Decline of carpophores of mycorrhizal fungi
in stands of Pinus sylvestris

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Pinus sylvestris

Proefschrift

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Woord vooraf

Iedereen die bijgedragen heeft tot deze publicatie en mijn promotie wil ik van harte bedanken.

Theunis Limonard en Jan Barkman hebben veel werk verricht om het onderzoeksvoorstel te schrijven, en hebben op deze manier de fundamenten voor deze publicatie gelegd. Als dagelijks begeleider ben ik Theunis bijzonder veel dank verschuldigd voor zijn interesse en steun. Mijn beide promotoren, Prof. Dekker en Barkman, stelden mij in staat te promoveren. Ik dank jullie voor de uitgebreide en zinvolle discussies die ik met jullie gevoerd heb.

Naast bovengenoemd team hebben eveneens André Schaffers en Eef Arnolds mij veelvuldig bijgestaan. André heeft als doctoraalstudent, vrijwilliger en dienstweigeraar mij in staat gesteld het veldonderzoek aanzienlijk uit te breiden. Zowel met André als met Eef voerde ik veel discussies over de aanpak en de resultaten van dit onderzoek.

Belangrijk zijn ook de bijdragen geweest van Berry Diekema en Yvon van Haasteren, die gedurende twee respectievelijk één jaar als analist bij dit onderzoek betrokken zijn geweest. Zonder hun hulp zouden de experimenten en uitgebreide mycorrhizatellingen onmogelijk geweest zijn.

De kritische massa werd verder in belangrijke mate vergroot door de bijdragen van doctoraalstudenten: Jos Wintermans, Petra Ket en Ellen ter Stege. Ik heb bewondering voor het enthousiasme en de durf waarmee Jos Wintermans en Petra Ket het toen voor mij nog bijna onbetreden pad van het experimentele ectomycorrhiza-onderzoek aanpakten. Ellen ter Stege heeft veel hooi op haar vork genomen door zowel in belangrijke mate mee te helpen bij de veldbemestingsproef als bij het mycologische onderzoek van de eerste generatie terreinen.

De velen die dachten dat het veldwerk bestond uit het vrijblijvend zoeken van eetbare paddestoelen en die dit wel eens mee wilden maken, hebben dit altijd moeten bekopen met een dag hard werken; maar gezellig was het wel.

Het begassingsexperiment met zwaveldioxide heb ik uitgevoerd met Ludger van der Eerden en Thom Dueck: een fraai voorbeeld van een mutualistische symbiose.

De veldkennis van Ir. C.P. van Goor en zijn inzicht in de herkomstenproblematiek van de groveden in Nederland is van groot belang geweest bij de selectie van de terreinen voor het veldonderzoek.

De vakgroep Fytopathologie heb ik ervaren als een groep mensen waar iedereen aandacht voor elkaar heeft. Ik kwam er altijd met plezier. Het is niet mogelijk op deze plaats enkelen van de vakgroep te bedanken; hiermee zou ik anderen tekort doen. Een uitzondering wil ik echter maken voor Rob van der Vossen, die mij heel wat tijd bespaard heeft bij het zoeken naar praktische hulpmiddelen en ideeën. Iedereen van

harte bedankt voor de prettige samenwerking!

Op de vakgroep Bosbouw ben ik in staat gesteld mijn proefschrift af te ronden.

Het LEB-fonds en BION hebben het mij mogelijk gemaakt laboratoria in Schotland en de U.S.A. te bezoeken, alsmede twee mycorrhiza-congressen (in U.S.A. en Praag) bij te wonen. Deze reizen hebben bijgedragen tot mijn huidige inzicht in het mycorrhiza-onderzoek. Het LEB-fonds heeft bovendien de produktie van deze dissertatie gesubsidieerd.

In de loop van het onderzoek is aan de Landbouwuniversiteit een mycorrhiza overleggroep ontstaan. De discussies die hier plaatsvonden waren voor mij erg nuttig.

Ik heb de interesse die mijn ouders tentoongespreid hebben voor mij in het algemeen en dit onderzoek in het bijzonder altijd als een grote steun ondervonden. Ook de discussies met Eef Arnolds, Theunis Limonard en André Schaffers over het nut van dit onderzoek, van ander onderzoek en over geheel andere zaken zijn voor mij erg belangrijk geweest. Gedurende de eerste jaren van mijn onderzoek hebben mijn toenmalige huisgenoten Nico Heinsbroek en Bernie Jenster als geduldige praatpaal gefungeerd. De opmaak van de tabellen was in handen van Jan Breembroek. Ludger van der Eerden vervaardigde de tekening op de omslag. Bij de vervaardiging van de figuren en van de uiteindelijke lay-out van de tekst is mijn broer Koos behulpzaam geweest. Thom Dueck heeft de Engelse tekst gecorrigeerd. Door Ton Gorissen werd de laatste correctie van het manuscript verricht. En tot slot bedank ik hier graag Jan Breembroek, wiens aanstekelijke, tomeloos positieve kijk op het leven mij over de bergen en door de dalen van het schrijven van dit proefschrift geholpen heeft.

Aad Termorshuizen

STELLINGEN

1. De meeste wetenschappers die werken met planten, realiseren zich niet dat ze bijna altijd ook werken of dienen te werken met mycorrhiza's.

2. In definities van de term mycorrhiza waarin het mutualistische karakter van de symbiose belicht wordt is geen plaats voor de orchideeënmycorrhiza's.

3. De mycoflora in stuifzandgebieden kan het best beheerd worden door plaatselijke spontane opslag van bomen enerzijds toe te staan, maar er anderzijds zorg voor te dragen dat de bodem niet geheel vastgelegd wordt door de vegetatie.

4. Een verlaging van de stikstofinput in de Nederlandse bossen zal spoedig een positief effect hebben op de paddestoelenflora van mycorrhizavormende schimmels. Een geheel herstel zal echter pas kunnen plaatsvinden wanneer de luchtverontreiniging gedecimeerd is, de onnatuurlijke overmaat aan stikstof uit het bosoecosysteem verdwenen is en de vitaliteit van het bos hersteld is.
    Dit proefschrift.

5. Voordat bosbemesting in Nederland op grote schaal toegepast gaat worden ter bestrijding van de effecten van de stikstofdepositie moet het effect van bosbemesting op mycorrhiza's onderzocht worden, omdat een negatief effect grote gevolgen kan hebben op de vochtvoorziening van de bossen op de zandgronden in droge jaren.

6. Er wordt te weinig aandacht besteed aan het fysiologische en oecologische verschil tussen gedifferentieerde en ongedifferentieerde rhizomorfen van schimmels.

7. Bij de bepaling van naald- en bladverlies ter vaststelling van de boomvitaliteit in het nationaal vitaliteitsonderzoek van Staatsbosbeheer wordt er ten onrechte van uit gegaan dat bekend is wat de naald- en bladbezetting is onder "normale" omstandigheden.
    Anoniem, 1984-1987. Verslag van het landelijk vitaliteitsonderzoek, Staatsbosbeer.

8. De bestudering van natuurlijke processen in bodemoecosystemen in Nederland wordt essentieel beïnvloed door de hoge depositie van stikstofverbindingen.

9. De verspreidingspatronen van de in Nederland op houtige planten algemene soorten Honingzwammen (*Armillaria mellea, A. lutea* en *A. ostoyae*) worden in hoofdzaak bepaald door de zuurgraad van de bodem.

10. Geluidsoverlast is een onderschat probleem.

11. Rijkswaterstaat houdt bij het verzwaren van de rivierdijken te weinig rekening met de belangen van natuur, cultuur en bewoners.

12. Bij het opzetten van geïntegreerde landbouwsystemen zou een belangrijk doel moeten zijn een gunstig klimaat te scheppen voor de vesiculair arbusculaire mycorrhiza's.

13. Het toepassen van "alternatieve" landbouwmethoden op grote schaal is essentieel voor een minder vervuilde wereld en zal tegen relatief geringe kosten een positief *effect hebben op het milieu, de werkgelegenheid en de volksgezondheid.*

14. President Gorbatsjov van de Sovjet-Unie dient de Nobelprijs voor de vrede te krijgen.

15. Het gebruik van het woord "milieuvriendelijk" is bijna altijd ernstig misleidend en zou vervangen moeten worden door "minder milieu-onvriendelijk".

CONTENTS

Chapter 1

GENERAL INTRODUCTION

A decrease in the occurrence of carpophores of mycorrhizal fungi in the Netherlands and in other parts of Europe during this century has recently been reported. The subject of this study was to investigate the causes of this decrease.

A concise introduction on ectomycorrhizas will be presented in section 1.1, followed by the motives (section 1.2) and a hypothesis in section 1.3. The general approach of the present research is presented in section 1.4. Finally, the importance of this study will be considered in the scope of present-day mycorrhiza research in section 1.5.

1.1 Ectomycorrhiza: a short introduction

Mycorrhiza is the association of a fungus with living roots of a plant without causing damage to the plant. This association usually occurs during the whole life-span of the plant. Mycorrhizas possess characteristics which differentiate them morphologically from other plant infections. Usually both fungus and plant benefit from the mycorrhizal symbiosis: the fungus obtains carbohydrates from the plant and the host obtains water and mineral nutrients from the fungus.

Several mycorrhiza types which differ in morphology and physiology can be recognized. *Pinus sylvestris*, on which this research is focused, has only one type, viz. the ectomycorrhiza. Hence, we will deal here only with this type.

Ectomycorrhizas are characterized by the presence of a fungal layer called the Hartig net, which grows between the cortex cells of fine roots. In addition, a pseudoparenchymatic fungal mantle surrounds the fine roots. In ectomycorrhizas, penetration of the plant cells by the fungus does not occur, except in older stages of the mycorrhiza. The ectomycorrhizas are called ectendomycorrhizas if intracellular penetration occurs in a relatively young stage of mycorrhizal development (Harley, 1983). However, an objective distinction between ectomycorrhiza and ectendomycorrhiza is difficult, and because they have several characteristics in common, ectendomy-corrhiza is regarded here as a subtype of ectomycorrhiza. Ectomycorrhizas are extremely common and occur in many forest tree species.

Practically all ectomycorrhizal fungi belong to the *Basidiomycetes* and *Ascomyce-tes*. All ectomycorrhizal fungi are ecologically obligate symbionts, *i.e.* they grow under field-conditions only if they have formed mycorrhizas. Consequently, if carpophores of

these fungi are present, the mycorrhizas must also be present. Analysis of the aboveground carpophores is therefore a relatively easy way to identify a part of the population of mycorrhizal fungi present in a forest. However, it gives an incomplete picture of the species present, because fructification is dependent on season and weather. In addition, mycorrhizal fungi do not necessarily fructify every year, some mycorrhizal fungi fructify belowground (*e.g. Rhizopogon* Fr. & Nordh. *em.* Tul. *spp.*) or form very small, easily overlooked carpophores (*Ascomycetes spp.*) and finally some fungi do not form carpophores at all (*e.g. Cenococcum geophilum* Fr.).

Evidence of the mycorrhizal status of a fungal species can only be given if an isolate of the fungus has been shown to form mycorrhiza under axenical conditions. However, it is widely accepted on the basis of field observations that species from *e.g. Cortinarius* Fr., *Inocybe* Fr. and *Russula* Pers.: S.F.Gray form ectomycorrhiza (Trappe, 1962), although they have not yet been grown in culture.

The significance of ectomycorrhizas for the trees is (1) better provision of water and nutrients, and (2) increased resistance to pathogens. The increased provision of water and nutrients is thought to be caused by the fact that the absorption surface of the mycelium of mycorrhizal fungi is much larger than that of the plant root. The mechanisms involved in the protection afforded by mycorrhizal fungi to pathogens are supposed to be caused by (1) the fungal mantle, which serves as a physical barrier for the pathogens, (2) the decreased root exudation compared with that of non-mycorrhizal roots, and (3) the production of antibiotics by mycorrhizal fungi. In return, the mycorrhizal fungus obtains carbohydrates from the plant. One should best view the nature of a mycorrhiza as a dynamic but stable balance, where fungus and plant are able to withdraw nutrients from each other. The result is a mutualistic symbiosis.

From an ecological point of view it is important that ectomycorrhizas increase the tolerance of the host to stress. Effects of mycorrhizal infection are therefore more clear under circumstances of stress, for instance, drought or nutrient stress.

The main problems in ectomycorrhiza research are (1) the obvious difficulty of performing experiments under controlled conditions with full-grown trees and hence, the necessity to generalize the results of laboratory experiments with young plants; (2) the inability to grow many species of ectomycorrhizal fungi.

For more details, the introduction by Jackson & Mason (1984) and the standard-work by Harley & Smith (1983) are recommended.

1.2 Motives for the research

The mycoflora has changed drastically in the Netherlands during this century, as was shown by Arnolds (1985a & 1988). Arnolds (1985a) compared 15 excursion reports from

the period 1912-1954 with 15 equivalent reports from the period 1973-1982 and reported
a significant (P<5%) decline in the localities with fungal carpophores of 55 mycorrhizal
species, whilst not a single species showed a significant increase (table 1). In the first
period, an average of 71 ectomycorrhizal fruiting species were found per excursion,
compared to 38 species in the second period (Arnolds, 1988). On the other hand, a
number of saprophytic and parasitic fruiting species showed a significant increase,
while others significantly declined (table 1). In the first period, a total of 79 sapro-
trophic fruiting species was reported per excursion, compared to 87 in the second
period (Arnolds, 1988). The decline of carpophores of ectomycorrhizal fungi has
occurred in spite of the increased afforested area in the Netherlands, the increased
knowledge of fungal taxonomy and the strongly increased intensity of mycofloristic
research.

TABLE 1

Numbers of fruiting species of three ecological groups of fungi with a significant
(P<5%) increase or decline in numbers of localities between 1912-1954 and 1973-1982
(after Arnolds, 1988).

| Ecological group | No. of species | | |
	sign. increase	sign. decline	studied
Mycorrhizal fungi	0	55	126
Lignicolous fungi	13	4	94
Saprophytes on litter, humus, dung and fungi	7	13	94

The most spectacular decline was reported for the hydnaceous fungi, *Cantharellus*
cibarius Fr., *Cortinarius spp.*, *Dermocybe* (Fr.) Wünsche *spp.*, *Suillus* Micheli: S.F. Gray
spp. and *Tricholoma* (Fr.) Staude *spp.* (Arnolds, 1985a). Of a total of 800 ectomy-
corrhizal macrofungi, 56 (*i.e.*, 7%) are classified as extinct (Arnolds, 1989a). A detailed
study on the 21 native species of hydnaceous fungi in the Netherlands revealed that
eight species have not been observed since 1973 and are regarded to be extinct, six
species were not observed in at least 90% of the localities and the remaining seven
species were not observed in 58-87% of the localities (Arnolds, 1989a).

A decline of carpophores of mycorrhizal fungi from other countries in Europe is
reported as well (Derbsch & Schmitt, 1987; Fellner, 1988; Arnolds, in preparation), and
lists of threatened species have been published (Derbsch & Schmitt, 1984; Winterhoff &
Krieglsteiner, 1984; Wojewoda & Lawrynowicz, 1986; Arnolds, 1989a). Derbsch &
Schmitt (1987) gathered data on the supply of carpophores of *Cantharellus cibarius*,
Boletaceae spp. and *Armillaria mellea* (Vahl:Fr.) Kumm. at the market in Saarbrücken
between 1956 and 1975 (fig. 1). The mycorrhizal *Cantharellus cibarius* and *Boletaceae*
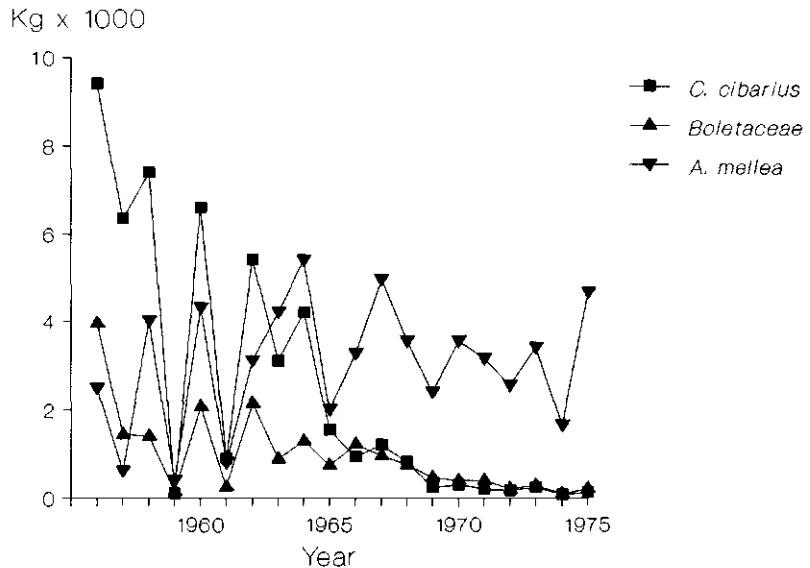
Figure 1. The supply of *Cantharellus cibarius*, *Boletaceae spp.* and *Armillaria mellea* at the market in Saarbrücken between 1956 and 1975 (after Derbsch & Schmitt, 1987).

spp. showed a drastic decline, in contrast to the non-mycorrhizal *A. mellea*, which did not decline at all.

According to Arnolds (1985a & 1988), the decline in the Netherlands has mainly taken place on acid sandy soils. On calcareous sandy soils and clayey soils the decline was absent or less prominent for most species. Fungi associated with coniferous trees declined more strongly than those associated with deciduous trees (Arnolds, 1985a). This might be caused by the fact that coniferous trees in the Netherlands are mainly restricted to acid sandy soils, in contrast to deciduous trees.

The decline of *Cantharellus cibarius* appears to have begun around 1960 (Jansen & Van Dobben, 1987). Before 1950, this species was extremely common, nowadays it is decidedly rare. A decrease in the number of localities of *C. cibarius* within three decades is reported to be as high as 49% in the province of Noord-Brabant. Jansen & Van Dobben (1987) and De Vries *et al.* (1985) reported that presently carpophores of mycorrhizal fungi occur most frequently in sites with a fairly thin humus layer.

Several hypotheses have been formulated to explain the decline of carpophores of mycorrhizal fungi. Most of them are related to the effects of air pollution on soil chemistry and on tree vitality. Other hypotheses relate to the succession of forests,

ageing of trees, changes in forest management, picking of mushrooms and pollution by heavy metals (*cf.* Jansen & Van Dobben, 1987; Arnolds, 1985a & in preparation.).

Jansen & Van Dobben (1987) excluded the possibility of excessive picking as explanation for the decline of *C. cibarius.* Most of the other fungi could not have declined due to increased mushroom collecting because they are inedible. Moreover, most edible fungi are seldom collected in the Netherlands. The possible effect of heavy metals on mycorrhizal fungi was excluded by Jansen & Van Dobben (1987) because of the low concentrations in the soil.

