientific Programmes Report no. 150, CSIR, Pretoria.

Van Breemen N, Van Dijk HFG (1988) Ecosystem effects of atmospheric deposition of nitrogen in The Netherlands. *Environmental Pollution* **75**: 283-308.

Van den Driessche R (1971) Response of conifer seedlings to nitrate and ammonium sources of nitrogen. *Plant and Soil* **34**: 421-439.

Van Dijk HFG, Roelofs JGM (1988) Effects of excessive ammonium deposition on the nutritional status and condition of pine neeldes. *Physiologia Plantarum* **73**: 494-501.

Van Miegroet H, Johnson DW, Cole DW (1990) Soil nitrification as affected by N fertility and changes in forest floor C/N ratio in four forest soils. *Canadian Journal of Forestry Research* **20**: 1012-1019.

Versveld DB, Donald DGH (1991) Litterfall and nutrient release in mature *Pinus radiata* in the South-western Cape. *South African Forestry Journal* **156**: 61-69.

Vitousek PM (1981) Clear-cutting and the nitrogen cycle. *In*: Clark FE, Rosswall T (Eds) *Terrestrial Nitrogen Cycles. Processes, Ecosystem Strategies and Management impacts. Ecological Bulletin* **33**: 631-642.

Vitousek PM, Shearer G, Khol DH (1989) Foliar ^{15}N natural abundance in Hawaiian rainforest: patterns and possible mechanisms. *Oecologia* **78**: 383-388.

Vitousek PM, Aber J, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tillman GD (1997) Human alterations of the global nitrogen cycle: Sources and consequences. *Ecological Applications* **7**: 737–750.

Wada Y, Hattori H (1978) Nitrogen isotope effects in the assimilation of inorganic nitrogenous compounds. *Geomicrobiology Journal* **1**: 85-101.

Wallander H, Arnebrandt K, Ostrand F, Karen O (1997) Uptake of ^{15} N-labelled alanine, ammonium and nitrate in *Pinus sylvestris* l. Ectomycorrhiza growing in forest soil treated with nitrogen, sulphur and lime. *Plant and Soil* **195**: 329-338.

Waring RH, Schlesinger WH (1985) Forest Ecosystems: Concepts and Management. Academic Press, New York, USA.

Warren CR, Adams MA (2000) Trade-offs between the persistence of foliage and productivity in two *Pinus* species. *Oecologia* **124**: 487-494.

Weetman GF (1989) Graphical vector analysis technique for testing stand nutritional status. *In* Dyck WJ, Mees CA (Eds.), *Research Strategies for Long-Term Site Productivity*. Proceeding, IEA/BE A3 Workshop, Seattle, Washington, August, 1988.

Wienand KT, Stock WD (1995) Long-term phosphorus fertilization effects on the litter dynamics of an age sequence of *Pinus elliottii* plantations in the southern Cape of South Africa. *Forest Ecology And Management* **75**: 135-146.

Williams MW, Baron JS, Caine N, Sommerfield R, Sanford R (1996) Nitrogen saturation in the Rocky Mountains. *Environmental Science and Technology* **30**: 640-646.

Wilson EJ, Skeffington RA (1994a) The effects of excess nitrogen deposition on young Norway spruce trees. Part I The soil. *Environmental Pollution* **86**: 146-152.

Wilson EJ, Skeffington RA (1994b) The effects of excess nitrogen deposition on young Norway spruce trees. Part II The vegetation. *Environmental Pollution* **86**: 153-161.

Wilson EJ, Emmett BA (1998) Factors influencing nitrogen saturation in forest ecosystems: advances in our understanding since the mid 1980's. *In*: Lanyan A, Wilson EJ (Eds), *The Impacts of Nitrogen Saturation on Natural and Semi-natural Ecosystems*. Chapman and Hall.

Wookey PA, Ineson P, Mansfield TA (1991) Effects of atmospheric sulphur dioxide on microbial activity in decomposing forets litter. *Agriculture, Ecosystems and Environment* **33**: 263-280.

Wormald TJ (1975) *Pinus patula*. Tropical Forestry Papers 7: 172. Commonwealth Forestry Institute, Oxford.