1.3 Hypothesis

It seems likely that the decline of carpophores of mycorrhizal fungi is linked with the decrease of tree vitality in Europe (Schütt & Cowling, 1985), because the decline has not been observed for saprophytic species (*cf.* sect. 1.2). A reduced host vitality most probably results in a reduction of mycorrhiza vitality and consequently, of that of the mycorrhizal fungus. Another possibility is that the air pollutants affect the mycorrhizal fungi via changes in the soil, which in turn, may also reduce tree vitality. However, irrespective whether air pollution primarily acts on the tree or on the fungus, it will always affect the symbiotic partner indirectly.

The working hypothesis therefore, was that the decline of the mycorrhizal mycoflora was caused by air pollution, either affecting the fungus via decreased tree vitality, or via changes in soil chemistry. Air pollution was expected to be the main factor affecting the fructification of mycorrhizal fungi because (1) the vitality of Dutch forests is decreasing drastically, which is generally ascribed to air pollution, (2) the decline of carpophores of mycorrhizal fungi mainly occurred on acid, poorly buffered sandy soils (Arnolds, 1985a), and (3) this decline was strongest in the more polluted, southern part of the country (Arnolds, 1985a).

1.4 General approach

The aim of the present research was to investigate whether a relationship could be found between the parameters air pollution, tree vitality, frequency of carpophores and quantity and quality of ectomycorrhizas. This relationship was studied in field observations, a field experiment, and in two laboratory experiments. Field observations and experiments each have important disadvantages: only indications can be obtained from field observations, and the conclusions from laboratory experiments are usually

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difficult to apply to the field situation. Therefore, it was considered essential to combine these approaches.

The regional variation in the present mycorrhizal mycoflora in the field was studied. The hypothesis was that this variation would parallel the known variation in air pollution. Another important question was whether the decrease in number of carpophores was related to a similar decline of mycorrhizas. For the causal interpretation of the correlations found in field work, experiments were carried out.

The research was focused on *Pinus sylvestris* L., because (1) its vitality was decreasing sharply in the Netherlands (Anonymous, 1984, 1985, 1986c, 1987), (2) the decline of the mycorrhizal mycoflora of coniferous species is more evident than for deciduous species (Arnolds, 1985a), (3) *P. sylvestris* is the only native conifer which possesses ectomycorrhizas and (4) plantations of *P. sylvestris* of the same age and on the same soil type can be found throughout the country, *i.e.* in both strongly and weakly polluted areas.

1.4.1 Field observations

The main part of the field observations was concerned with quantifying carpophores of mycorrhizal fungi and observations of tree vitality. It was supposed that counting carpophores (per species) would estimate the quantitative occurrence of the species present. There were several methodological reasons to concentrate the work on the carpophores, instead of direct observations on the mycorrhizas:

(1) In principle, the number of carpophores can be determined more objectively, whilst the number of mycorrhizas is dependent on the sampling method, viz. stand and plot choice, sampling date, number of samples, sample size, sample site (random, stratified), variation within the plots (trees, vegetation, soil) and sample depth. Only the first three variables (stand and plot selection and sample date) are of importance for an analysis of carpophores. However, the estimation of species which fructify in the soil, such as *Rhizopogon* species, is like the mycorrhizas dependent on the sampling method.

The high fluctuations in annual and seasonal fructification of carpophores were attempted to eliminate by sampling each plot several times during several years, a method to be recommended for mycorrhiza sampling.

(2) The mycorrhizal fungus involved in the symbiosis can not be usually identified. Although our knowledge of the morphology of mycorrhizas has been increased considerably by the work of Agerer (1987) and his colleagues, the majority of mycorrhizas is still unidentifiable, even at the genus level. Information on the number of mycorrhizas only (without identification) is insufficient if the fungal species

involved interact with environmental changes in different ways, which is likely to be the case. On the other hand, observations on mycorrhizas reveal species which never fructify (*e.g.*, *Cenococcum geophilum*), as well as species which do not happen to fructify during the research. An example is *Hebeloma* Kumm. *spec.* in a Douglas-fir stand in the Netherlands, which did not fructify for three years, while the typical mycorrhizas of this genus were recognized (Jansen & De Vries, 1988). Yet, many more species can be registered by the "carpophore method" than by the "mycorrhiza method".

(3) Quantification methods of mycorrhizas differ between laboratories. The difference between mycorrhizal and non-mycorrhizal roots is not sharp. Mycorrhizal units are interpreted differently and the distinction between dead and living roots is often unclear.

(4) A moderately or good sampling method requires an enormous amount of work. It was possible to cover at least three times as many plots with the "carpophore method" as would have been possible if mycorrhizas had been observed with the same intensity.

Naturally, most information is obtained by studying the carpophores as well as the mycorrhizas. With the "carpophore method", much more information can be obtained about the species composition, and mycorrhizas of all fungi are studied with the "mycorrhiza method", including the non-fruiting and hypogeous fungi. Therefore, the two approaches have been combined in this study. For practical reasons and because the original problem of this research concerned occurrence of carpophores, most emphasis was placed on the observations of carpophores. To study the relation between the occurrence of carpophores and mycorrhiza parameters, one sampling was made of the mycorrhizas in each plot.

At the time this research was planned, the existence of a clear succession of the *mycorrhizal mycoflora* became apparent (Mason *et al.*, 1982). In order to study different stages of mycorrhizal succession, two age classes of stands were involved in this research.

As the mycorrhizal mycoflora may differ strongly between different habitats of the same tree species (Jansen, 1981), only stands on dry sandy soils beyond the direct influence of ground water were selected. To reduce variation, plots were selected containing as few other ectomycorrhizal undergrowth as possible, and the undergrowth possessing ectomycorrhizas was removed (viz. *Betula* L. *spp.*, *Quercus robur* L. and *Q. rubra* L.). Finally, redundant variation was avoided by selecting only stands of a good provenance, as described in section 6.2.

1.4.2 Laboratory experiments

The laboratory experiments were carried out on mycorrhizal and, in one experiment, non-mycorrhizal seedlings. The following general ideas were considered essential for the experimental work:

(1) Fungal isolates were only made from carpophores collected in young stands, in order to exclude the possibility of fungi being adapted to old trees.

(2) Only those fungal species were used which were relatively easy to isolate and which formed mycorrhizas with young seedlings within three months.

(3) The effects of treatments were studied on plants which had already acquired mycorrhizas. Another possibility would have been to study the effects of treatments on the mycorrhization of plants, by treating the plant before and after addition of the fungal symbiont. The effects of treatments on already existing mycorrhizas are studied by the first method, and effects on newly synthesized mycorrhizas by the latter. The first method is probably a better simulation of reality, because the formation of mycorrhizas in nature usually occurs by mycorrhizal fungi which have already formed mycorrhizas in other places. In the alternative method, an unnatural situation is created in which the mycorrhizal fungus starts without having infected a host.

(4) The mycorrhizas were synthesized in culture tubes in order to prevent infection by unwanted mycorrhizal fungi, so-called 'nursery fungi' (*e.g. Thelephora terrestris* Ehrh.:Fr.). After the seedlings had acquired mycorrhizas, they were transplanted into plastic pots. From this moment on, the possibility of infection by other fungal species existed, but the chance of successful infection was thought to be reduced by the axenic synthesis in culture tubes. After transplantation, plants were allowed to acclimatize for several weeks.

(5) The pots used were large enough to allow free root growth during the experiment. Too small pots hinder root growth and the exploration of the soil by mycorrhizal fungus. The latter is probably essential to obtain a more or less naturally functioning mycorrhizal symbiosis. This is because the positive effect of mycorrhizal fungi on plant nutrition is thought to be caused by the fact that the mycelial absorption surface is much larger than that of the plant roots (*cf.* section 1.1).

(6) The soil used was sand, practically without humus. The sand was taken from a drift-sand area with occasional *P. sylvestris*. The sand was taken from places where no vegetation occurred. The soil was pasteurized instead of sterilized to reduce the possibility of the formation of fungus-inhibiting substances, which was already reduced by the practical absence of organic matter.

1.5 Relation to international mycorrhiza research

Mycorrhiza research on the basis of observations of carpophores is an indirect approximation of the problem. Until recently, this kind of research was highly underestimated. For instance, Harley & Smith (1983) treated aspects of carpophore research in a part of only one chapter. However, very interesting results were obtained by the group of Mason and Dighton, who reported the existence of a succession of mycorrhizal fungi in the course of stand development on the basis of carpophore observations (Mason *et al.*, 1982; Dighton & Mason, 1985).

Methods to study communities of macrofungi on the basis of carpophores (mycocoenology) have been thoroughly developed since 1963 at the Biological Station of the Agricultural University at Wijster (Barkman, 1987). Although the central subject was to study the mycofloras (including saprophytes and parasites) in different plant communities rather than the ecology of mycorrhizal fungi, the methods are the same. The methodology in mycocoenology is comprehensively treated by Arnolds (1981).

The only place with experience in experimental ectomycorrhiza research in the Netherlands was the department of Phytopathology of the Agricultural University, where this study was carried out. Experiments with inoculated axenically grown plants are well-known, and the type of experiments elaborated on in this study is not special (*cf.* section 1.4.2). However, study of the effects of air pollutants (including their deposition) on mycorrhizas is a relatively new branch of mycorrhiza research.

Chapter 2

RESPONSES OF MYCORRHIZAL AND NON-MYCORRHIZAL *PINUS SYLVESTRIS* SEEDLINGS TO FUMIGATION WITH SO_2 ALONE OR IN COMBINATION WITH NH_3

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2.1 Introduction

In this century, the occurrence of carpophores of mycorrhizal fungi has declined drastically in the Netherlands. Of the 126 mycorrhizal species investigated, 55 species have declined significantly, while none of the species has increased its occurrence significantly (Arnolds, 1988). On the other hand, the numbers of carpophores of saprophytic and parasitic fungi have remained constant or have increased slightly. Arnolds (1988) discussed the possible causes of this phenomenon and concluded that the decline of carpophores of mycorrhizal fungi is mainly attributed to acid precipitation and its effects on soil chemistry and the vitality of trees.

In mature stands of *Pinus sylvestris* (50-80 years), the number of carpophores of mycorrhizal fungi per plot (1050 m^2) showed a highly negative correlation with the 98-percentile SO_2 concentration and with the NH_3 deposition, while showing a positive correlation with several tree parameters, *e.g.* mean needle occupation per plot (Termorshuizen & Schaffers, 1987). The correlations with air pollution and tree vitality might be explained by (1) a decrease of the carbohydrate supply to the roots or (2) action via the soil, either acidification by SO_2 and NH_3 or eutrophication by NH_3, or both.

Our objectives in this study were to investigate (1) the effect of air pollution on the sensitivity of *P. sylvestris* inoculated with two mycorrhizal fungi and (2) the mechanisms through which air pollution affects mycorrhizal development.

The correlations mentioned above between the number of carpophores and SO_2 pollution in mature stands were not found in young stands (5-10 years) (Termorshuizen & Schaffers, 1987). We hypothesized that the disturbance of the upper soil-layer at the time of planting temporarily created more advantageous circumstances for the development of mycorrhizal fungi in young stands. Another possibility is that young plants are more tolerant to air pollution. It is interesting therefore to compare the results with young plants in the present fumigation experiment with the results of our field-work.

2.2 Material & methods

Experimental design

During 7 weeks, seedlings of *Pinus sylvestris* L. were exposed to 6 levels of SO_2 (0, 32, 66, 130, 260 and 520 μg m^{-3}) and to 2 levels of SO_2 (66 and 260 μg m^{-3}) combined with 100 μg m^{-3} NH_3. The seedlings were inoculated with the mycorrhizal fungus *Laccaria proxima* (Boud.) Pat. (Li = *L. proxima* inoculated), with *Paxillus involutus* (Batsch) Fr. (Pi = *P. involutus* inoculated), or non-inoculated (ni).

The surface of half of the Li- and ni-pots was covered with a layer of activated charcoal in order to absorb the pollutants and prevent them from entering the soil. In a pilot study, the charcoal layer prevented almost 100% of the SO_2 from entering the soil.

The different treatments and the number of plants per treatment are presented in table 2. Because of capacity problems not all possible combinations were carried out.

Plant, fungus and soil materials

Seeds of *P. sylvestris*, qualified as topseed, were obtained from the seed garden "Grubbenvorst".

The mycorrhizal fungi were isolated from carpophores in the autumn of 1986. *Laccaria proxima* and *Paxillus involutus* were collected from young (approx. 7 yr) stands of *Pinus sylvestris*. Both species were isolated on modified Melin-Norkrans agar medium (Marx, 1969).

The soil used was an extremely poor sand practically without humus, originating from a drift-sand area near Kootwijk. The soil had a pH(CaCl$_2$) of 4.3 and contained per kg soil 2.2 mg N-NO$_3$, 1.8 mg N-NH$_4$ and 0.3 mg P, analysed in a 0.01 M CaCl$_2$ extract (Houba *et al.*, 1986a). After pasteurization (25 min, 60°C), the humidity of the soil was maintained at 6% (w/w). In order to reduce evaporation, the surfaces of the pots were covered with a 1 cm layer of washed gravel.

TABLE 2

The treatments and the number of plants per treatment. ni = non-inoculated seedlings, Li = Laccaria proxima inoculated seedlings, Pi = Paxillus involutus inoculated seedlings, +C = soil covered with activated charcoal.

treatment	ni	ni+C	Li	Li+C	Pi
no fumigation	5	6	3	4	8
SO_2[a]	5	4	4	4	8
SO_2[b] + 100 μg m^{-3} NH_3	5	0	4	0	8

[a] 32, 66, 130, 260 and 520 μg m^{-3}
[b] 66 and 260 μg m^{-3}

The gravel in the charcoal-treated pots was then covered with 70 g of Norit RB-1 activated charcoal (ca. 1 cm thick layer). The acidic charcoal was neutralized by washing it in running water for 3 days, after which no change in the pH could be measured.

Preparation of the mycorrhizal plants

Culture tubes (length 145 mm, diam. 21 mm) were filled with 18 cm^3 of a 5:1 (v:v) mixture of vermiculite and peat. The mixture was then moistened with 1 ml distilled water and the tubes were autoclaved twice for 20 min. at 121°C. Twelve ml Norkrans nutrient solution (Norkrans, 1949) was then added after which the tubes were again autoclaved, and two plugs of mycelium were placed in each of the tubes.

P. sylvestris seeds were soaked in sterile distilled water for 1 night and surface sterilized the next day for 30 min. in 30% H_2O_2. After the fungi started to grow into the substrate, one seed was placed in each tube. After 7 weeks, uncontaminated seedlings which had developed mycorrhizas were transplanted into 650 ml plastic pots (height 12 cm, diam. 9.5 cm) containing 700 g pasteurized sand.

Plants were allowed to acclimatize for 2 weeks in a climate room with climatic conditions similar to those in the fumigation chambers (see below).

Plants were watered with demineralized water supplemented with 80 μmol l^{-1} Cl^- in the form of sea salt (*i.e.* the natural Cl^- concentration occurring in unpolluted rainwater near Wageningen). In order to prevent leaching of the pollutants accumulated in the charcoal layer, water was injected directly into the gravel layer.

Exposure to the pollutants

The experiment was started February 11, 1987 and terminated April 1, 1987.

Fumigation took place in fully controlled stainless steel fumigation chambers. The climatic conditions were maintained at 20 ± 0.5°C and 70 ± 3% RH during the day and 16 ± 0.5°C and 85 ± 3% RH during the night. Artificial light (450 μE m^{-2} s^{-1}) was supplied by HPL-N (mercury) and SON-T (sodium) lamps for a diurnal 16 hour light period.

Ambient air was passed via charcoal filters (1.5 s contact time) through fumigation chambers (3.3 m^3) at the rate of 80 cm s^{-1} (7 m^3 min^{-1}). SO_2 was continuously supplied to the ventilation air by thermal mass flow controllers (Brooks 5850 TR) to final concentrations of 0, 32, 66, 130, 260 and 520 μg m^{-3} SO_2. SO_2 concentrations in the fumigation chambers were sequentially monitored (Teco 43) and regulated by a HP 1000-40 computer and joined to a HP data acquisition system. NH_3 was continuously injected in a similar manner and after being passed through a stainless steel tube heated to 800°C to oxidize NH_3, it was monitored with a NO_x monitor (CSI 1600).

Measurement of aboveground parameters

Prior to terminating the exposure to the pollutants, 4 Pi-plants from the 0, 32, 260 and 520 μg m^{-3} SO$_2$ treatments were used for the measurement of photosynthesis. Directly after the selected plants were removed from the fumigation chambers, photosynthesis was measured at 22.4°C, 74% RH and a light intensity of 230 μE m^{-2} s^{-1} (400-700 nm).

At the end of the experiment the plants were observed macroscopically for damage symptoms. Further measurements included determination of the maximum and mean needle length and estimation of the percentage of secondary needles. The shoot was then dried and weighed.

Measurement of underground parameters

The root systems were stored in a glutaraldehyde buffer (Alexander & Bigg, 1981) for further analysis. Soil samples were air dried after which the pH was measured in a 10 g sample shaken 24 hr in a 100 ml 0.01 M CaCl$_2$ solution.

The number of root tips, divided into non-mycorrhizal and mycorrhizal, was counted. Mycorrhizal roots were defined as roots with a Hartig net between at least some of the cortical cells. Non-mycorrhizal root tips were divided into short (<2 cm) and long roots (>2 cm).

The mycorrhizal roots were divided into forked and unforked mycorrhizas, according to their branching. The individual tips of forked mycorrhizas were not counted. The mycorrhizal roots were also classified based on their appearance. The first class (so-called well-developed mycorrhizas), possessed a smooth, relatively thick mantle, so that the root cells were not visible under a 12x magnification. The poorly-developed second class mycorrhizas either possessed a dented, more or less wrinkled mantle or no distinct mantle.

Statistics

Results were tested for significant differences using Student's t-test.

2.3 Results

Mycorrhiza development (table 3)

No mycorrhizas were found in the ni-plants. At all treatments, the Pi-plants showed a significantly smaller mycorrhizal frequency than the Li-plants (P<1%). The mycorrhizal frequency of Li-plants remained at a very high level (93-97%) in all treatments, whereas the mycorrhizal frequency of the Pi-plants decreased significantly from the 130 μg m^{-3} SO$_2$ treatment onwards. Addition of 100 μg m^{-3} NH$_3$ to 66 or 260 μg m^{-3} SO$_2$

TABLE 3

Mycorrhizal frequency (= total number of mycorrhizas relative to the total number of short roots), the relative number of forked mycorrhizas (= forked / total number of mycorrhizas * 100%), the relative number of well-developed mycorrhizas (= well-developed / total number of mycorrhizas * 100%) in the Li- and Pi-plants and the number of sclerotia per Pi-plant. Asterisks indicate a significant difference with the control. * = P<2.5%, ** = P<1%.

Treatment	Mycorrhizal frequency		Forked mycorrhizas (%)		Well-developed mycorrhizas (%)		No. sclerotia
	Li	Pi	Li	Pi	Li	Pi	Pi
0 SO_2	97±1	78±16	24±11	36±15	89± 7	75±16	55±40
32 SO_2	97±1	73±22	28±10	36±14	89± 5	84±13	56±22
66 SO_2	97±1	73± 8	28±12	43±11	90± 6	74±11	76±31
66 SO_2+NH_3	97±1	32±24**	27± 6	26±20	83± 5	45±24**	44±39
130 SO_2	96±2	45±33*	28±10	27±15	82±10	65±32	41±25
260 SO_2	97±1	44±18**	31± 8	29± 8	77± 6	64±22	57±43
260 SO_2+NH_3	96±2	30±10**	28± 9	37±16	83± 5	55±10**	36±22
520 SO_2	93±8	33±21**	23± 7	27±17	80± 8	75±18	19±12*

significantly reduced the mycorrhizal frequency of the Pi-plants compared to the treatments without NH_3 (P<5%).

The percentage of forked mycorrhizas of the Li-plants was not affected by the fumigation treatments, while that of the Pi-plants tended to be negatively affected, although not significantly.

In almost all treatments more than 50% of the mycorrhizas were classified as well-developed. The relative number of well-developed mycorrhizas of Li-plants tended to decrease slightly with increasing SO_2 concentrations. On the other hand, the relative number of well-developed mycorrhizas of Pi-plants was not affected by the SO_2 treatments alone, but was significantly reduced by the SO_2 + NH_3 treatments (P<1%).

The number of sclerotia formed by *P. involutus* decreased significantly only in the highest SO_2 treatment by a factor 2-3 compared with the other treatments. The combined SO_2 + NH_3 treatments compared to SO_2 alone reduced the number of *P. involutus* sclerotia, but these differences were not significant.

Plant growth (table 4)

No macroscopical symptoms on the shoots were observed.

The total dry weights of the non-fumigated plants were highest for the ni-plants, intermediate for the Pi-plants and lowest for the Li-plants, the latter group being significantly different with the ni-plants at P<5%. The ni-plants showed the lowest shoot dry weights and the highest root dry weights. The order of root biomass

TABLE 4

Shoot-root ratios and plant dry weights (mg) of the Li-. Pi- and ni-seedlings of P. sylvestris following exposure to SO_2 (μg m^{-3}) and NH_3 (100 μg m^{-3}). Asterisks indicate a significant difference with the control.
* = P<2.5%, ** = P<1%.

Treatment	Shoot-root ratio			Plant dry weight		
	Li	Pi	ni	Li	Pi	ni
0 SO_2	1.0±0.1	1.0±0.5	0.7±0.1	161± 9	184±51	186±66
32 SO_2	1.1±0.3	0.7±0.4	0.5±0.2	146±29	192±34	181±34
66 SO_2	1.0±0.2	1.1±0.3	0.8±0.2	171±29	199±36	224±54
66 SO_2+NH_3	1.1±0.1	0.9±0.2	0.6±0.3	183±21	186±59	203±71
130 SO_2	1.1±0.1	0.9±0.2	0.6±0.1	148±40	183±32	187±87
260 SO_2	1.5±0.2*	1.0±0.2	0.5±0.3	149±19	198±47	224±54
260 SO_2+NH_3	1.6±0.4*	1.2±0.2	0.6±0.0	183±21	211±23	203±71
520 SO_2	1.9±0.3*	1.2±0.4	1.1±0.6	92±22**	163±34	179±76

production is ni > Pi > Li. Only the root dry weights in the Li-group were significantly different (P<2.5%) from the control group. The shoot-root ratios were significantly lowest for the ni-plants (P<2.5%), and highest for the Li-plants.

The fumigation treatments did not affect the plant dry weights, except for Li-plants in the highest SO_2 treatment. The shoot-root ratios generally increased at the higher SO_2 treatments, but were significant only for the Li-plants from the 260 μg m^{-3} SO_2 treatment onwards (P<2.5%).

Photosynthesis (table 5)

Large effects due to the fumigation treatments were observed on the photosynthesis of the Pi-plants (table 5). The amount of photosynthesized CO_2 per g fresh weight of the needles decreased significantly by 47% and 70% in the 260 and 520 μg m^{-3} SO_2 treatments, respectively.

Soil pH

The pH of the soil was 4.3 ± 0.2 for all treatments.

TABLE 5

Photosynthesis per g fresh needles of Pi-plants after fumigation of P. sylvestris seedlings with SO_2. Asterisks indicate significant difference with the control.
** = P<1%.

Treatment	mg CO_2 g^{-1} h^{-1}
0 SO_2	0.53 ± 0.12
32 SO_2	0.50 ± 0.09
260 SO_2	0.28 ± 0.06**
520 SO_2	0.16 ± 0.07**

Activated charcoal effects

Activated charcoal covered the soil surface of the ni- and Li-plants only. No effects of the addition of charcoal were found, neither for the plant, its mycorrhizas, nor for the pH of the soil.

2.4 Discussion

The fumigations had different effects on the two fungal species. The mycorrhizal frequency of *L. proxima* was not decreased, while on the other hand, the mycorrhizal frequency of *P. involutus* was inhibited from 66 μg m^{-3} SO$_2$ combined with 100 μg m^{-3} NH$_3$, or from 130 μg m^{-3} SO$_2$ alone onwards (table 3). These SO$_2$ concentrations are comparable with the 95- and 98-percentile concentrations occurring in the Netherlands (Anonymous, 1986ab).

The question now arises how *P. involutus* is affected, and why *L. proxima* is not affected by SO$_2$. That SO$_2$ affected mycorrhizas via the soil is unlikely, because no changes in the soil pH were detected, and the charcoal did not influence the fumigation effects.

The negative effect of SO$_2$ on the mycorrhizal frequency of Pi-plants may well be explained by the inhibition of photosynthesis, possibly in combination with a decrease in carbohydrate transport (McLaughlin *et al.*, 1982; Lorenc-Plucinska, 1986), which decreases the availability of carbohydrates for the mycorrhizal fungi. Likewise, shading or defoliating trees results in a decreased mycorrhizal frequency (*e.g.* Ekwebalam & Reid, 1983) and a decreased number of carpophores of mycorrhizal fungi (Last *et al.*, 1979).

In contrast to the mycorrhizal frequency of the Pi-plants the plant dry weight was not affected by SO$_2$, except for the Li-plants at the highest SO$_2$ treatment. Comparable results were obtained in vital, mature stands of *P. sylvestris*, where a significant negative correlation of the SO$_2$ concentration with the number of carpophores of mycorrhizal fungi was found (*cf.* Termorshuizen & Schaffers, Ch. 6). At the same time, the average stand vitality showed a small negative correlation with the SO$_2$ concentration. However, the correlation of the SO$_2$ concentration with the mycorrhizal frequency was very weak, indicating that the formation of carpophores is hampered before the development of mycorrhizas is affected.

The results from the present study as well as from the field observations indicate that the assimilate-dependent structures which are most distant from the photosynthetic apparatus (*i.e.*, the mycorrhizal fungus and the mycorrhiza) are the first to be affected if the production and transport of carbohydrates is reduced. This seems to be due to both a decrease in the photosynthesis and a higher retention of carbohydrates

by the needles. Lorenc-Plucinska (1986) and Gorissen & Van Veen (1988) reported a much stronger inhibition of assimilate transport than of the photosynthesis itself in seedlings of *P. sylvestris*, regardless of the concentration used (650-1950 μg m^{-3} SO_2 for 5 hr).

In contrast to the mycorrhizal frequency, the percentage of forked mycorrhizas of *P. involutus* was not affected by the fumigation treatments. This indicates that at all fumigation levels, once established mycorrhizas are able to continue their growth at the expense of new infections.

Because *L. proxima* mycorrhizas were not affected by the pollution treatments, the carbohydrate demand of *L. proxima* is apparently much lower than that of *P. involutus*, or, alternatively, *L. proxima* is a stronger sink for carbohydrates than *P. involutus*. Indirect evidence for a lower carbohydrate demand of *L. proxima* is the absence of rhizomorphs and sclerotia in *L. proxima* and the formation of much smaller carpophores than *P. involutus* (Dighton & Mason, 1985). On the other hand, Stenström & Unestam (1987) and Gagnon *et al.* (1988) ascribe relatively high sink capacities to *Laccaria* species (*L. proxima* and *L. bicolor* (Maire) P.D.Orton, respectively) compared with other mycorrhizal fungi. This may also be true in this experiment, where lower dry weights of Li-plants compared to the Pi-plants were found. The lower dry weight of inoculated plants compared to ni-plants might be an initial effect of mycorrhizal formation caused by the absorption of photosynthates by the mycorrhizal fungus (*e.g.* Dighton *et al.*, 1987; Gagnon *et al.*, 1988).

The negative effect of NH_3 added to the SO_2 fumigations on *P. involutus* mycorrhizas might be caused by direct uptake of the shoot as well. Kaupenjohann *et al.* (1989) suggested that the negative effects found on *P. sylvestris* needles were due to NH_3 from a near-by hen-house. Dueck *et al.* (in press) and Van der Eerden *et al.* (1989) reported physiological disturbances in NH_3-polluted young plants of *P. sylvestris* and *Pseudotsuga menziesii*, respectively. Another possible pathway of NH_3, via the soil, is the subject of a following study (*cf.* Ch. 3 & 4).

In this study, we showed that SO_2 can affect the mycorrhizas of *P. sylvestris* seedlings in a relatively short time. We further obtained indications that the effects are highly dependent on the fungal symbiont. This may well explain the fact that some mycorrhizal fungi did not decline this century in the Netherlands (Arnolds, 1988).

2.5 Summary

The effects of a range of SO_2 concentrations from 0 to 520 μg m^{-3} (including two concentrations of SO_2 combined with 100 μg m^{-3} NH_3) on 9 week old seedlings of *Pinus sylvestris* were studied. Seedlings were non-inoculated or inoculated with either

Paxillus involutus or *Laccaria proxima*. In order to study the effect of SO_2 fumigation on the mycorrhiza via the soil, half of the non-inoculated pots and half of the pots inoculated with *L. proxima* were covered with a layer of activated charcoal.

Exposure to SO_2 for 7 weeks decreased the photosynthesis of the plants significantly. Mycorrhizal formation of *L. proxima* was not affected by SO_2, whilst that of *P. involutus* decreased significantly from 130 μg m^{-3} SO_2 or 66 μg m^{-3} SO_2 + 100 μg m^{-3} NH_3 onwards. Activated charcoal did not influence the results and the pH of the soil showed no differences between the treatments.

The results are compared with field observations, and the possible mechanisms involved are discussed.

Chapter 3

EFFECTS OF AMMONIUM AND NITRATE ON MYCORRHIZAL SEEDLINGS OF *PINUS SYLVESTRIS*

3.1 Introduction

Under natural conditions, nitrogen is a growth-limiting factor in forest ecosystems. In the Netherlands however, nitrogen input in forests has reached levels which are very likely deleterious to trees (Nihlgård, 1985; Roelofs, 1985). The mean deposition levels for nitrogen oxides (mainly from industries and traffic) and ammonia (mainly from farmyard manure) in 1986 in the Netherlands were 22 and 19 kg N ha^{-1} yr^{-1} respectively (Anonymous, 1988). Local extremes up to 64 kg ha^{-1} yr^{-1} (Van Breemen *et al.*, 1982) can be largely ascribed to ammonia because of its relatively high dry deposition rate (Asman & Maas, 1987).

The dry deposition of ammonia on leaves may increase growth of epiphyllic algae and may enhance fungal and insect attacks (Nihlgård, 1985). Plants can take up ammonia and ammonium sulphate particles directly from the air through the stomata, which results in leaf damage (Gmur *et al.*, 1983). In the soil, ammonia is rapidly changed into ammonium compounds. Deposition of ammonia can therefore be seen as an ammonium fertilization effect. According to Nihlgård (1985), an increased uptake of nitrogen increases growth and conversion of carbohydrates into amino acids, which in turn decreases frost hardiness, carbohydrate transport to the roots and mycorrhizas, and increases leaching of nutrients from the leaves.

Although the effects of different forms of nitrogen on mycorrhizas have often been investigated and discussed, only low concentrations have usually been considered. In the present study, we examined the effects of high depositions of nitrogen on mycorrhizas in the following laboratory experiment and a field experiment (Termorshuizen, Ch. 4). In the laboratory experiment we studied the effects of ammonium and nitrate on already established mycorrhizas formed by *Pinus sylvestris* after inoculation with either *Paxillus involutus* or *Suillus bovinus*.

3.2 Material & Methods

Preparation of the mycorrhizal plants

Seeds of *Pinus sylvestris* L. were obtained from the seed garden "Grubbenvorst". They were soaked in sterile distilled water for one night and surface sterilized the next day in 30% H_2O_2 for 30 min. Subsequently, the seeds were allowed to germinate on sterile 1.0% agar containing 0.5% glucose.

Mycorrhizal fungi were isolated from carpophores in the autumn of 1985. *Paxillus involutus* (Batsch:Fr.) Fr. and *Suillus bovinus* (L.:Fr.) O.Kuntze were collected from a 6-year-old stand of *P. sylvestris* and were isolated on modified Melin-Norkrans agar medium (Marx, 1969).

Culture tubes (length 145 mm, diam. 21 mm) were filled with 25 cm^3 vermiculite, moistened with 2 ml distilled water and then autoclaved twice for 20 min. at 121°C. Thirteen ml MMN nutrient solution (Marx, 1969) was then added after which the tubes were autoclaved again for 20 min. at 121°C. After the last autoclaving, two plugs of mycelium and one germinated seed were placed in each of the tubes.

After 10 weeks, uncontaminated seedlings which had acquired mycorrhizas were transplanted into plastic pots (height 17.5 cm, diam. 18 cm) containing 3300 g pasteurized sand. In order to reduce evaporation, the surfaces of the pots were covered with a 1 cm layer of gravel.

The soil used was an extremely poor sand practically without humus, originating from a drift-sand area near Kootwijk (pH($CaCl_2$) 4.3, 2.2 mg kg^{-1} N-NO_3, 1.8 mg kg^{-1} N-NH_4 and 0.3 mg kg^{-1} P). Soil analysis was performed according to Houba *et al.* (1986a, 1986b) in a 0.01 $CaCl_2$ extract. After pasteurization (25 min, 60°C), soil moisture was maintained at 5-7% (w/w).

Treatments

The experiment took place in a glasshouse without temperature or air humidity control. Soil moisture was maintained at 5-7% (w/w) after weighing the pots once, or during periods of warm weather, twice a week. After acclimatizing for 6 weeks, plants were watered weekly with ammonium sulphate or sodium nitrate in distilled water, equalling 0, 0.49, 2.5, 4.9 and 19.6 mg N wk^{-1} pot^{-1} or 0, 10, 50, 100 and 400 kg N ha^{-1} yr^{-1} (recalculation based on the pot surface). For reasons of simplicity, the treatments in kg N ha^{-1} yr^{-1} will be used here and will be abbreviated to kg. The solutions were adjusted with HCl to pH 4.5.

A weekly fertilization was chosen instead of a single fertilization because the former simulates the field situation better and the plant and mycorrhizal fungus would likely suffer from the sudden change in nutrition following a single fertilization. Ammonium sulphate was used because this approaches the reality better than ammonium

tartrate or ammonium phosphate. Sodium nitrate was used because it is generally considered to affect the plant less than hydrogen, potassium or magnesium combined with nitrate (Richards, 1965).

The fertilizations started May 19, 1986 and the plants were harvested November 23, 1986 (26 weeks).

Measurement of parameters

After terminating the experiment, shoot length, number of branches per shoot, percentage of secondary needles and needle color was estimated. The needles and stems were dried separately for 48 hr at 80°C and weighed. The root system was carefully removed from the soil, gently washed and stored in a glutaraldehyde buffer (Alexander & Bigg, 1981) until further analysis took place.

The root length and the number of root tips were determined on a random part of the fragmented root system (1-5 cm fragments). The samples consisted of at least 40 random subsamples, and at least 20% of the root system. The root tips were divided into non-mycorrhizal and mycorrhizal.

It was necessary to develop a working definition for mycorrhizal roots because intermediate types were observed between typical mycorrhizal roots (with a clearly visible mantle and aerial mycelium) and non-mycorrhizal roots (with many, approx. 5 mm long root hairs). Short roots with one or two poorly developed root hairs always possessed a Hartig net between some of the cortical cells. Short roots with more than two poorly developed root hairs often had no Hartig net. Therefore, all short roots possessing less than three root hairs were called mycorrhizal, and those with more than two root hairs non-mycorrhizal.

The mycorrhizal roots were divided into forked and unforked mycorrhizas according to their branching. The individual tips of forked mycorrhizas were not counted.

Non-mycorrhizal root tips were divided into short (<2 cm) and long (>2 cm) roots.

N, P, K and Mg content of the needles of two seedlings per treatment were analysed according to Novozamsky *et al.* (1988).

3.3 Results

Plant parameters

The dry weight of *P. involutus*-inoculated plants increased significantly (P<1%) to the nitrogen treatments, with an optimum at 50 kg (fig. 2a). Ammonium stimulated growth more than nitrate, except at the 400 kg treatment. The dry weight of *S. bovinus*-inoculated plants showed no significant change due to the treatments except an increase (P<5%) at the 100 kg ammonium treatment. The changes in biomass production can be ascribed to changes in the shoot biomass. However, for both fungal treatments, fertilization from 50 kg onwards negatively affected the root length. The root length/weight ratio decreased up to a factor 2.6 and 1.6 for the *P. involutus* and *S. bovinus* inoculated plants, respectively (fig. 2b). This decrease was significant in *P. involutus*-inoculated plants (P<5%) for all treatments, except for the 10 kg ammonium treatment, and was only weakly significant for the highest treatments (P<7%) for the *S. bovinus*-inoculated plants. Accordingly, roots from the higher nitrogen treatments had thicker roots than those of the control.

The average shoot length at the end of the experiment was 11 cm for the *P. involutus*-inoculated plants and 7.5 cm for the *S. bovinus*-inoculated plants. The shoot length was only positively affected by the 100 kg nitrate treatment of the *P. involutus*-inoculated plants (P<5%). There were no effects on the percentage of secondary needles or on the number of branches. Except for one plant (treatment *P. involutus*, 400 kg ammonium) no changes in the needle color were observed.

Mycorrhiza parameters

The mean number of *P. involutus* mycorrhizas per treatment varied from ca. 1000 to 6000 per plant, increasing with both 10 kg treatments and then decreasing (fig. 4a). The decrease was significant from the 100 kg ammonium treatment onwards, and for the highest nitrate treatment. The two highest treatments showed significantly more mycorrhizas in the nitrate treatments than in the ammonium treatments (P<1%). The total number of *S. bovinus* mycorrhizas per plant ranged from ca. 500 - 2500, and showed no clear differences between the nitrogen levels.