Wright RF, Roelofs JGM, Bredemeier M, Blanck K, Boxman AW, Emmett BA, Gunderson P, Hultberg H, Kjonaas OJ, Moldan M, Tietema A, Van Breemen M, Van Dijk HFG (1995) NITREX: responses of coniferous forest ecosystems to experimentally changed deposition of nitrogen. *Forest Ecology and Management* **71**: 163-169.

Zar JH (1984) Biostatistical analysis. Second Edition. Prentice-Hall Inc., New Jersey.

Zimmerman MH (1978) Hydraulic architecture of some diffuse porous trees. *Canadian Journal of Botany* **56:** 2286-2295.

Appendix I : Summary of Statistics

Table 9.1. *ANOVA table for tree nutrient parameters. The highlighted lines denotes significance at* p<*0.05.*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
N	Leafage	1	0.04	36	0.04	1.09	0.30
	Plantage	2	0.10	36	0.04	2.63	0.09
	Treatment	1	1.04	36	0.04	26.65	0.00
	Leafage x Plantage	2	0.01	36	0.04	0.22	0.80
	Leafage x Treatment	1	0.00	36	0.04	0.01	0.91
	Plantage x Treatment	2	0.02	36	0.04	0.47	0.63
	Leafage x Plantage x Treatment	2	0.00	36	0.04	0.13	0.88
P	Leafage	1	0.01	36	0.00	33.98	0.00
	Plantage	2	0.00	36	0.00	2.09	0.14
	Treatment	1	0.00	36	0.00	2.92	0.10
	Leafage x Plantage	2	0.00	36	0.00	4.57	0.02
	Leafage x Treatment	1	0.00	36	0.00	0.09	0.77
	Plantage x Treatment	2	0.00	36	0.00	2.34	0.11
	Leafage x Plantage x Treatment	2	0.00	36	0.00	0.92	0.41
Cu	Leafage	1	11.68	36	27.13	0.43	0.52
	Plantage	2	15.52	36	27.13	0.57	0.57
	Treatment	1	48.54	36	27.13	1.79	0.19
	Leafage x Plantage	2	5.68	36	27.13	0.21	0.81

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	p-level
	Leafage x Treatment	1	25.97	36	27.13	0.96	0.33
	Plantage x Treatment	2	0.63	36	27.13	0.02	0.98
	Leafage x Plantage x Treatment	2	51.27	36	27.13	1.89	0.17
K	Leafage	1	0.63	36	0.04	15.77	0.00
	Plantage	2	0.09	36	0.04	2.35	0.11
	Treatment	1	1.69	36	0.04	42.54	0.00
	Leafage x Plantage	2	0.17	36	0.04	4.21	0.02
	Leafage x Treatment	1	0.01	36	0.04	0.35	0.56
	Plantage x Treatment	2	0.01	36	0.04	0.25	0.78
	Leafage x Plantage x Treatment	2	0.04	36	0.04	0.89	0.42
Mn	Leafage	1	351197.00	36	471283.90	0.75	0.39
	Plantage	2	5331225.00	36	471283.90	11.31	0.00
	Treatment	1	5247.00	36	471283.90	0.01	0.92
	Leafage x Plantage	2	78744.00	36	471283.90	0.17	0.85
	Leafage x Treatment	1	192442.00	36	471283.90	0.41	0.53
	Plantage x Treatment	2	226067.00	36	471283.90	0.48	0.62
	Leafage x Plantage x Treatment	2	17589.00	36	471283.90	0.04	0.96
Fe	Leafage	1	880.14	36	4532.97	0.19	0.66
	Plantage	2	19925.88	36	4532.97	4.40	0.02
	Treatment	1	1259.36	36	4532.97	0.28	0.60
	Leafage x Plantage	2	5300.94	36	4532.97	1.17	0.32