The reduction in total number of mycorrhizas at high nitrogen levels was much more than the decrease in plant dry weight. Consequently, the number of mycorrhizas per unit shoot dry weight (fig. 4b) decreased significantly, for the *P. involutus* (P<1%) and *S. bovinus*-inoculated (P<5%) plants, and for ammonium and nitrate from the 50 kg treatments onwards. The largest difference occurred between the 10 and 50 kg treatments. The negative effect of nitrate on the number of mycorrhizas per unit shoot
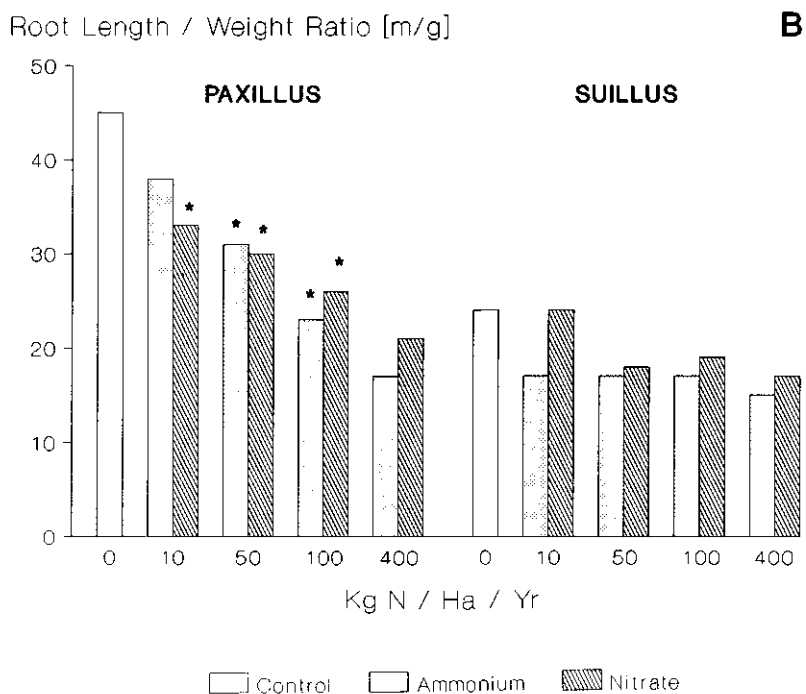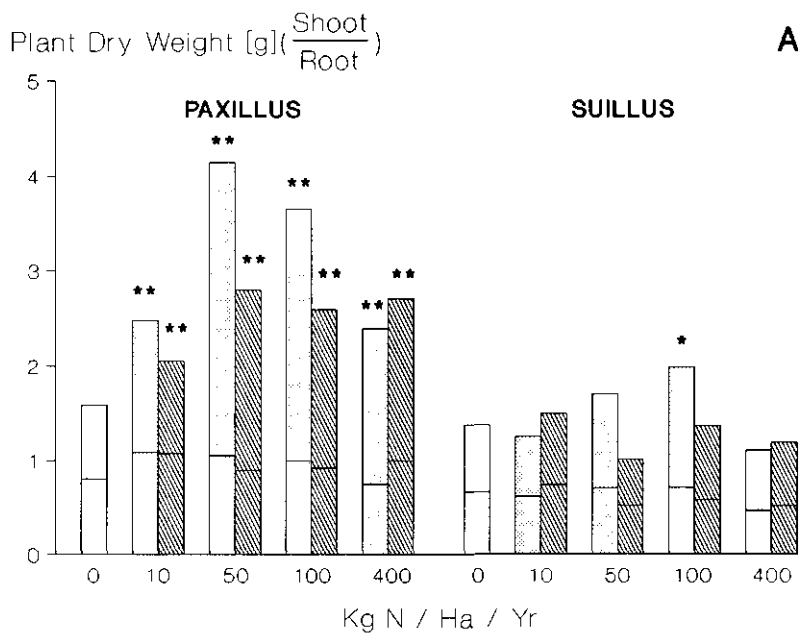
Figure 2. The effect of ammonium sulphate and sodium nitrate on (a) total plant dry weight [g] (the lower part of the bars represents the root dry weight) and (b) root length/weight ratio [m/g]. Asterisks indicate a significant difference from the control. ** = P<1%, * = P<5%.

dry weight was somewhat smaller at the 100 and 400 kg treatments than the effect of ammonium.

Although the mycorrhizal frequency of *P. involutus*-inoculated plants was significantly decreased at the higher ammonium treatments (P<1%) compared to the control, ca. 40% of the root system was still mycorrhizal (fig. 3). On the other hand, mycorrhizal frequency of *P. involutus*-inoculated plants was only slightly reduced due to the nitrate treatments. The mycorrhizal frequency of *S. bovinus*-inoculated plants showed an insignificant decrease.

We determined the percentage of forked mycorrhizas relative to the total number of mycorrhizas to obtain a parameter for the growth of individual mycorrhizas. The relative number of forked mycorrhizas varied and was not affected by the treatments.

The number of sclerotia produced by *P. involutus* was drastically decreased by ammonium and nitrate treatments from 50 kg treatments onwards (P<1%, table 6). The number of sclerotia at the 10 kg treatments was higher than in the control, significantly so (P<1%) for the ammonium treatment.
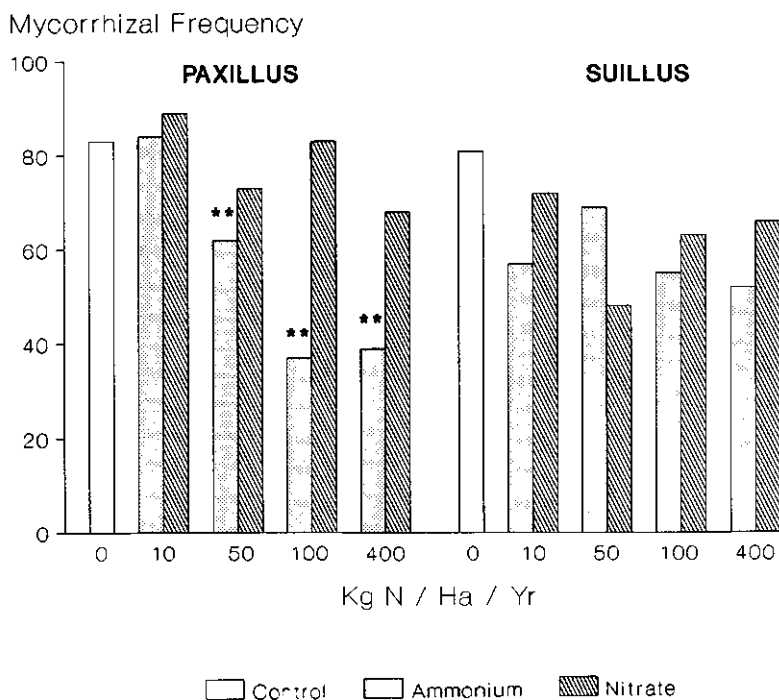
Figure 3. The effect of ammonium sulphate and sodium nitrate on the mycorrhizal frequency (= total number of mycorrhizas / total number of (mycorrhizal and non-mycorrhizal) root tips * 100%). Asterisks indicate a significant difference from the control. ** = P<1%, * = P<5%.
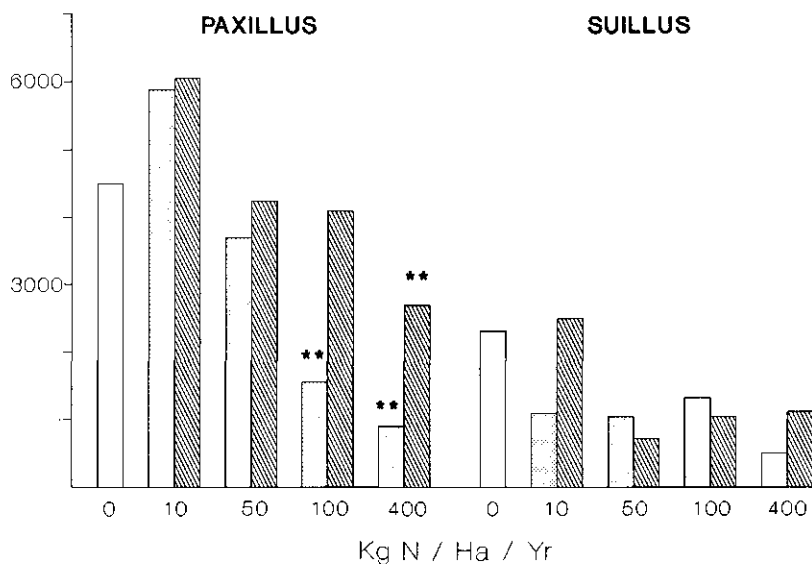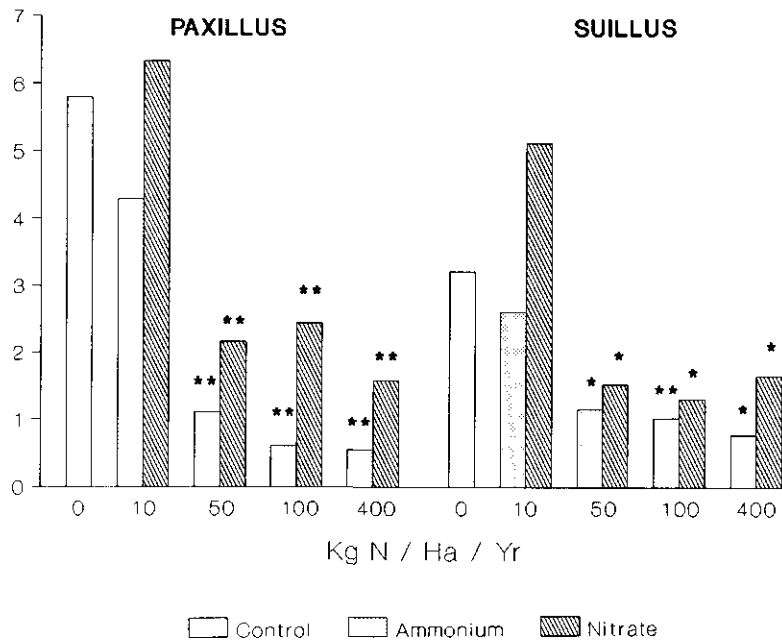
Figure 4. The effect of ammonium sulphate and sodium nitrate on (a) the total number of mycorrhizas per plant and (b) the number of mycorrhizas per mg shoot dry weight. Asterisks indicate a significant difference from the control. ** = P<1%, * = P<5%.

TABLE 6

The mean number of P. involutus sclerotia per plant (± S.D.). Asterisks indicate a significant difference from the control. ** = P<1%, * = P<5%.

Treatment	Level (kg N/ha/yr)
	0	10	50	100	400
Control	178± 79				
Ammonium		581±185**	5± 3**	3±5**	13±15**
Nitrate		288±197	20±26**	7±9**	4±15**

Concentration of nutrients in the needles

The nitrogen concentration in the needles was positively correlated with the amount of nitrogen input (table 7). Needles of plants treated with ammonium contained higher nitrogen concentrations than those treated with nitrate, and needles of *S. bovinus*-inoculated plants contained more nitrogen than *P. involutus*-inoculated plants. The concentration of P, K and Mg in the needles generally decreased at higher treatments (table 7). At the 400 kg treatment, P concentration is decreased ca. 50% compared to the control. The same holds for K concentrations in ammonium-treated needles. In the nitrate treatments however, a slight decrease in the K concentration appeared only in *P. involutus*-inoculated plants, and increased in *S. bovinus*-inoculated plants at the highest nitrate treatment. The Mg concentration in the needles did not change significantly from the control.

Soil pH

There was only a very small fertilization effect on soil pH. Compared to the control, the highest treatments differed -0.1 and 0.1 pH units for the ammonium and nitrate treatments, respectively.

TABLE 7

Total N, P, K and Mg content of Pinus sylvestris needles (mmol kg^{-1} dry weight).

Treatment (kg N/ha/yr)		\multicolumn{4}{c}{Paxillus involutus}	\multicolumn{4}{c}{Suillus bovinus}
		N	P	K	Mg	N	P	K	Mg
Control	0	1152	80	280	69	1602	97	271	65
Ammonium	10	1180	70	262	54	2175	44	264	57
	50	1785	59	178	59	2958	33	232	44
	100	2084	43	170	35	2691	46	211	39
	400	2830	39	152	44	3421	25	160	42
Nitrate	10	1245	76	256	63	1322	62	253	62
	50	1487	58	231	59	1641	37	293	53
	100	1436	60	196	68	2328	38	285	56
	400	1861	40	214	53	2764	24	366	43

3.4 Discussion

The results show that fertilization with ammonium as well as with nitrate has a significant positive effect on the shoot dry weight of *P. involutus*-inoculated plants, with an optimum at 50 kg. The effects on shoot dry weights of *S. bovinus*-inoculated plants were much smaller (ammonium treatments) or absent (nitrate treatments). Evidently, the positive effect will be found only if the nitrogen content of the soil is very low. The decrease of plant dry weights at high fertilization rates are likely to be caused by the decrease of mycorrhizal formation and deficiency of other nutrients.

The reason for the increased weight of the roots per unit of length is unclear. It was not caused by a decreased number of mycorrhizal roots, because roots from the highest treatments were clearly thicker than those of the control. Comparable observations were made by Olsthoorn *et al.* (in preparation), who found an 1.8 decrease of the root length/weight ratio in one-year-old Douglas fir seedlings treated with 60 mMol $(NH_4)_2SO_4$ per plant for five months (ca. 336 kg N ha^{-1} yr^{-1}).

The ammonium fertilized plants produced higher dry weights than the nitrate fertilized plants, caused by a preferential uptake of ammonium (table 7), which is confirmed by most authors (France & Reid, 1983).

In contrast to the reaction of plant dry weight, the mycorrhizas were more inhibited by ammonium than by nitrate, indicating an indirect effect by nitrogen via the internal nutritional status of the plant (Björkman, 1942). Following Björkman's hypothesis, mycorrhizal formation decreases if the absorption of nitrogen by the plant increases. This causes an increased conversion of carbohydrates into amino acids, reducing the supply of carbohydrates to the mycorrhizas.

Björkman's hypothesis is in agreement with our results. In the case of nitrate nutrition, lower nitrogen concentrations in the needles were found, making relatively more carbohydrates available to the mycorrhizal fungus, which results in a higher mycorrhizal frequency and a higher number of mycorrhizas than in the case of ammonium nutrition.

Ammonium and nitrate did not affect the percentage of forked mycorrhizas. Once established, mycorrhizas are probably able to continue growth at the expense of new infections and in the case of *P. involutus*, the formation of new sclerotia.

Our results do not agree with those of some authors, who report that mycorrhizal fungi prefer ammonium, barely or not at all absorbing nitrate. Bigg (in Alexander, 1983) reported a so-called "direct" toxic effect of nitrate on mycorrhizas by showing that mycorrhizal formation decreased under the influence of nitrate nutrition in a symbiosis where both phyto- and mycobiont separately were able to absorb nitrate. However, this effect could also have been caused by changes in the N content of the plant, as explained above by Björkman's hypothesis. Rudawska (1986) reported negative effects of

nitrate compared to ammonium on the occurrence of mycorrhizas in potted *P. sylvestris* seedlings.

Nitrogen nutrition research has always been subject to conflicting results. For example, Krajina *et al.* (1973), Bigg & Daniel (1978) and Bledsoe & Zasoski (1983) reported positive effects on the growth of Douglas-fir seedlings fertilized with nitrate, in comparison to ammonium fertilization, but Van den Driessche (1971, 1975) reported a larger growth response due to ammonium. Later, Van den Driessche (1978) reported that ammonium was more effective than nitrate on Douglas-fir seedlings at soil pH 7, and that nitrate was increasingly more effective than ammonium at soil pH 5.5 or less. These conflicting results may be caused by differences in the nitrogen nutrition of mycorrhizal fungal species and their isolates. Usually, mycorrhizal fungi are not taken into account in nitrogen nutrition studies of woody plants, and if so, the variation between species and isolates is not considered.

The variation between different mycobionts was clearly demonstrated by Lundeberg (1970), who found large variations in the nitrogen utilization between species and between isolates of the same mycorrhizal species growing in pure culture. Although the results from these pure culture studies cannot be extended to the field situation, Lundeberg's research (1970) definitely indicates that mycorrhizal fungal species apparently differ highly in their nitrogen nutrition strategy. This is also demonstrated by the differences between the *P. involutus*- and *S. bovinus*-inoculated plants in our present research. Differences in carbohydrate demands between mycorrhizal fungal species (and between isolates of the same species) might also explain differences in the effects of nitrogen on mycorrhizal plants and fungi. Mycorrhizal fungal species with a relatively low carbohydrate demand will be less inhibited by nitrogen fertilization than species with a relatively high carbohydrate demand. Subsequent research into the nitrogen nutrition of mycorrhizas and the variation between species and isolates of mycorrhizal fungi is therefore necessary.

We conclude that ammonia deposition on soil negatively affects mycorrhizas. Inhibition of mycorrhizal formation occurs at lower nitrogen levels than the inhibition of tree growth. The nitrogen level where inhibition of mycorrhizas starts depends on the availability and form of mineral nitrogen, the mycorrhizal fungal species, the tree species and the carbohydrate economy of the plant. The present rate of nitrogen deposition in the Netherlands might very well affect mycorrhizas.

3.5 Summary

Potted *Pinus sylvestris* seedlings with established mycorrhizas of *Paxillus involutus* and *Suillus bovinus* were fertilized weekly with ammonium sulphate and sodium nitrate for 26 weeks in order to simulate the nitrogen pollution in the Netherlands. The seedlings were fertilized with 0, 0.49, 2.5, 4.9 and 19.6 mg N wk^{-1} pot^{-1}, which equals 0, 10, 50, 100 and 400 kg N ha^{-1} yr^{-1}. The dry weight of the *P. involutus*-inoculated plants was positively affected by both fertilizations with an optimum at 50 kg N ha^{-1} yr^{-1}. The ammonium treatments had a larger positive effect than nitrate, especially for *P. involutus*-inoculated plants. The root length/weight ratio of *P. involutus*-inoculated plants decreased significantly due to fertilizations, indicating thicker roots. The number of mycorrhizas and the mycorrhizal frequency were negatively affected by the nitrogen treatments, especially ammonium. The nitrogen content in the needles was higher in ammonium-treated plants than in nitrate-treated plants. The effects on seedlings inoculated with *P. involutus* were more pronounced than on those inoculated with *S. bovinus*. Mycorrhizas appeared to be affected by nitrogen after it has been taken up.

Chapter 4

THE INFLUENCE OF NITROGEN FERTILIZATION ON ECTOMYCORRHIZAS AND THEIR FUNGAL CARPOPHORES IN YOUNG STANDS OF *PINUS SYLVESTRIS*.

4.1 Introduction

The mycorrhizal mycoflora has decreased severely this century in the Netherlands (Arnolds, 1988) as well as in other European countries (Fellner, 1988; Arnolds, in preparation). Although it appears likely that the decrease is linked to air pollution or decreased tree vitality, the mechanism has not yet been elucidated.

Termorshuizen & Schaffers (1987) reported that the occurrence of carpophores of mycorrhizal fungi in 50 to 80-year-old stands of *Pinus sylvestris* was negatively correlated to the NH_3 deposition and ambient NO_x concentration, and to a lesser extent to the ambient SO_2 air concentration. However, these correlations could not be found for carpophores in 5 to 10-year-old stands. Many more carpophores and fruiting species of mycorrhizal fungi appeared to occur in young stands than in 50 to 80-year-old stands. It was concluded that the conditions for fructification of mycorrhizal fungi are more favourable in young stands than in old ones. Termorshuizen & Schaffers (Ch. 6) assumed that this was due to the high nitrogen losses caused by previous clear-cutting and soil ploughing, the lower interception of air pollutants by smaller trees and the higher need of young trees for external nitrogen. Mycorrhizas and their fungal carpophores in young stands might therefore be tolerant to the actual levels of ambient nitrogen pollution, but they might suffer at higher levels of nitrogen pollution. A nitrogen fertilization experiment was carried out in order to study the sensitivity of the mycorrhizas and their fungal carpophores to increased nitrogen pollution, in two young stands of *P. sylvestris*.

4.2 Material & methods

Two stands of *P. sylvestris* L. were selected, one near Dwingeloo (northern Nether-lands) planted in 1980 and the other near Liessel (southern Netherlands) planted in 1979. Both soils were dry sandy soils without horizon development. The canopy closure

in the Dwingeloo and Liessel stand was 90% and 98%, respectively. The tree height in 1986 was approx. 3-3.5 m in both stands. The herbal understory vegetation covered about 50% of the area and consisted mainly of *Deschampsia flexuosa* (L.) Trin. in both stands. Woody plants possessing ectomycorrhizas were removed.

In each stand, 15 plots measuring 15x20 m^2 were laid out with 2.5 m paths left. The plots were annually fertilized from 1986 to 1988 with $(NH_4)_2SO_4$ or $NaNO_3$ (30 and 60 kg N ha^{-1} yr^{-1}), each on three plots, in addition to three control plots receiving no fertilizers. Each year half of the fertilizer was applied in May and the other half in August.

Each year from the end of September until the middle of November, the plots were searched three times for carpophores of mycorrhizal fungi. The caps of the carpophores were removed after each visit in order to prevent recounting the carpophores.

Root samples were randomly taken from each plot in October 1987 (ten samples) and 1988 (seven samples) from the upper 13 cm with a 2.5 cm diameter auger (volume 63.8 cm^3), after removing the litter layer. The samples were cleaned and the root length, mycorrhizal frequency (= 100% * no. of mycorrhizas/(no. of mycorrhizas + no. of non-mycorrhizal root tips)) and number of mycorrhizas were estimated. A mycorrhiza is defined here as a (dichotomously) branched or unbranched short root possessing a Hartig net.

An analysis of variance was carried out to examine the effect of nitrogen level and nitrogen form on mycorrhizas. Because the effects of nitrogen form were absent, the analysis was repeated with the nitrogen level as the only factor. The controls were compared to the treatments using Student's t-test on ln-transformed data (number and dry weight of carpophores).

4.3 Results

The mycorrhizal mycoflora in both stands consisted for more than 50% of *Lactarius rufus* (Scop.:Fr.) Fr. Other species contributing more than 2.5% of the total number of carpophores were *Laccaria proxima* (Boud.) Pat., *Suillus bovinus* (L.:Fr.) O.Kuntze and *Inocybe lacera* (Fr.:Fr.) Kummer in Liessel, and *Paxillus involutus* (Batsch:Fr.) Fr., *Laccaria proxima, Lactarius hepaticus* Plowr. *in* Boud. and *Hygrophorus hypothejus* (Fr.:Fr.) Fr. in Dwingeloo. During the three years, 8717 carpophores were observed in Liessel and 1544 in Dwingeloo.

The number and dry weight production of carpophores and the number of fruiting species involved were nearly always negatively affected by the fertilization level. However, due to the large degree of variation, few effects were significant (fig. 5-9).

DWINGELOO

No. Carpophores

1986   1987++   1988

LIESSEL

No. Carpophores

1986++   1987   1988

■ Control □ Ammonium ▨ Nitrate
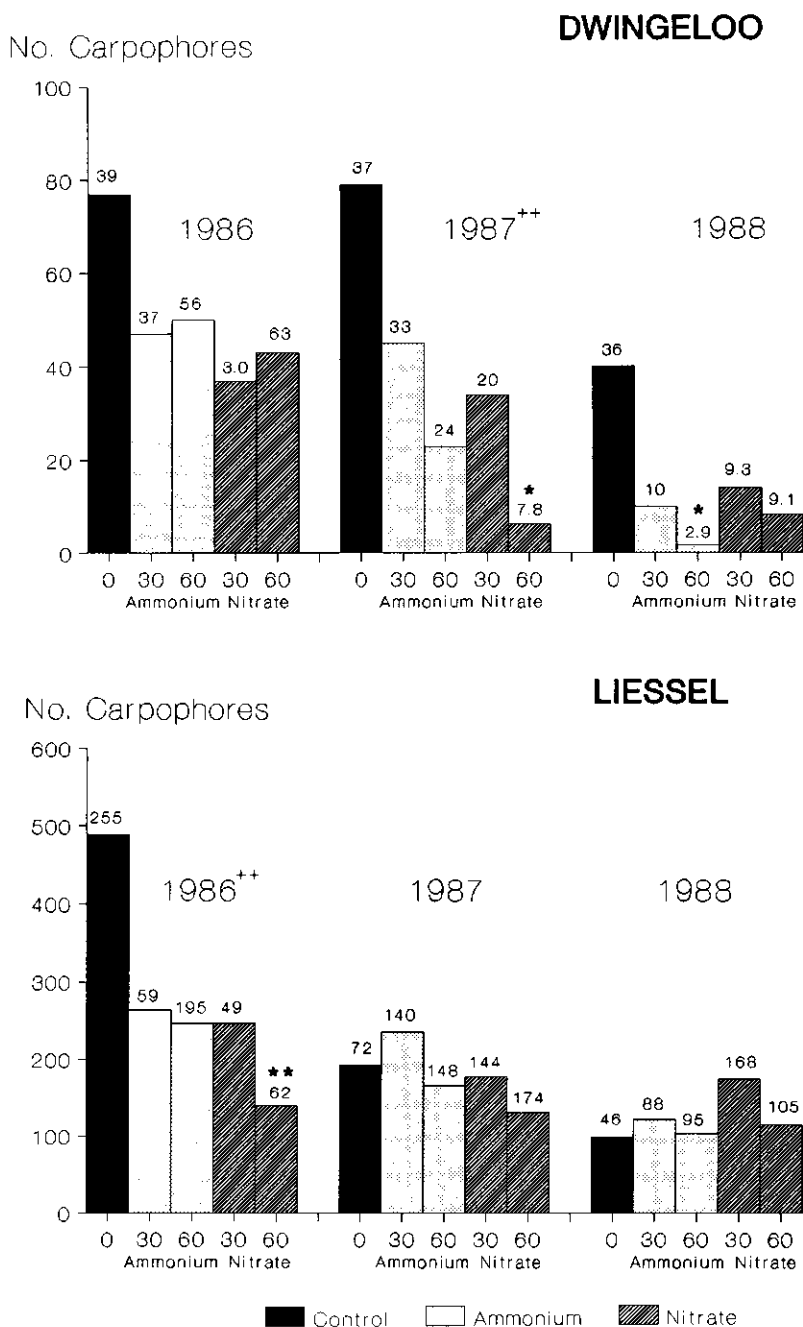
Figure 5. The effect of $(NH_4)_2SO_4$ and $NaNO_3$ on the number of carpophores of mycorrhizal fungi in the plots at Dwingeloo and at Liessel. Standard deviations are indicated above each bar and asterisks indicate a significant difference from the control according to Student's t-test and '+' or '++' behind the year indication indicate a significant F value of the nitrogen level in that year. *, $^+$ = $P < 10\%$, **, $^{++}$ = $P < 5\%$.
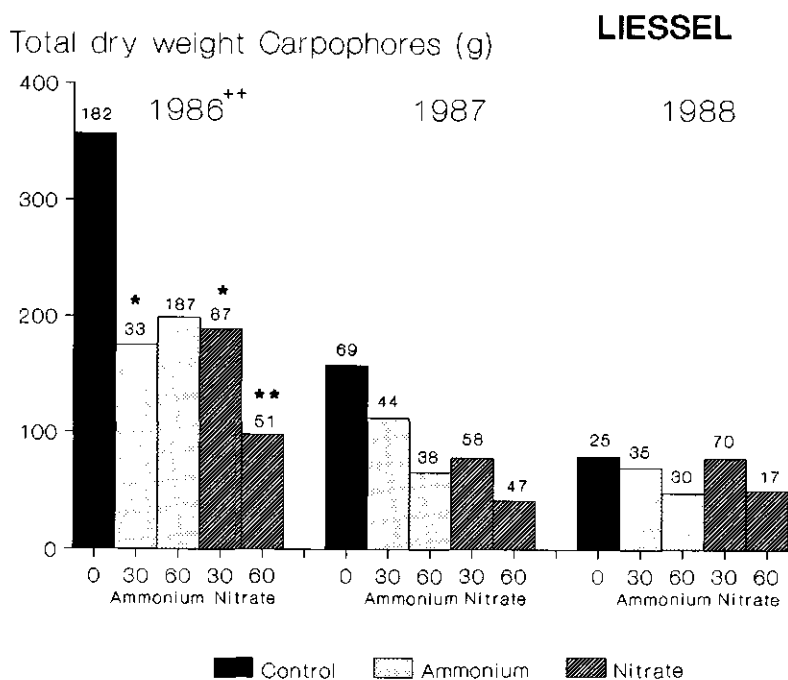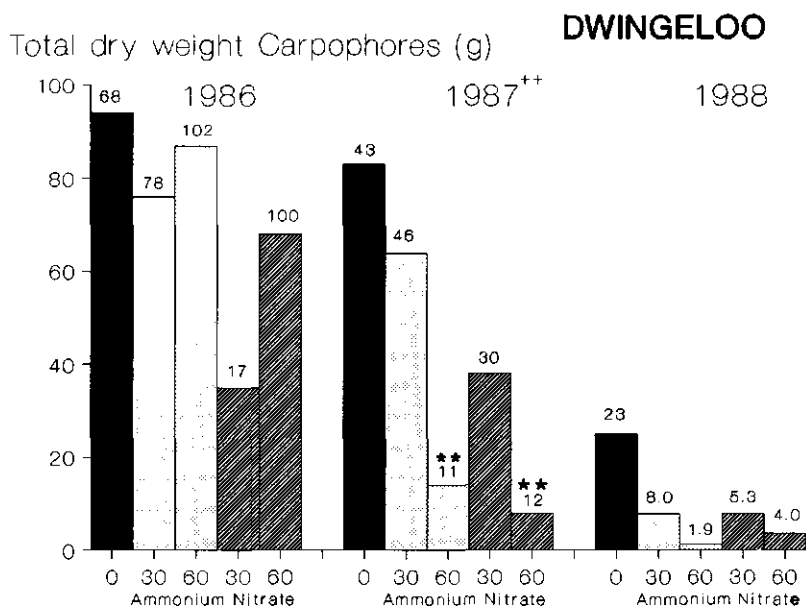
Figure 6. The effect of $(NH_4)_2SO_4$ and $NaNO_3$ on the dry weight production of carpophores of mycorrhizal fungi in the plots at Dwingeloo and at Liessel. For further explanation see figure 5.

No. Fruiting Species — DWINGELOO
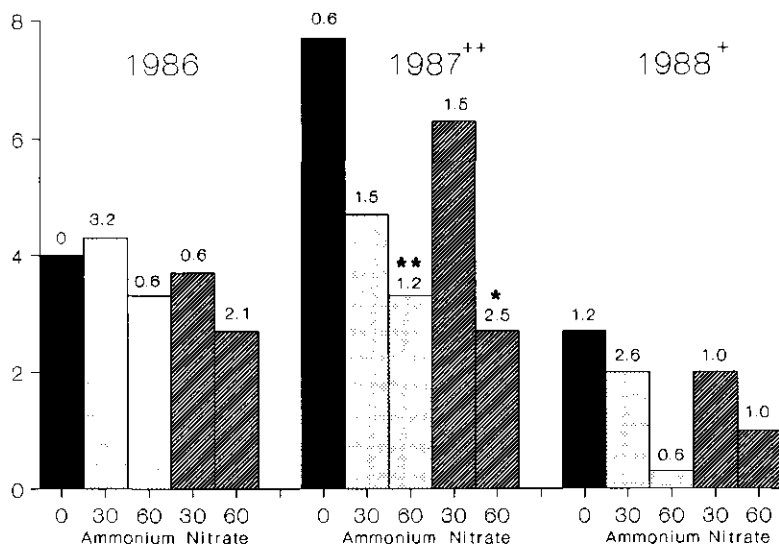
No. Fruiting Species — LIESSEL
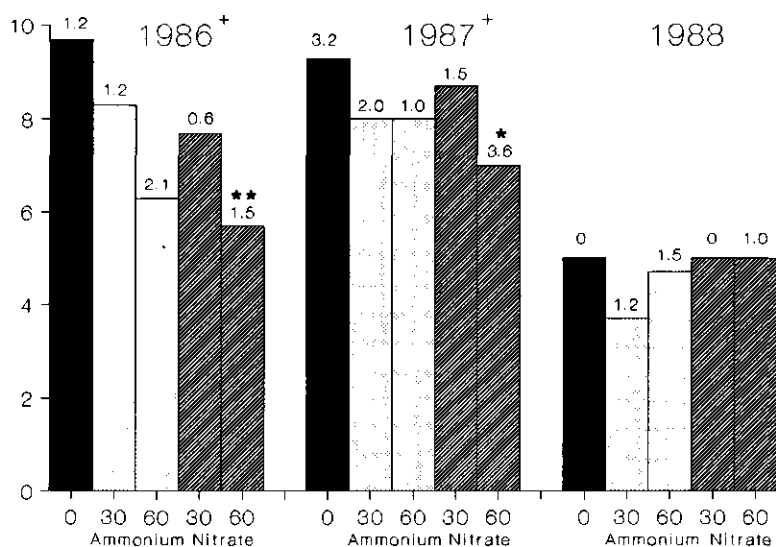
Control   Ammonium   Nitrate

Figure 7. The effect of $(NH_4)_2SO_4$ and $NaNO_3$ on the number of fruiting species of mycorrhizal fungi in the plots at Dwingeloo and at Liessel. For further explanation see figure 5.
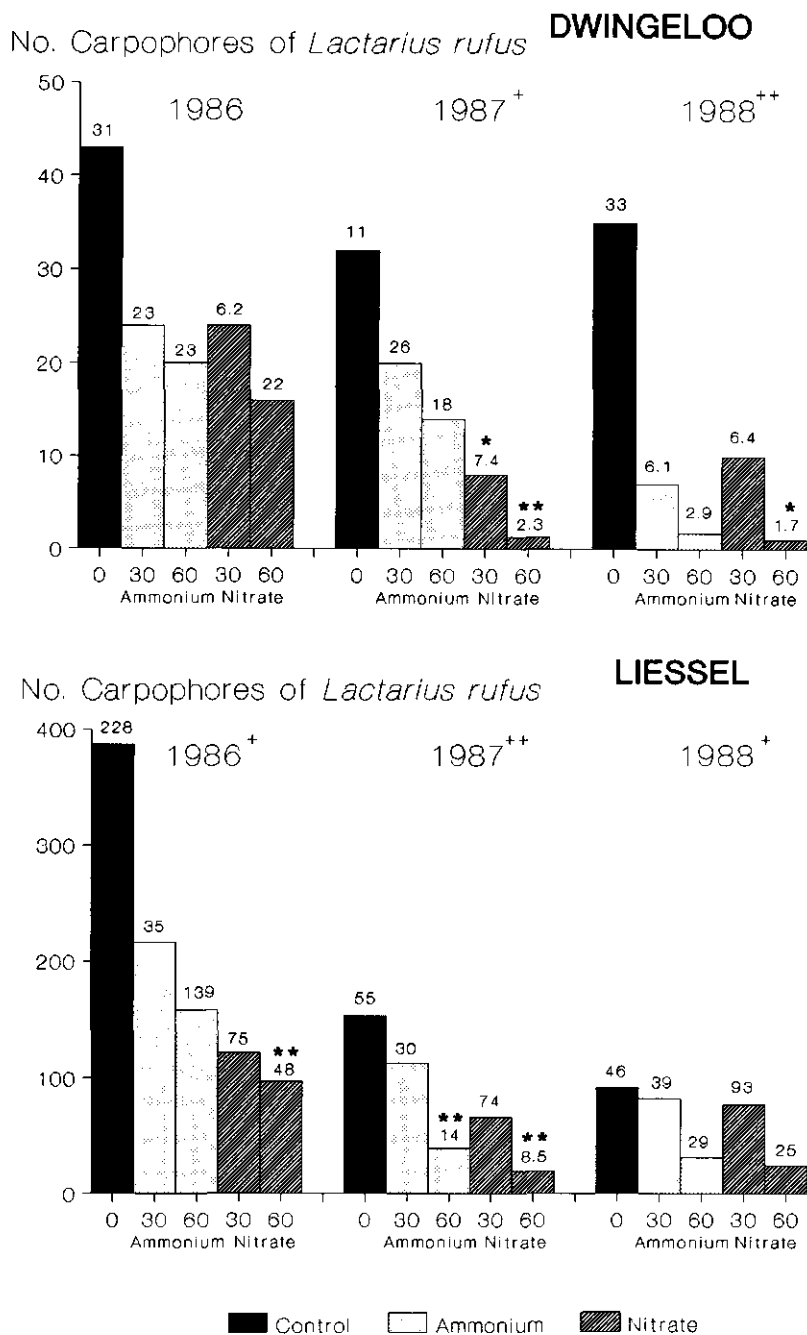
Figure 8. The effect of $(NH_4)_2SO_4$ and $NaNO_3$ on the number of carpophores of Lactarius rufus in the plots at Dwingeloo and at Liessel. For further explanation see figure 5.