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Leafage x Treatment	1	2281.42	36	4532.97	0.50	0.48
	Plantage x Treatment	2	1669.76	36	4532.97	0.37	0.69
	Leafage x Plantage x Treatment	2	1581.56	36	4532.97	0.35	0.71
Mg	Leafage	1	0.02	36	0.00	8.91	0.01
	Plantage	2	0.00	36	0.00	1.80	0.18
	Treatment	1	0.00	36	0.00	2.64	0.11
	Leafage x Plantage	2	0.00	36	0.00	0.84	0.44
	Leafage x Treatment	1	0.00	36	0.00	0.12	0.73
	Plantage x Treatment	2	0.00	36	0.00	0.16	0.85
	Leafage x Plantage x Treatment	2	0.00	36	0.00	1.12	0.34
Al	Leafage	1	222695.30	36	126787.70	1.76	0.19
	Plantage	2	87428.90	36	126787.70	0.69	0.51
	Treatment	1	101801.70	36	126787.70	0.80	0.38
	Leafage x Plantage	2	433468.00	36	126787.70	3.42	0.04
	Leafage x Treatment	1	38364.10	36	126787.70	0.30	0.59
	Plantage x Treatment	2	70256.00	36	126787.70	0.55	0.58
	Leafage x Plantage x Treatment	2	312795.40	36	126787.70	2.47	0.10
Ca	Leafage	1	0.02	36	0.01	3.32	0.08
	Plantage	2	0.01	36	0.01	1.03	0.37
	Treatment	1	0.02	36	0.01	2.50	0.12
	Leafage x Plantage	2	0.00	36	0.01	0.60	0.55

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Leafage x Treatment	1	0.03	36	0.01	4.21	0.05
	Plantage x Treatment	2	0.01	36	0.01	2.18	0.13
	Leafage x Plantage x Treatment	2	0.00	36	0.01	0.12	0.89

***Table 9.2**. ANOVA table for tree nutrient ratios. The highlighted lines denotes significance at p<0.05.*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
N:P	Leafage	1	74.51	36	4.97	15.00	0.00
	Plantage	2	1.87	36	4.97	0.38	0.69
	Treatment	1	92.77	36	4.97	18.67	0.00
	Leafage x Plantage	2	9.50	36	4.97	1.91	0.16
	Leafage x Treatment	1	4.35	36	4.97	0.88	0.36
	Plantage x Treatment	2	6.67	36	4.97	1.34	0.27
	Leafage x Plantage x Treatment	2	0.69	36	4.97	0.14	0.87
N:Ca	Leafage	1	69.66	36	6.67	10.45	0.00
	Plantage	2	33.12	36	6.67	4.97	0.01
	Treatment	1	176.30	36	6.67	26.44	0.00
	Leafage x Plantage	2	13.13	36	6.67	1.97	0.15
	Leafage x Treatment	1	3.08	36	6.67	0.46	0.50
	Plantage x Treatment	2	1.32	36	6.67	0.20	0.82
	Leafage x Plantage x Treatment	2	2.72	36	6.67	0.41	0.67
Ca:Al	Leafage	1	0.02	36	0.00	12.34	0.00

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Plantage	2	0.00	36	0.00	0.41	0.67
	Treatment	1	0.00	36	0.00	1.35	0.25
	Leafage x Plantage	2	0.00	36	0.00	1.20	0.31
	Leafage x Treatment	1	0.02	36	0.00	9.18	0.00
	Plantage x Treatment	2	0.00	36	0.00	2.26	0.12
	Leafage x Plantage x Treatment	2	0.00	36	0.00	1.32	0.28
N:Mg	Leafage	1	2.53	36	5.13	0.49	0.49
	Plantage	2	26.60	36	5.13	5.19	0.01
	Treatment	1	92.66	36	5.13	18.08	0.00
	Leafage x Plantage	2	0.52	36	5.13	0.10	0.90
	Leafage x Treatment	1	13.17	36	5.13	2.57	0.12
	Plantage x Treatment	2	9.23	36	5.13	1.80	0.18
	Leafage x Plantage x Treatment	2	2.17	36	5.13	0.42	0.66
N:K	Leafage	1	0.60	36	0.10	6.02	0.02
	Plantage	2	0.64	36	0.10	6.44	0.00
	Treatment	1	1.32	36	0.10	13.39	0.00
	Leafage x Plantage	2	0.09	36	0.10	0.93	0.40
	Leafage x Treatment	1	0.10	36	0.10	1.01	0.32
	Plantage x Treatment	2	0.30	36	0.10	3.03	0.06
	Leafage x Plantage x Treatment	2	0.00	36	0.10	0.03	0.97

***Fig. 9.3**. ANOVA table for nutrients of the litter layer. The highlighted lines denotes significance at p<0.05..*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
P	Litter type	2	0.00	18	0.00	19.86	0.00
	Plant Age	1	0.00	18	0.00	1.35	0.26
	Litertype x Plant Age	2	0.00	18	0.00	2.39	0.12
N	Litter type	2	0.29	18	0.01	24.25	0.00
	Plant Age	1	0.22	18	0.01	18.03	0.00
	Litertype x Plant Age	2	0.01	18	0.01	0.59	0.57
Cu	Litter type	2	2013.67	18	1598.13	1.26	0.31
	Plant Age	1	1642.77	18	1598.13	1.03	0.32
	Litertype x Plant Age	2	1157.21	18	1598.13	0.72	0.50
Mn	Litter type	2	3174464. 0	18	542090.30	5.86	0.01
	Plant Age	1	18616. 0	18	542090.30	0.03	0.86
	Litertype x Plant Age	2	106949. 0	18	542090.30	0.20	0.82
Fe	Litter type	2	370712.90	18	7117.92	52.08	0.00
	Plant Age	1	47678.80	18	7117.92	6.70	0.02
	Litertype x Plant Age	2	15611.20	18	7117.92	2.19	0.14
Mg	Litter type	2	0.01	18	0.00	7.20	0.01
	Plant Age	1	0.00	18	0.00	2.48	0.13
	Litertype x Plant Age	2	0.00	18	0.00	0.31	0.74
Al	Litter type	2	754214.40	18	49486.66	15.24	0.00
	Plant Age	1	654.90	18	49486.66	0.01	0.91

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Litertype x Plant Age	2	10287.00	18	49486.66	0.21	0.81
Ca	Litter type	2	0.09	18	0.01	6.60	0.01
	Plant Age	1	0.00	18	0.01	0.17	0.68
	Litertype x Plant Age	2	0.00	18	0.01	0.18	0.84
K	Litter type	2	0.11	18	0.00	24.90	0.00
	Plant Age	1	0.34	18	0.00	78.35	0.00
	Litertype x Plant Age	2	0.00	18	0.00	0.01	0.99

***Fig. 9.4**. ANOVA table for soil pH ($CaCl_2$). The highlighted lines denotes significance at p<0.05.*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
4-year-old site	Year	2	0.01	66	0.01	0.86	0.43
	Treatment	1	0.00	66	0.01	0.07	0.79
	Year x Treatment	2	0.03	66	0.01	2.21	0.12
14-year-old site	Year	2	0.02	64	0.02	0.77	0.47
	Treatment	1	0.01	64	0.02	0.53	0.47
	Year x Treatment	2	0.00	64	0.02	0.05	0.95
22-year-old site	Year	2	0.02	66	0.01	2.46	0.09
	Treatment	1	0.01	66	0.01	1.00	0.32
	Year x Treatment	2	0.02	66	0.01	1.91	0.16

***Fig. 9.5**. ANOVA table for soil chemistry of field experiment. The highlighted lines denotes significance at p<0.05.*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
EC	Site Age	1	55094.96	18	3807.08	14.47	.01
	Treatment	2	3166.96	18	3807.08	.83	.45
	Site Age x Treatment	2	102.05	18	3807.08	.03	.97
Acid	Site Age	2	4.63	18	0.87	5.31	0.02
	Treatment	1	1.71	18	0.87	1.96	0.18
	Site Age x Treatment	2	0.43	18	0.87	0.49	0.62
Na	Site Age	2	0.00	18	0.00	4.18	0.03
	Treatment	1	0.00	18	0.00	0.34	0.57
	Site Age x Treatment	2	0.00	18	0.00	1.12	0.35
K	Site Age	2	0.04	18	0.00	20.69	0.00
	Treatment	1	0.07	18	0.00	33.02	0.00
	Site Age x Treatment	2	0.01	18	0.00	4.94	0.02
Ca	Site Age	2	0.01	18	0.00	6.12	0.01
	Treatment	1	0.00	18	0.00	1.27	0.27
	Site Age x Treatment	2	0.00	18	0.00	1.62	0.23
Mg	Site Age	2	0.03	18	0.00	32.09	0.00
	Treatment	1	0.00	18	0.00	0.63	0.44
	Site Age x xTreatment	2	0.00	18	0.00	0.91	0.42
Total Bases	Site Age	2	0.27	18	0.01	19.25	0.00
	Treatment	1	0.11	18	0.01	7.50	0.01
	Site Age x Treatment	2	0.01	18	0.01	0.88	0.43