No. Carpophores of *Laccaria proxima* **DWINGELOO**

1986   1987   1988
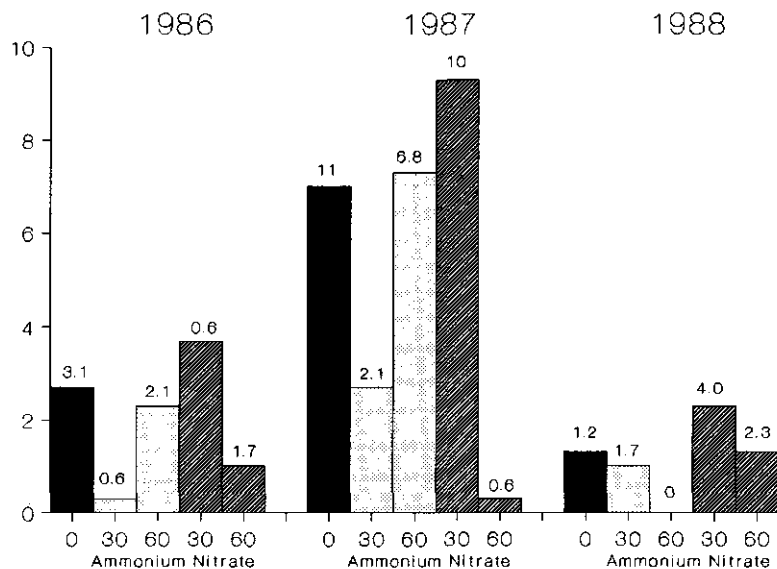
No. Carpophores of *Laccaria proxima* **LIESSEL**

1986   1987   1988

Control   Ammonium   Nitrate
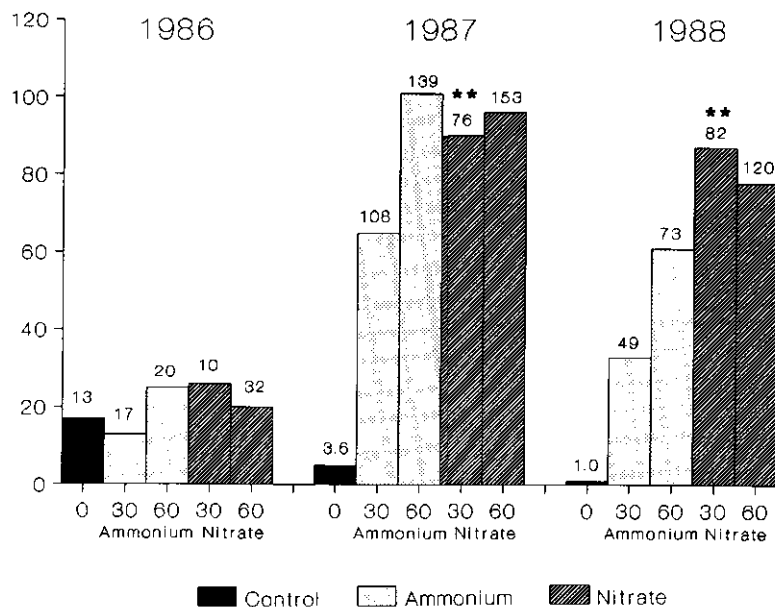
Figure 9. The effect of $(NH_4)_2SO_4$ and $NaNO_3$ on the number of carpophores of Laccaria proxima in the plots at Dwingeloo and at Liessel. For further explanation see figure 5.

The dry weight production of carpophores was affected to a greater extent than the number of carpophores. The effects were most clear in Dwingeloo, although comparable trends in Liessel appeared in 1986 and 1987. No effects on the number and production of carpophores or on the number of fruiting species were observed in Liessel in 1988. There was no evidence of a cumulative effect on the number and production of carpophores or on the number of fruiting species over the years.

There were no indications for a systematic change in species composition, except for two species. *Lactarius rufus* reacted negatively to the nitrogen level in Dwingeloo as well as in Liessel (fig. 8), while *Laccaria proxima*, which showed a remarkable positive reaction to the nitrogen level in Liessel in 1987 and 1988 (fig. 9). However, the latter effect was only statistically significant for the 30 kg $N\text{-}NO_3$ ha^{-1} yr^{-1} treatment. *L. proxima* occurred in very small quantities in Dwingeloo, and did not react to the treatments.

The mean mycorrhizal frequency was higher than 99% in all plots (fig. 10). No differences between the treatments were noted in the total number of mycorrhizas or mycorrhizas per unit of root length (fig. 10). There were about 3.5 times more mycorrhizas per unit of soil volume in Liessel than in Dwingeloo, although the number of mycorrhizas per unit of root length was slightly higher in Dwingeloo.

No differences between the ammonium and nitrate treatments were observed.

A severe infection by *Lophodermium seditiosum* Mintar, Staley & Millar occurred in the stand near Dwingeloo in 1986 and 1987. The mean needle age class was less than 1.0, whereas that in Liessel was 2.0. While recovering from the *L. seditiosum* infection in 1988, the stand in Dwingeloo showed a mean needle age class of 2.0, whereas that in Liessel was 2.7.

4.4 Discussion

The number of fruiting species and the number of carpophores generally decrease after nitrogen fertilization (*Pinus sylvestris*: Ohenoja, 1988; Ritter & Tölle, 1978; Shubin, 1988; Wästerlund, 1982; *Pinus taeda* L.: Menge & Grand, 1978). Likewise, nitrogen fertilization in pot experiments usually inhibits mycorrhizal formation (Richards, 1965; Termorshuizen & Ket, Ch. 3). Björkman (1942) suggested that this negative effect was caused by the increased conversion of carbohydrates into amino acids, reducing the supply of carbohydrates to the mycorrhizas.

On the other hand, several studies showed an increase in the number of carpophores of *Paxillus involutus* after fertilizations up to 240 kg N ha^{-1} (Laiho, 1970 ($CaNH_4NO_3$); Ohenoja, 1988 (Urea); Hora, 1959 (($NH_4)_2SO_4$)). Also, the number of carpophores of *Laccaria bicolor* (Maire) P.D. Orton (Ohenoja, 1988) and *Lactarius rufus*
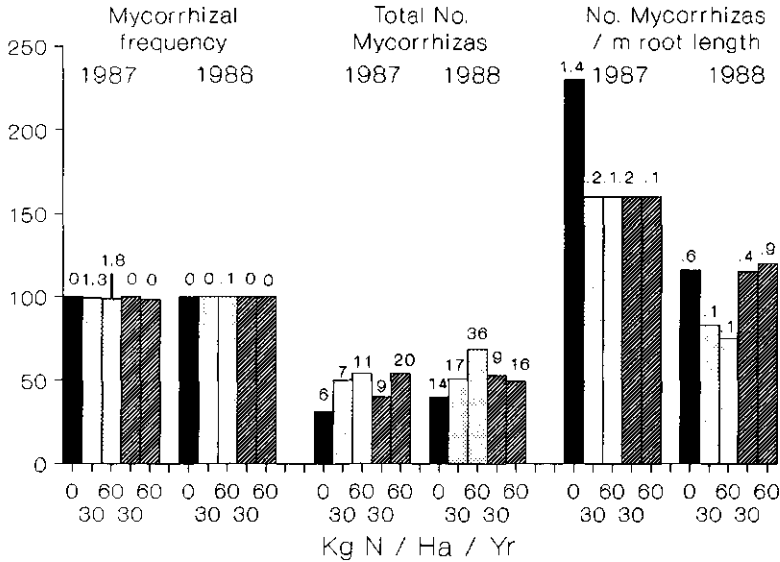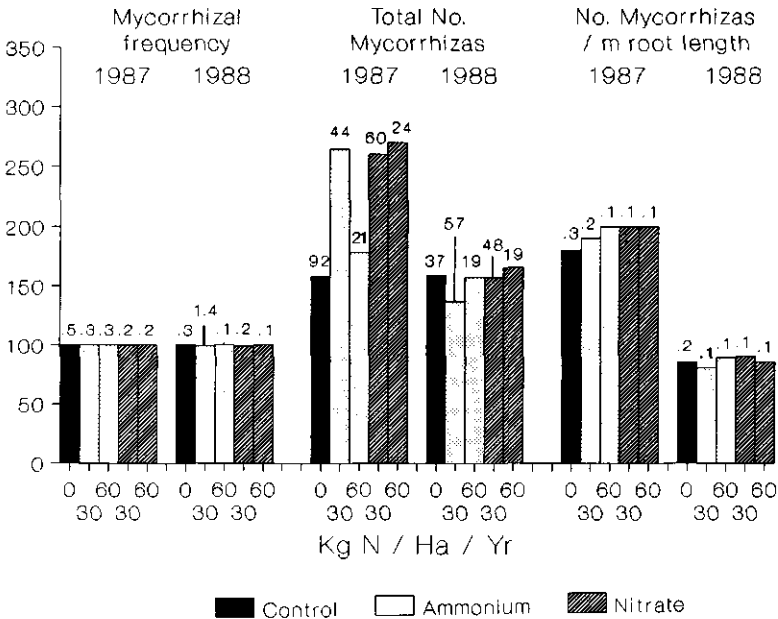
Figure 10. The mycorrhizal frequency, number of mycorrhizas per 100 cm³ and number
of mycorrhizas per 100 cm of root length in Dwingeloo and Liessel, in 1987 and 1988.

(Hora, 1959) have been reported to increase with nitrogen fertilization. Conflicting results might have their origin in differences in soil fertility. However, in studies where the total mycorrhizal mycoflora was taken into account, the total number of carpophores was still decreased, irrespective of the reaction of the individual species. Apparently differences exist between mycorrhizal species in the sensitivity to ammonium and nitrate and consequently, according to Björkman (1942), to carbohydrate availability. The relatively tolerant species will take the places of the more sensitive species. This seems to have occurred in Liessel, where the number of carpophores of *Laccaria proxima* increased considerably in 1987 and 1988 in the fertilized plots. Interestingly, *L. proxima* is one of the few mycorrhizal species in the Netherlands which showed an (insignificant) increase this century (Arnolds, 1985a). On the other hand, *Lactarius rufus* showed an (insignificant) decline.

The results indicate that the mycorrhizas are not influenced quantitatively by the nitrogen fertilization, in contrast to their fungal carpophores. Menge & Grand (1978) reported an 88% decrease of the number of carpophores and a 14% decrease in the number of mycorrhizas per unit of soil volume in 11-year-old, fertilized (112 kg N ha^{-1} as NH_4NO_3) *Pinus taeda* L. plantations, compared to the control. Ritter & Tölle (1978) reported the complete elimination of the carpophores in 1977 in stands of *P. sylvestris*, where from 1974 to 1977 3000 kg N ha^{-1} was applied as liquid manure. The mycorrhizal frequency however, was still 55%, compared to 87% in the control. If effects of nitrogen fertilization on mycorrhizas can be explained in terms of changes in the carbohydrate availability, it seems quite logical that the formation of carpophores is hampered before the development of mycorrhizas decreases.

The stronger effect of fertilization at Dwingeloo might be explained by the lower vitality of the trees caused by infection with *Lophodermium seditiosum*. This needle-infecting fungus inhibits the production of photosynthates, further decreasing the carbohydrate availability for mycorrhizal fungi in addition to the influence of the nitrogen fertilization. This may also explain the fact that 5.6 times more carpophores were found in the stand near Liessel than in the stand near Dwingeloo.

The results indicate that nitrogen pollution might significantly contribute to the decline of the mycorrhizal mycoflora in the Netherlands.

4.5 Summary

Previous research indicated that young stands of *Pinus sylvestris* L. have a richer mycorrhizal mycoflora than old ones. In old stands a strongly negative correlation was found between the ammonia deposition and the mycorrhizal mycoflora, which could not be found in young stands. The sensitivity of mycorrhizas and their fungal carpophores

to increased nitrogen pollution was tested by a nitrogen fertilization experiment in two stands of *P. sylvestris* of 6- and 7-years-old. In the years 1986 to 1988 plots (15x20 m^2) were fertilized each year with $(NH_4)_2SO_4$ or $NaNO_3$, at rates of 0, 30 and 60 kg N ha^{-1} yr^{-1}. Treatments were carried out in triplicate. The number of fruiting species and the number and dry weight of carpophores were both decreased by the higher fertilization levels, but the mycorrhizal frequency and the number of mycorrhizas per unit of soil volume were not affected. In conclusion it seems that the carpophore formation is much more sensitive to nitrogen fertilization than the mycorrhizal formation. This is possibly due to carbohydrate deficiency of the mycorrhizal fungi.

Chapter 5

SUCCESSION OF MYCORRHIZAL FUNGI IN STANDS OF *PINUS SYLVESTRIS* IN THE NETHERLANDS

Submitted to Vegetation Science, author A.J. Termorshuizen.

5.1 Introduction

Succession in the mycorrhizal mycoflora in tree stands in monoculture has been recognized by several authors (*Pinus contorta* Dougl., *Picea sitchensis* (Bong.) Carr.: Dighton *et al.*, 1986; *Betula* L. *spp.*: Mason *et al.*, 1982; *Pinus radiata* D. Don.: Chu-Chou, 1979; *Pinus sylvestris* L.: Termorshuizen & Schaffers, 1987). The group of Dighton and Mason introduced the terms 'early-stage' and 'late-stage' fungi, referring to fungi which predominate and fruit during the first years following the tree planting (*e.g. Thelephora terrestris* Ehrh.:Fr.) and fungi which are usually found later in the course of plantation development (*e.g. Russula* Pers. *spp.* and *Amanita* Pers. *spp.*).

According to Dighton & Mason (1985), the succession of mycorrhizal fungi can possibly be explained by changes in carbohydrate supply from the host tree and by an increase in the litter and humus layer. A change in the supply of carbohydrates might be caused by a decrease of net photosynthesis (Hintikka, 1988), tree vitality (Termorshuizen & Schaffers, 1987), or by an altered distribution of photosynthates over root and shoot (Hintikka, 1988). Furthermore, the mycorrhizas might be affected by an increased internal recycling of nutrients as the trees age (Miller *et al.*, 1979). Succession may also be influenced by allelopathic effects of litter (Rose *et al.*, 1983; Perry & Choquette, 1987), or of plants (Robinson, 1972) and by competition for nutrients with saprophytic fungi (Gadgil & Gadgil, 1975) or with plants (Theodorou & Bowen, 1971).

The phenomena of both 'early-stage' and 'late-stage' fungi apply only to the first 10-20 years of first rotation stands. The aim of the present study was to examine the relation between the composition of the mycorrhizal mycoflora and stand age of *P. sylvestris* plantations of different rotations.

5.2 Material & methods

A number of 35 stands of *Pinus sylvestris* L. were selected throughout the Netherlands (fig. 11), in which plots measuring 1050 m^2 each were set out. Selection was confined
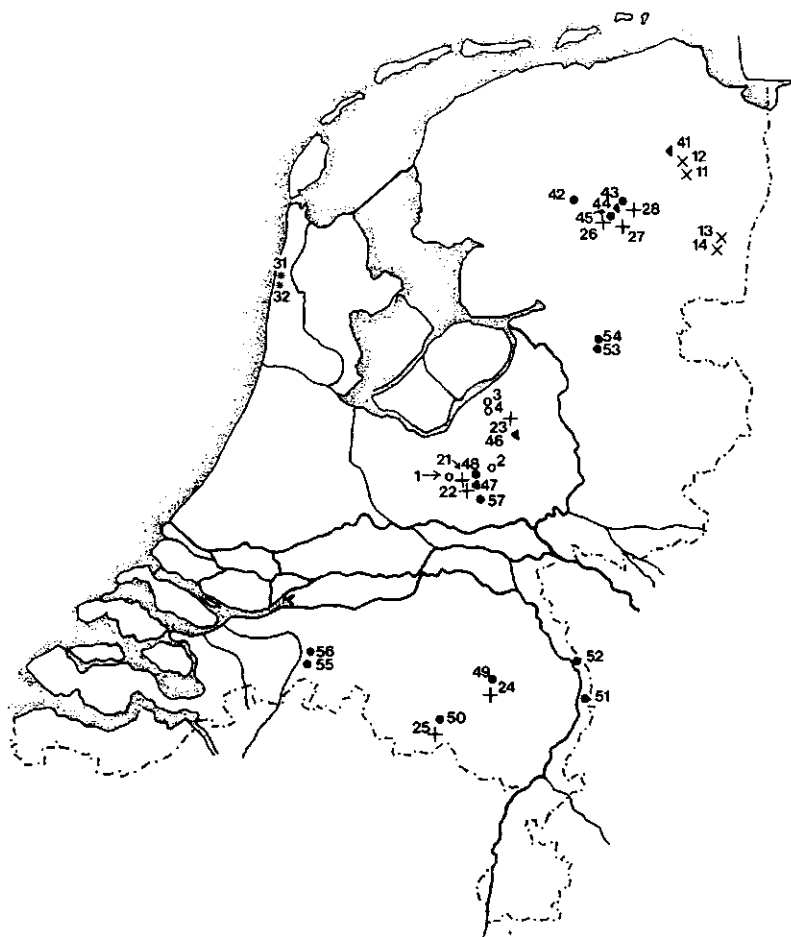
Figure 11. Location of the Y1-s (o), Y1-p (x), Y2 (+), OW-n (•), OW-h (*) and OP (◄) stands in the Netherlands. For abbreviations of the stand types see table 8.

to homogeneous stands on sandy soils beyond the direct influence of the ground water, supporting as few other ectomycorrhizal plants as possible. Undergrowth possessing ectomycorrhizas was removed. Only stands of a good provenance, based on morphological characteristics of the trees were selected, as explained in a previous publication (Termorshuizen & Schaffers, 1987).

Young (4-13 years) and old (50-80 years) stands were selected. In each age class, stands of three types were selected on the basis of the number of rotations and the soil type. The young stands had either grown spontaneously on drift sands (1st rotation, type Y1-s), were planted and 1st rotation on non-forest soil (type Y1-p) or planted and 2nd or 3rd rotation (type Y2). Two of the Y1-p stands were situated on formerly arable land, and the other two on drained moor-peat. The old stands were

TABLE 8

Age classes and stand types under study and abbreviations used.

	Code	Description
Age classes	Y	Young (4-13 yr)
	O	Old (50-80 yr)
Stand types	Y1-s	Young, 1st rotation and self-sown
	Y1-p	Young, 1st rotation and planted
	Y2	Young, 2nd or 3rd rotation and planted
	OW-h	Old, planted on soil without horizon development
	OW-n	as OW-h, but very thin humus layer and practically no herbs
	OP	Old, planted on soil with pronounced podsol

distinguished on the basis of their occurrence on soils with or without a pronounced podsol (type OP: old with podsol and types OW-h and OW-n: old without podsol). The presence of a podsol corresponds to a long forest history and the absence thereof corresponds with first rotation forests. Stands of type OW-n were characterized by the almost complete absence of a herb layer and very thin humus layer, in contrast to the stands of type OW-h where a well-developed herb and humus layer were present. All the old stands were planted.

The two age classes and the selected stand types are listed with their descriptions in table 8. The number of stands under study in 1986 and 1987 and some of the plot characteristics are also presented (table 9).

During the autumns of 1986 and 1987, the plots were systematically searched three (1986) resp. four (1987) times at three-week intervals for carpophores of mycorrhizal fungi. In order to avoid counting the same carpophore twice, the caps were removed.

TABLE 9

Number of plots under study and some stand type characteristics. For abbreviations of the stand types see table 8.

		Stand type					
		Y1-s	Y1-p	Y2	OW-n	OW-h	OP
No. Plots	1986	2	4	8	2	13	4
	1987	4	4	8	2	12[a]	4
Age (yr)[b]		8-13	4-10	6-11	60	50-80	50-80
Humus layer (cm)		0.5	0.5	0.5-2.0 & mixed	0.5-1.0	1-3	3-5
Tree height (m)		3-5	2.5-4.5	3-5	5-10	10-20	15-20
No. trees plot^{-1}		300-500	250-400	350-450	120-265	40-110	40-80
Canopy closure		85-95	35-98	80-95	90-95	45-80	50-85

[a] One plot was clear cut before the 1987 observations were started.
[b] In 1986.