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
Total bases, except K	Site Age	2	0.10	18	0.01	13.18	0.00
	Treatment	1	0.00	18	0.01	0.45	0.51
	Site Age x Treatment	2	0.01	18	0.01	1.20	0.32
Cu	Site Age	2	7.30	18	0.34	21.47	0.00
	Treatment	1	0.41	18	0.34	1.21	0.29
	Site Age x Treatment	2	0.62	18	0.34	1.82	0.19
Zn	Site Age	2	0.11	18	0.11	0.94	0.41
	Treatment	1	0.15	18	0.11	1.35	0.26
	Site Age x Treatment	2	0.51	18	0.11	4.53	0.03
Mn	Site Age	2	4.70	18	5.36	0.88	0.43
	Treatment	1	18.96	18	5.36	3.54	0.08
	Site Age x Treatment	2	3.46	18	5.36	0.64	0.54
Fe	Site Age	2	587700.50	18	26001.36	22.60	0.00
	Treatment	1	114816.70	18	26001.36	4.42	0.05
	Site Age x Treatment	2	121653.20	18	26001.36	4.68	0.02
Organic C	Site Age	2	35.11	18	1.58	22.18	0.00
	Treatment	1	1.14	18	1.58	0.72	0.41
	Site Age x Treatment	2	0.36	18	1.58	0.22	0.80
Available P	Site Age	2	1087.13	18	34.44	31.56	0.00
	Treatment	1	104.17	18	34.44	3.02	0.10
	Site Age x Treatment	2	14.04	18	34.44	0.41	0.67

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
Total P	Site Age	2	2.98	18	0.52	5.69	0.01
	Treatment	1	0.03	18	0.52	0.06	0.80
	Site Age x Treatment	2	0.51	18	0.52	0.97	0.40
Total N	Site Age	2	115.73	18	2.08	55.71	0.00
	Treatment	1	13.70	18	2.08	6.59	0.02
	Site Age x Treatment	2	0.85	18	2.08	0.41	0.67

Fig. 9.6 *ANOVA table for litter mass remaining. The highlighted lines denotes significance at* $p<0.05$.

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
Litter mass remaining	Site Age	2	301.38	66	62.52	4.828	.01
	Treatment	1	2.28	66	62.52	.036	.85
	Site Age x Treatment	2	129.79	66	62.52	2.08	.13

Fig. 9.7 *ANOVA table for mycorrhizal variables. The highlighted lines denotes significance at* $p<0.05$.

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
Root Density	Age	2	0.16	32	0.09	1.75	0.19
	Treatment	1	0.07	32	0.09	0.77	0.39
	Age x Treatment	2	0.00	32	0.09	0.00	1.00
Total	Age	2	3.45	32	4.00	0.86	0.43
	Treatment	1	14.56	32	4.00	3.64	0.07
	Age x Treatment	2	17.49	32	4.00	4.37	0.02
Species 2 v. Species 3	Age	2	0.54	31	3.03	0.18	0.84

	Treatment	1	14.53	31	3.03	4.79	0.04
	Age x Treatment	2	2.43	31	3.03	0.80	0.46
Specific Root Density	Age	2	0.34	26	1.45	0.23	0.80
	Treatment	1	4.69	26	1.45	3.24	0.08
	Age x Treatment	2	5.86	26	1.45	4.05	0.03

Fig. 9.8 *ANOVA table for nitrogen and carbon isotopic variables. The highlighted lines denotes significance at* $p<0.05$.

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
$\delta^{15}N$ in Organic Soil Layers	Organic Soil Layer	4	87.24	113	0.51	169.43	0.00
	Site Age	2	18.82	113	0.51	36.56	0.00
	Treatment	1	0.24	113	0.51	0.47	0.49
	Organic Soil Layer x Site Age	8	2.88	113	0.51	5.60	0.00
	Organic Soil Layer x Treatment	4	1.17	113	0.51	2.27	0.07
	Site Age x Treatment	2	1.73	113	0.51	3.37	0.04
	Organic Soil Layer x Site Age x Treatment			113	0.51		
$\delta^{15}N$ in Curent and One-year-old Leaves	Leaf age	1	2.68	39	0.27	9.92	0.00
	Site Age	2	1.21	39	0.27	4.47	0.02
	Treatment	1	0.04	39	0.27	0.15	0.70
	Leaf Age x Site age	2	0.24	39	0.27	0.87	0.43
	Leaf Age x Treatment	1	0.00	39	0.27	0.00	0.99
	Site Age x Treatment	2	5.96	39	0.27	22.08	0.00

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Leaf Age x Site Age x treatment	2	0.25	39	0.27	0.92	0.41
C:N Ratio	Organic Soil Horizons	4	1531.14	55	32.14	47.64	0.00
	Site Age	2	294.27	55	32.14	9.16	0.00
	Orgaic Soil Horizons x Site Age	8	232.40	55	32.14	7.23	0.00
Carbon Isotopes	Soil Organic Horizon	5	164.40	133	0.40	408.08	0.00
	Site Age	2	8.94	133	0.40	22.18	0.00
	Soil Organic Horizon x Site Age	10	4.79	133	0.40	11.89	0.00
Carpophores	Site Age	2	0.51	18	0.10	4.88	0.02
	Treatment	1	0.43	18	0.10	4.08	0.06
	Site Age x Treatment	2	0.00	18	0.10	0.03	0.97

***Fig. 9.9**. ANOVA table for seedling growth parameters. The highlighted lines denotes significance at p<0.05.*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	p-level
Final Height	Dosage	1	2413.02	138	302.47	7.98	0.01
	Ratio	2	291.86	138	302.47	0.96	0.38
	Dosage x Ratio	2	391.31	138	302.47	1.29	0.28
Height Increment	Dosage*	1	6859.69	137	522.83	13.12	0.00
	Ratio	2	538.16	137	522.83	1.03	0.36
	Dosage x Ratio	2	53.48	137	522.83	0.10	0.90
Final Collar Diameter	Dosage	1	213.35	138	108.56	1.97	0.16
	Ratio	2	294.90	138	108.56	2.72	0.07
	Dosage x Ratio	2	4.67	138	108.56	0.04	0.96
Collar Diameter Increment	Dosage	1	938.59	138	1477.08	0.64	0.43
	Ratio	2	2041.67	138	1477.08	1.38	0.25
	Dosage x Ratio	2	9626.76	138	1477.08	6.52	0.00
Crown Diameter Increment	Dosage	1	2216.45	138	293.96	7.54	0.01
	Ratio	2	1539.19	138	293.96	5.24	0.01
	Dosage x Ratio	2	2.09	138	293.96	0.01	0.99
Colour	Dosage	1	2936.32	136	358.26	8.20	0.00
	Ratio	2	1207.15	136	358.26	3.37	0.04
	Dosage x Ratio	2	642.79	136	358.26	1.79	0.17
Root:Shoot Ratio	Dosage	1	10981.38	86	985.94	11.14	0.00
	Ratio	2	139.53	86	985.94	0.14	0.87

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Dosage x Ratio	2	730.98	86	985.94	0.74	0.48
Root Weight	Dosage	1	1326.87	85	1150.87	1.15	0.29
	Ratio	2	1404.41	85	1150.87	1.22	0.30
	Dosage x Ratio	2	1899.49	85	1150.87	1.65	0.20
Shoot Weight	Dosage	1	2727.91	90	696.97	3.91	0.05
	Ratio	2	1765.38	90	696.97	2.53	0.09
	Dosage x Ratio	2	1556.62	90	696.97	2.23	0.11

***Fig. 9.10**. ANOVA table for foliar chemistry of the seedling experiment. The highlighted lines denotes significance at* p<*0.05.*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
K	Dosage	1.00	23420.18	18	2815.13	8.32	0.01
	Ratio	2.00	88059.34	18	2815.13	31.28	0.00
	Dosage x Ratio	2.00	6626.55	18	2815.13	2.35	0.12
Ca	Dosage	1.00	1346.55	18	445.31	3.02	0.10
	Ratio	2.00	857.13	18	445.31	1.92	0.17
	Dosage x Ratio	2.00	182.78	18	445.31	0.41	0.67
Mg	Dosage	1.00	1108.28	18	358.37	3.09	0.10
	Ratio	2.00	374.27	18	358.37	1.04	0.37
	Dosage x Ratio	2.00	115.18	18	358.37	0.32	0.73
Mn	Dosage	1.00	653.48	18	87.40	7.48	0.01
	Ratio	2.00	3.08	18	87.40	0.04	0.97
	Dosage x Ratio	2.00	307.04	18	87.40	3.51	0.05