Taxonomy and nomenclature are according to Moser (1983) and Jülich (1984), except for the taxonomy of *Dermocybe* (Fr.) Wünsche where Høiland (1984) was followed.

Comparison of the mycofloristic composition of the plots was principally based on differences in species composition, and relatively little weight was given to differences in numbers of carpophores of each species by using transformed figures, in which 0 means 0 carpophores, 1 means 1-9 carpophores, 2 means 10-99 carpophores, 3 means 100-999 carpophores and 4 means more than 1000 carpophores. For all comparative analyses used, the total number of carpophores of each species in the most productive year was taken.

To identify the mycofloristic associated groups of plots, the transformed data matrix was subjected to a Bray and Curtis simple ordination technique, after Van der Maarel (1979), using a matrix of indices of dissimilarity. Indices of dissimilarity of the transformed data matrix were calculated according to Sørensen (Van der Maarel, 1979). The use of other indices of dissimilarity according to Jaccard, Barkman, Van der Maarel and Moravec (Whittaker, 1973) hardly influenced the results.

In addition, the transformed data matrix was subjected to a divisive clustering technique known as two-way indicator species analysis (TWINSPAN). TWINSPAN is based on a reciprocal averaging algorithm, and divisions are made on the basis of species presence and abundance (Van Tongeren, 1987).

Preferential occurrence of fungal species in one (group of) stand type(s) was tested using the Chi square test (Snedecor & Cochran, 1980). Species which occurred more frequently in one (group of) stand type(s) at P<2.5% were referred to as differential species for that (group of) stand type(s). Species which occurred in only one (group of) stand type(s) were referred to as exclusive species for that (group of) stand type(s). Exclusive species may therefore occur in one plot only and are not automatically differential at the same time.

A second Bray and Curtis ordination was carried out on the basis of composition and cover percentages of the green vegetation. The cover percentages were transformed to a scale ranging from 0 to 9 according to Van der Maarel (1979).

The relation between the composition of the mycorrhizal mycoflora and the composition of the green vegetation was studied using a matrix of indices of dissimilarity after Sørensen, based on the presence/absence of species only. One matrix was based on the mycorrhizal mycoflora in the plots and the other was based on the green vegetation. The index of dissimilarity between two stand types was calculated as the mean of the indices of dissimilarity between the plots of the one stand type with those of the other stand type. Subsequently the indices of dissimilarity which were based on the mycorrhizal mycoflora were compared with those based on the green vegetation.

In October 1987 ten root samples were randomly taken in each plot from the upper 13 cm with a 2.5 cm diameter auger (volume 63.8 cm^3), after removing the litter

layer. The major part of litter and sand was removed with a 1 mm sieve. Roots were subsequently cleaned in a small glass box containing water. During cleaning, the dead roots (defined as roots which are desiccated, shrunken and highly fragile) were removed. After cleaning, the roots were stored in a glutaraldehyde buffer (Alexander & Bigg, 1981) until further analysis.

The root length in each soil sample was determined and the number of root tips was counted. The root tips were divided into non-mycorrhizal and mycorrhizal. Non-mycorrhizal root tips were divided into short (<2 cm) and long (>2 cm) roots. The mycorrhizas were classified on the basis of their appearance. The first class of mycorrhizas (the "well-developed" mycorrhizas), possessed a smooth, relatively thick mantle, so that root cells were not visible at a magnification of 12x. The second class of mycorrhizas ("poorly-developed") either possessed a dented and more or less wrinkled mantle or no visible mantle. No attempts were made to identify the mycorrhizas in any way. All root data were recalculated to a volume of 100 cm^3.

5.3 Results

Number of species, carpophores and dry weight production
The number of carpophores and the number of species per plot were considerably higher for the young plots than for the old plots, except for the OW-n plots (fig. 12). The smallest number of carpophores was found in the OW-h plots and the smallest number of species in the OP and OW-h plots (table 10). In both years the number of species in the Y1-s plots was higher than in the Y2 and Y1-p plots, and in the Y2 plots higher than in the Y1-p plots.

The carpophore dry weight production per plot roughly coincided with the number of carpophores. The average carpophore dry weight in the Y1-s stand types was 1.2-5.2 times higher than in the other young stand types, and 1.6-61 times higher than in the other old stand types (table 10).

The number of carpophores differed considerably between the two years in all stand types, showing a mean decrease of 130%, 310% and 310% for the OW-h, OP and Y1-p plots, respectively, and a mean increase of 120%, 140% and 290% for the OW-n, Y1-s and Y2 plots, respectively. The number of species was higher in 1987 than in 1986, viz. 130% for the old plots and 160% for the young plots.
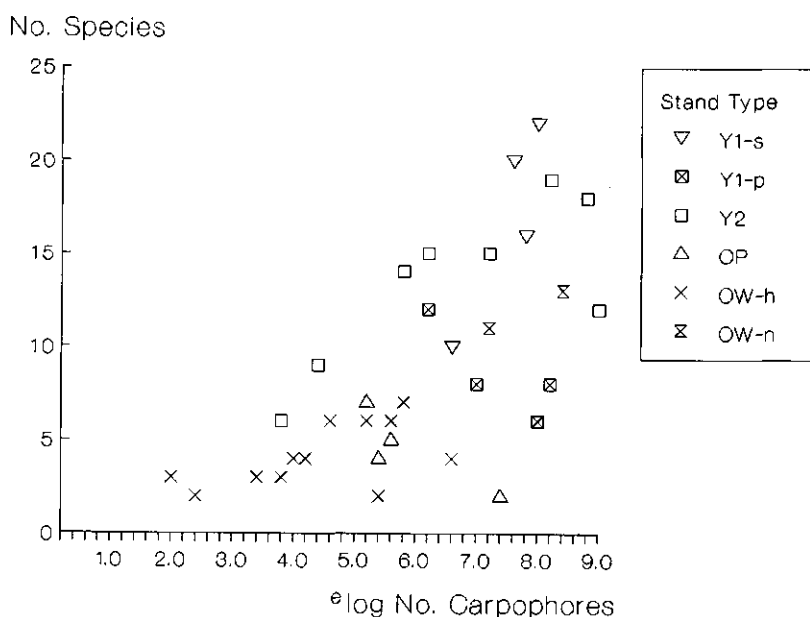
No. Species

Figure 12. The total number of species and maximum number of carpophores per plot. For abbreviations of the stand types see table 8.

Species

A total of 42 species was found (table 11). Five species were found in all stand types (table 12). A number of 17 species appeared to occur significantly (P<2.5%) more in one (group of) stand type(s) than in the other stand types (=differential species). Only three of these species occurred in the old plots, viz. *Cortinarius obtusus*, *Lactarius hepaticus* and *Russula ochroleuca*. The other 14 species appeared to be differential for one or more young stand types. Of the differential species, 11 were also classified as exclusive species. In addition, 13 exclusive species were found which were not differential, all occurring in the young plots. *Tricholoma* was exclusively present with 4 species in plots of the Y1-s stand type, and absent in the other stand types. Of the young stand types, Y1-s had much more exclusive species than the other types. Of the five species occurring in all plots *Laccaria proxima* and *Paxillus involutus* were found in all stand types without showing preference for one stand type.

Composition of the mycoflora

After three divisions the TWINSPAN analysis resulted in six clusters (table 11). The first division separated the young and old plots.

In the young plots, the second division separated the two Y1-p plots which were situated on former arable land (cluster 1, table 11), and the third division separated the Y1-s plots (cluster 2) from the other young plots (cluster 3). The two Y1-p plots

TABLE 10

Mean number (± S.D.) of fruiting species, number of carpophores and total dry weight of carpophores [g] per plot in the different stand types and the average carpophore dry weight (W/C, [g]) per stand type. In cases of two observations the range is mentioned between brackets. For abbreviations of the stand types see table 8.

Stand type	1986				1987			
	Species	Carpophores	Weight	W/C	Species	Carpophores	Weight	W/C
Y1-s	14 (3)	1617 (725)	2336 (1835)	1.44	16 ±4.3	2212±1056	2260± 972	1.02
Y1-p	4.3±1.9	2091±1659	1945±1876	.93	7.0±2.7	670± 198	437± 262	.65
Y2	7.9±4.1	953± 972	464± 542	.49	14 ±2.7	2775±2454	1225±1225	.44
OW-n	9.0 (1)	2509 (3577)	1435 (1384)	.57	13 (1)	2968 (3244)	1451 (857)	.49
OW-h	3.0±1.7	135± 192	57± 62	.42	3.1±1.8	104± 143	37± 44	.36
OP	2.0±1.2	591± 727	183± 214	.31	4.5±2.1	190± 52	70± 3	.37

TABLE II

Mycorrhizal mycoflora of the plots manually arranged. Figures refer to transformed numbers of carpophores, in which 0 means 0 carpophores, 1 means 1 to 9 carpophores, 2 means 10 to 99 carpophores, etc. Plots with the same cluster numbers (third line) belong to identical clusters according to the TWINSPAN cluster (contd. facing page)

Stand age: YOUNG

	Species	12	11	4	3	2	1	25	24	22	21	23	26	27	13	14	28
	Stand type:	lp	lp	ls	ls	ls	ls	2	2	2	2	2	2	2	lp	lp	2
	TWINSPAN cluster:	1	1	2	2	2	2	3	3	3	3	3	3	3	3	3	3
?	Hebeloma velutipes	3	2														
?	Cortinarius saniosus		2														
-*	Suillus luteus	2	2	2	1	1	1							1			
-	Tricholoma equestre			3	4	3	2										
-	Tricholoma imbricatum			2	2	2	3										
-	Lactarius glyciosmus			2	1												
-*	Boletus edulis			1		1											
-	Tricholoma portentosum				1	1											
?	Inocybe mixtilis						1										
-*	Amanita gemmata				1												
-*	Tricholoma robustum				2												
-*	Cortinarius mucosus			1													
-*	Chroogomphus rutilus		1	1									1				
=	Hebeloma mesophaeum	2	1	2						1							
=	Inocybe lacera		1	2	1	2	2	2	2	1							
-	Hygrophorus hypothejus		2	2	2	2		2						1			
?	Inocybe carpta		1	2	2	1	1	1		1	2	1		1			
?	Inocybe brevispora	1		2	2			2				1	1	2	2	1	1
+	Laccaria laccata		2										1			1	
-	Rhizopogon luteolus			2	2	2	2			2	2	1					
?	Cortinarius fusisporus				3			3		3	2	2					
-*	Coltricia perennis							2	1								
=	Amanita rubescens							2	1			1	1				
?	Cortinarius flexipes											2	1				
?	Inocybe umbrina												1	1			
-*	Suillus bovinus			3	2	2		1	3	2	2	2		1	4	4	
-	Amanita muscaria			2	2	1	2	1	1	1							
-	Gomphidius roseus			2					1	1				1			
-*	Dermocybe croceocona			2	2	1		2	1	4	2	2		2			
-*	Suillus variegatus			2						2	2						
-	Cortinarius obtusus																
?	Inocybe lanuginosa							1									
+	Russula emetica							2									
-*	Dermocybe semisanguinea							3	2	3	3	2		2			
+	Scleroderma citrinum							2	2	2		2					1
-	Lactarius rufus			3	3	2	2	4	2	4	2	2	1	2	1	2	1
=	Lactarius helvus														2	2	2
+	Laccaria proxima	3	2	3	3	3	2	2	3	4	4	2	2	2	3	2	2
=	Paxillus involutus			2	1	2		2	1	2	2	2	2	2	2	1	1
+	Xerocomus badius		1	2				2	2	1		1	1	1			
+	Lactarius hepaticus							2	2	3	3	2	2	2			
+	Russula ochroleuca												1	1			

TABLE 11 (contd.)

analysis. The change in occurrence during this century in the Netherlands (Arnolds, 1985a) is indicated left of the list of species: -*: significant decrease (P<5%), -: insignificant decrease, =: no change, +: insignificant increase and ?: change unknown. Abbreviations of the stand types: 1s=Y1-s, 1p=Y1-p, 2=Y2, Wh=OW-h, Wn=OW-n, P=OP.

Stand age: **OLD** (spanning all plot columns)

31	32	43	45	46	48	52	47	50	42	51	53	44	56	49	57	55	41	54	Species (Plot number / Stand type / TWINSPAN cluster)
Wn	Wn	Wh	Wh	P	Wh	Wh	P	Wh	Wh	Wh	Wh	P	Wh	Wh	Wh	Wh	P	Wh	Stand type
4	4	5	5	5	5	5	6	5	5	5	5	5	5	5	6	5	5	5	TWINSPAN cluster
																			Hebeloma velutipes
																			Cortinarius saniosus
																			Suillus luteus
																			Tricholoma equestre
																			Tricholoma imbricatum
																			Lactarius glyciosmus
																			Boletus edulis
																			Tricholoma portentosum
																			Inocybe mixtilis
																			Amanita gemmata
																			Tricholoma robustum
																			Cortinarius mucosus
																			Chroogomphus rutilus
																			Hebeloma mesophaeum
																			Inocybe lacera
																			Hygrophorus hypothejus
																			Inocybe carpta
																			Inocybe brevispora
																			Laccaria laccata
	1																		*Rhizopogon luteolus*
																			Cortinarius fusisporus
																			Coltricia perennis
																			Amanita rubescens
																			Cortinarius flexipes
																			Inocybe umbrina
1	2	1	1																*Suillus bovinus*
1																			*Amanita muscaria*
	1																		*Gomphidius roseus*
1	2																		*Dermocybe croceocona*
	2																		*Suillus variegatus*
2	3																		*Cortinarius obtusus*
1	1	1	1																*Inocybe lanuginosa*
3	3	1		1															*Russula emetica*
2	2																		*Dermocybe semisanguinea*
			1																*Scleroderma citrinum*
2	3			1	1			2				1	1						*Lactarius rufus*
			2																*Lactarius helvus*
1		1	2	1	1	1	1	1	2	2	1	1	2	2	1				*Laccaria proxima*
2	2			1	1	1	1	2	1	1	1				2				*Paxillus involutus*
1	2	1	1	1	1	1	1	1			1								*Xerocomus badius*
3	4	2	3	2	2	2	2	3	2		2	2	1	2	1		4	2	*Lactarius hepaticus*
1		1	1		1				1	1				1			2	1	*Russula ochroleuca*

TABLE 12

List of observed ectomycorrhizal fungi grouped into: species found in all stand types, exclusive species (i.e. species found in one (group of) stand type(s), but absent in the other stand types), and differential species (i.e. species which occur significantly (P<2.5%) more in one (group of) stand type(s) than in the other stand types). For abbreviations of the stand types see table 8.

Species found in all stand types
Laccaria proxima
Lactarius hepaticus
Lactarius rufus
Paxillus involutus
Suillus bovinus

Exclusive ([e]) and differential ([d]) species (young stands)

Young (=Y1-s, Y1-p, Y2)	Type Y1 (=Y1-s, Y1-p)	Type Y1-s	Type Y1-p	Type Y2
Amanita muscaria[d]	Suillus luteus[de]	Amanita gemmata[e]	Cortinarius saniosus[e]	Amanita rubescens[e]
Chroogomphus rutilus[de]		Boletus edulis[e]	Hebeloma velutipes[e]	Coltricia perennis[e]
Cortinarius fusisporus[de]		Cortinarius mucosus[e]		Cortinarius flexuosus[e]
Dermocybe croceocona[d]		Inocybe cf. xanthomelas[e]		Inocybe umbrina[e]
Hebeloma mesophaeum[de]		Lactarius glyciosmus[e]		
Hygrophorus hypothejus[de]		Tricholoma equestre[de]		
Inocybe brevispora[de]		Tricholoma focale[e]		
Inocybe carpta[de]		Tricholoma imbricatum[de]		
Inocybe lacera[de]		Tricholoma portentosum[e]		
Lactarius rufus[d]				
Suillus bovinus[d]				

Exclusive ([e]) and differential ([d]) species (old stands)

Old (=OP, OW-h, OW-n)	Type OP	Type OW-h	Type OW-n
Lactarius hepaticus[d]	none	none	Cortinarius obtusus[de]
Russula ochroleuca[d]			

which were situated on drained moor-peat were classified in the same cluster as the Y2 plots (viz. cluster 3).

In the old plots the two OW-n plots were separated in the second division (cluster 4) and in the third division two OW-h plots (cluster 6) were singled out from the rest of the OP + OW-h plots (cluster 5). However, this latter division is doubtful because there were no clear differences between the mycorrhizal mycoflora of the plots of cluster 5 with those of cluster 6 (table 11).

Most TWINSPAN clusters were also recognizable in the Bray & Curtis ordination (fig. 13). However, in the ordination graph, TWINSPAN cluster 6 was not clearly separated from TWINSPAN cluster 5. It also appeared that the two Y1-p plots which were grouped together with the Y2 plots in cluster 3, are ordinated intermediately between the other Y1-p plots (cluster 1) and the Y2 plots.

The separation of the OW-n plots from the other old plots by TWINSPAN was clear in the ordination diagram. From the ordination diagram can be concluded that these plots contain a mycoflora which is more similar to the Y2 plots than to the other old plots.

The ordination diagram and the TWINSPAN analysis did not show any relation with soil type of the old plots.
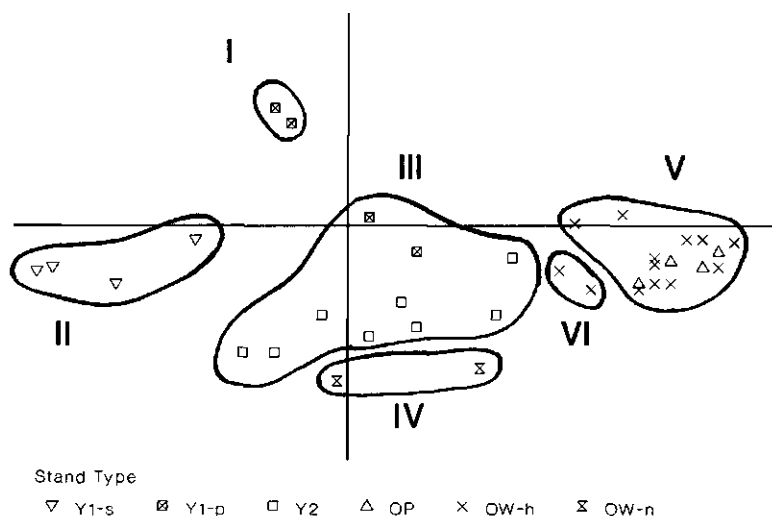
Figure 13. Ordination of the plots based on the mycoflora. ———— demarcates clusters recognized by TWINSPAN and roman numerals indicate TWINSPAN cluster numbers (cf. table 11).

Mycorrhizas

The root length and the number of mycorrhizas were higher in the Y1-s and Y2 plots than in the OP and OW-h plots (table 13). The Y1-p plots showed less mycorrhizas and a lower root length than the other young plots, but they were still higher than those of the OP and OW-h plots. The root length and the mycorrhiza parameters of the OW-n plots were comparable to those of the Y1-s and Y2 plots. On average 1.6 to 2.0 mycorrhizas occurred per cm root.