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
Fe	Dosage	1.00	2687.18	18	1611.51	1.67	0.21
	Ratio	2.00	8729.26	18	1611.51	5.42	0.01
	Dosage x Ratio	2.00	6353.59	18	1611.51	3.94	0.04
Cu	Dosage	1.00	8319.40	18	1297.25	6.41	0.02
	Ratio	2.00	1140.81	18	1297.25	0.88	0.43
	Dosage x Ratio	2.00	1762.74	18	1297.25	1.36	0.28
Al	Dosage	1.00	49.35	18	202.33	0.24	0.63
	Ratio	2.00	25.70	18	202.33	0.13	0.88
	Dosage x Ratio	2.00	469.84	18	202.33	2.32	0.13

***Fig. 9.11**. ANOVA table for soil chemistry of the seedling experiment. The highlighted lines denotes significance at* p*<0.05.*

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
pH	Dosage	1	0.26	18	1.85	0.14	0.71
	Ratio	2	0.00	18	1.85	0.00	1.00
	Dosage x Ratio	2	1.05	18	1.85	0.57	0.57
EC	Dosage	1.00	1.70	18.00	0.12	14.47	0.00
	Ratio	2.00	0.10	18.00	0.12	0.83	0.45
	Dosage x Ratio	2.00	0.00	18.00	0.12	0.03	0.97
Na	Dosage	1	358.31	18	288.55	1.24	0.28
	Ratio	2	168.66	18	288.55	0.58	0.57
	Dosage x Ratio	2	7.26	18	288.55	0.03	0.98
P	Dosage	1	30.44	18	194.38	0.16	0.70

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Ratio	2	242.27	18	194.38	1.25	0.31
	Dosage x Ratio	2	315.32	18	194.38	1.62	0.23
K	Dosage	1	112986.30	18	2873.53	39.32	0.00
	Ratio	2	161246.00	18	2873.53	56.11	0.01
	Dosage x Ratio	2	165157.40	18	2873.53	57.48	0.01
Acid	Dosage	1	6.77	18	11.22	0.60	0.45
	Ratio	2	32.35	18	11.22	2.88	0.08
	Dosage x Ratio	2	21.96	18	11.22	1.96	0.17
Ca	Dosage	1	0.04	18	267.17	0.00	0.99
	Ratio	2	137.82	18	267.17	0.52	0.61
	Dosage x Ratio	2	534.85	18	267.17	2.00	0.16
Mg	Dosage	1	148.51	18	487.12	0.30	0.59
	Ratio	2	308.90	18	487.12	0.63	0.54
	Dosage x Ratio	2	611.87	18	487.12	1.26	0.31
Cu	Dosage	1	0.26	18	42.53	0.01	0.94
	Ratio	2	1.11	18	42.53	0.03	0.97
	Dosage x Ratio	2	38.74	18	42.53	0.91	0.42
Zn	Dosage	1	548.60	18	558.54	0.98	0.33
	Ratio	2	21.28	18	558.54	0.04	0.96
	Dosage x Ratio	2	277.34	18	558.54	0.50	0.62
Mn	Dosage	1	86.59	18	98.19	0.88	0.36

Parameter	Interaction	Df Effect	MS Effect	Df Error	MS Error	F	*p*-level
	Ratio	2	890.94	18	98.19	9.07	0.00
	Dosage x Ratio	2	10.80	18	98.19	0.11	0.90
Fe	Dosage	1	6.53	18	457.56	0.01	0.91
	Ratio	2	398.86	18	457.56	0.87	0.44
	Dosage x Ratio	2	999.55	18	457.56	2.18	0.14
Org C	Dosage	1	313.37	18	24.86	12.61	0.00
	Ratio	2	10.82	18	24.86	0.44	0.65
	Dosage x Ratio	2	36.25	18	24.86	1.46	0.26
N	Dosage	1	599.40	18	30.12	19.90	0.00
	Ratio	2	6.23	18	30.12	0.21	0.82
	Dosage x Ratio	2	0.34	18	30.12	0.01	0.99
Al	Dosage	1	754.24	18	246.17	3.06	0.10
	Ratio	2	225.33	18	246.17	0.92	0.42
	Dosage x Ratio	2	538.09	18	246.17	2.19	0.14