On the other hand, the mycorrhizal frequency and the percentage of well-developed mycorrhizas showed small differences between the stand types. In all stand types the mycorrhizal frequency was on average higher than 96%.

The root length and the number of mycorrhizas showed very high standard deviations; those of the number of mycorrhizas per cm root and of the mycorrhizal frequency were much smaller.

TABLE 13

Root length and total number of mycorrhizas per 100 cm^3 soil, number of mycorrhizas per cm root length, mycorrhizal frequency and number of well-developed mycorrhizas relative to the total number of mycorrhizas of the soil samples (± S.D.). In cases of two observations the range is placed between brackets. For abbreviations of the stand types, see table 8.

Stand type	Root length (cm)	Total no. mycorrhizas	No. mycorrhizas/ cm root length	Mycorrhizal frequency (%)	Well-developed mycorrhizas (%)
Y1-s	137 ± 26	250 ± 53	1.9 ± 0.3	99.9 ± 0.1	12 ± 5.0
Y1-p	78 ± 39	138 ± 66	1.9 ± 0.4	98.8 ± 1.7	20 ± 6.8
Y2	104 ± 34	207 ± 67	2.0 ± 0.4	99.6 ± 0.5	21 ± 5.6
OW-n	135 (28)	228 (16)	1.7 (0.2)	100 (0)	22 (3.0)
OW-h	46 ± 21	90 ± 50	2.0 ± 0.5	96.7 ± 8.1	12 ± 6.8
OP	71 ± 21	114 ± 38	1.6 ± 0.3	99.8 ± 0.2	22 ± 2.4

Relation with changes in the Dutch mycoflora

The change in occurrence of the relevant species during this century in the Netherlands is indicated in table 11 (data after Arnolds, 1985a). It appeared that declining species were more common in young stand types (especially in the Y1-s type) than in old stand types OP and OW-h (fig. 14). In the Y1-s, Y1-p and Y2 plots 18, 6 and 11 declining species were found, respectively, and in the OP and OW-h plots 1 and 2 declining species, respectively. In the two old OW-n plots 8 declining species were found. In the OP plots the number of increasing species was higher than in the OW-h plots.

No. Species

No. Carpophores
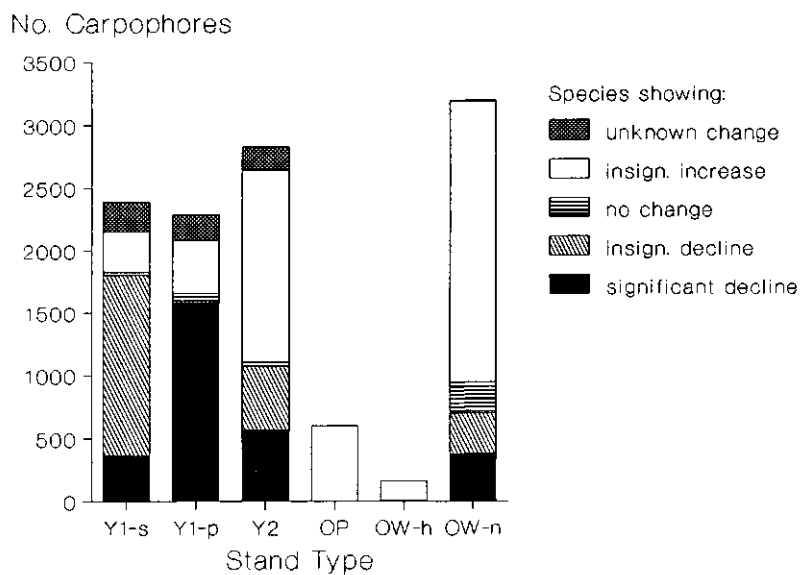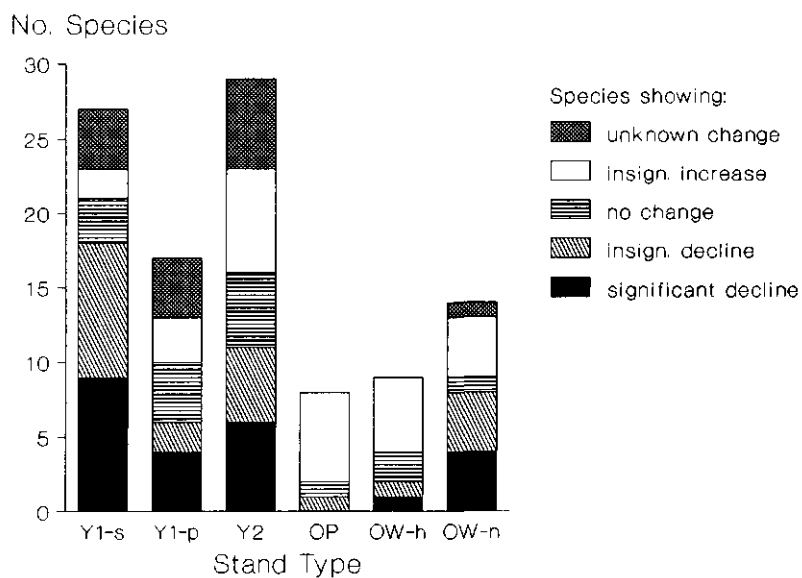
Figure 14. The occurrence of species classified according to their change this century in the Netherlands (Arnolds, 1985a). For abbreviations of the stand types see table 8.

TABLE 14

Green vegetation of the plots, arranged in the same order as in table 11. Figures refer to cover percentages: r: cover <1%, 1-2 individuals, +: cover <1%, 3-100 individuals, p: cover <1%, >100 individuals, a: cover 1-2%, b: 2-5%, 2a: 5-12.5%, (contd. facing page)

Stand age:	YOUNG															
Plot number:	12	11	4	3	2	1	25	24	22	21	23	26	27	13	14	28
Stand type:	1p	1p	1s	1s	1s	1s	2	2	2	2	2	2	2	1p	1p	2
Canopy closure (%):	35	97	95	90	90	85	95	80	85	95	95	95	90	95	98	90
Shrub layer cover (%):	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Herb layer cover (%):	50	5	<1	<1	<1	<1	1	50	11	33	54	50	55	80	65	17
Moss layer cover (%):	80	20	5	8	7	4	15	10	37	39	43	80	33	5	1	55
Holcus lanatus	2a	b											p			
Agrostis tenuis	2a	2a														
Rumex acetosella	2a															
Polytrichum piliferum		2b	b	2a	+											
Campylopus introflexus			a		b	+	2a		+						2	+
Cladonia spp.		+	+	+	+	+	a									
Vaccinium myrtillus					+			+	a	2b						
Rubus fruticosus							2a								b	
Polytrichum formosum	5a						+	a					r			2a
Deschampsia flexuosa	2a	+					a	3	2a	3	3	3	4			
Dicranum scoparium		p					+	3	2a	3	3	2a	2a			2a
Hypnum cupressiforme							+	a	a	a	3	3	3			3
Calluna vulgaris		+	+	+					a	a	a	+	2a	2a	2a	2a
Erica tetralix	+												p	2a	2a	+
Molinia caerulea														4	3	
Dryopteris carthusiana												r	r			r
Dryopteris dilatata																a
Corydalis claviculata												+	a			r
Galium saxatile																r
Pleurozium schreberi													2a			
Empetrum nigrum																
Goodyera repens																
Stellaria media																
Senecio sylvaticus																
Ilex aquifolium																
Chamaerion angustifolium																
Agrostis stolonifera																
Agrostis capillaris																
Festuca ovina																
Carex pilulifera																
Juncus squarrosus																
Vaccinium vitis-ideae							+									
Leucobryum glaucum																
Frangula alnus																
Prunus serotina																

Species, occurring in one plot only: *Aegopodium podagraria* (50: r), *Carex arenaria* (53: 2b), *Cerastium fontanum* (41: +), *Galeopsis tetrahit* (49: +), *Hieracium laevigatum* (45: r), *Juncus effusus* (41: +), *Nardus stricta* (42: a), (contd. facing page)

TABLE 14 (contd.)

2b: 12.5-25%, 3: 25-50%, 4: 50-75%, 5a: 75-90% and 5b: 90-100%. For abbreviations of the stand types see table 11.

OLD																			
31	32	43	45	46	48	52	47	50	42	51	53	44	56	49	57	55	41	54	Plot number
Wn	Wn	Wh	Wh		P	Wh	Wh		P	Wh	Wh	Wh	Wh		P	Wh	Wh	Wh	Wh	P	Wh	Stand type

Canopy closure (%): 95 90 70 50 50 55 80 70 80 45 70 40 55 45 80 45 58 85 60
Shrub layer cover (%): 0 0 0 0 0 0 <1 0 0 0 5 45 0 0 0 0 0 0 0
Herb layer cover (%): 2 <1 58 79 70 89 85 75 67 77 75 45 54 95 76 68 93 43 85
Moss layer cover (%): 25 17 48 29 60 18 80 10 16 8 15 70 49 7 26 26 9 48 80

Species	Values
Holcus lanatus	
Agrostis tenuis	r
Rumex acetosella	p
Polytrichum piliferum	
Campylopus introflexus	
Cladonia spp.	+ 2a + + +
Vaccinium myrtillus	3 b 3
Rubus fruticosus	a + + r + 4 r a r +
Polytrichum formosum	a b + + + a + a
Deschampsia flexuosa	3 4 2a 5a 5a 3 4 4 b 2b 3 5b 4 4 5a 3 5a
Dicranum scoparium	2a 2a 2b 2a a 2a + + 2a r 3 a b 2a b
Hypnum cupressiforme	2a a b 2b 3 2b b + b a r 2b b b 2b + 2b 4
Calluna vulgaris	+ + b + + + + b + b
Erica tetralix	+ + r a +
Molinia caerulea	a + + + + b + + r 2a a r
Dryopteris carthusiana	b + + + b + a + b a r + + b
Dryopteris dilatata	+ r b r + + b
Corydalis claviculata	b + + a b r b
Galium saxatile	+ + + + b +
Pleurozium schreberi	3 2a 3 + 5a 2a b + 2a 2a 2b 2a 2a 2b
Empetrum nigrum	+ b 3 b 2b
Goodyera repens	+ +
Stellaria media	+ r r +
Senecio sylvaticus	+ r +
Ilex aquifolium	r r
Chamaerion angustifolium	+ + r r + + r + + +
Agrostis stolonifera	+ a + +
Agrostis capillaris	+ r r +
Festuca ovina	2b + + r + b
Carex pilulifera	+ + + +
Juncus squarrosus	r r
Vaccinium vitis-ideae	2b
Leucobryum glaucum	+ +
Frangula alnus	r 3*
Prunus serotina	a r

Polytrichum juniperinum (1: b), *Rumex aecetosa* (42: r), *Sambucus nigra* (50: r), *Sorbus aucuparia* (shrub, 51: b).

---

* In shrub layer.

Composition of the green vegetation and its relation to the mycofloristic composition
Data on the green vegetation are presented in table 14. The most common species were *Deschampsia flexuosa*, *Calluna vulgaris*, *Dicranum scoparium* and *Hypnum cupressiforme*. Only *C. vulgaris* was found in all stand types.

The Y1-s and OW-n stand types were characterized by very low herb layer cover percentages and the presence of *Cladonia spp*. In the Y2 plots, less species occurred than in the old plots but no species occurred which differentiated them from the old stand types. The vegetation of the two Y1-p plots on drained moor-peat consisted mainly of *Molinia caerulea*, *Calluna vulgaris* and *Erica tetralix*. The vegetation of the two other Y1-p plots, which were situated on formerly arable land, differed strongly from all other plots (table 14).

The cover percentage of the herb layer was negatively correlated with the number of carpophores and the number of fungal species (fig. 16). The ordination on the basis of the green vegetation (fig. 15) separated the stand types less clearly, but in roughly the same way compared to the ordination on the basis of the mycoflora (*cf.* fig. 13). The indices of dissimilarity between the stand types based on the green vegetation were highly correlated with those based on the mycoflora (P<1%, fig. 17).

Stand Type
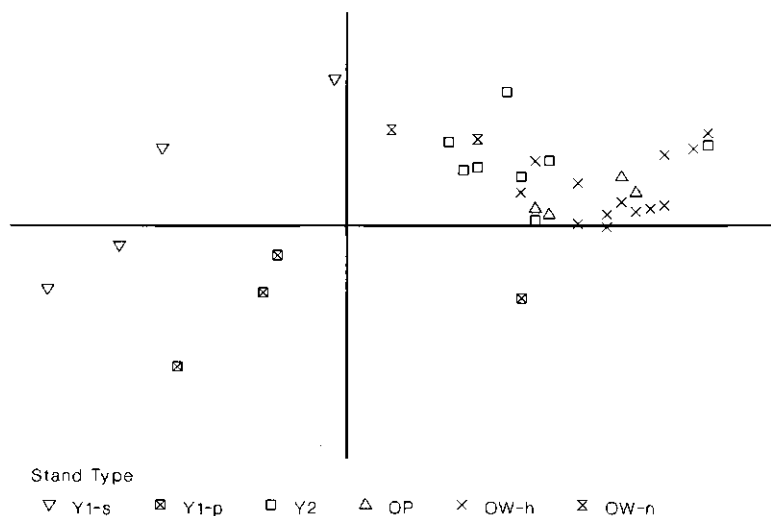▽ Y1-s   ⊠ Y1-p   □ Y2   △ OP   ✕ OW-h   ✗ OW-n

Figure 15. Ordination of the plots based on the green vegetation. For abbreviations of the stand types see table 8.
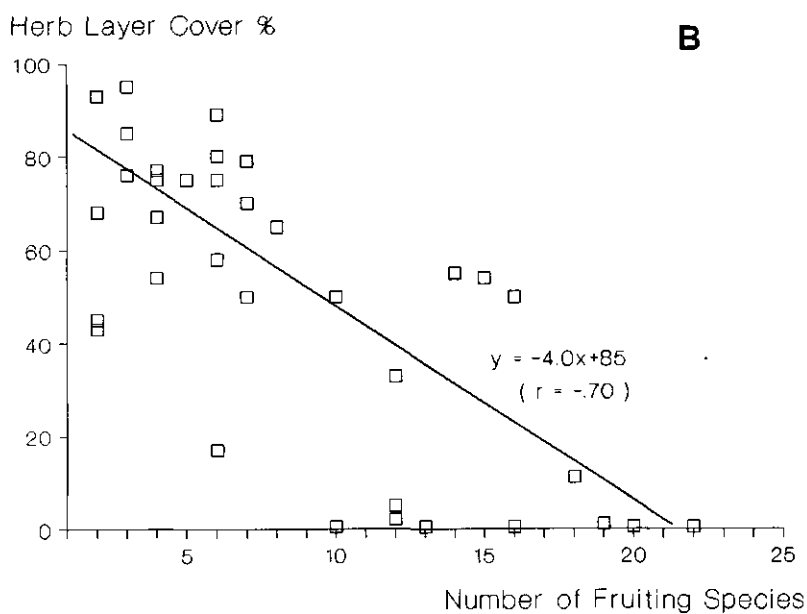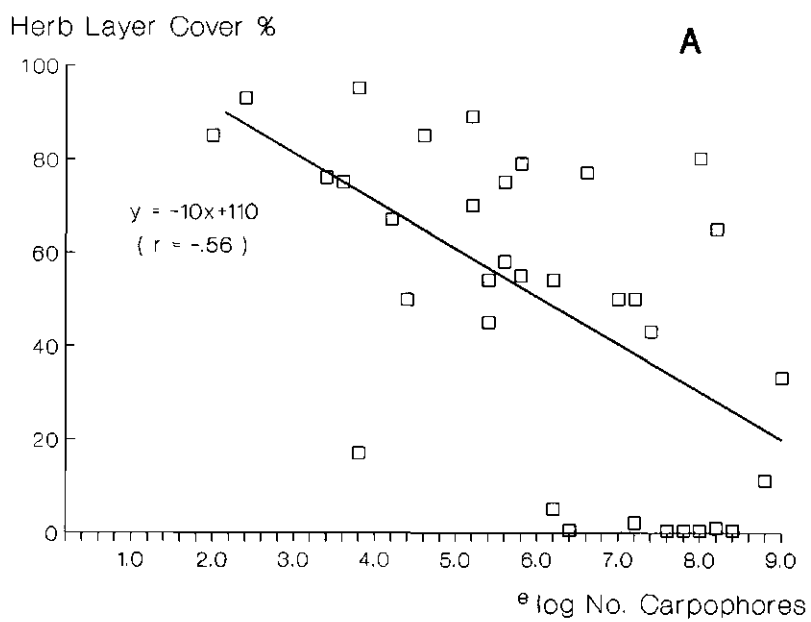
Figure 16. The relation between the herb layer cover percentage and (A) the maximum number of carpophores (^{e}log) and (B) the number of fruiting species per plot.

ID (Green Vegetation)
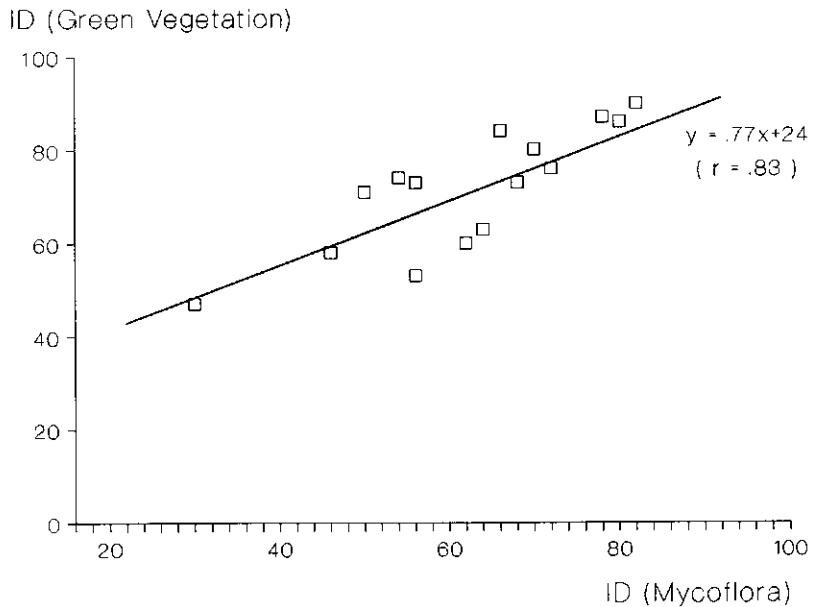
Figure 17. Indices of dissimilarity (after Sørensen) between all possible combinations of stand types, based on the mycoflora data, in relation to those based on the green vegetation data.

5.4 Discussion

Differences in mycorrhizal mycoflora were not only observed between stands of different age but also between stand types with the same tree age. The differences in the mycoflora must therefore be related to differences in the soil. This might have its origin in the humus content of the soil, because (1) the Y1 plots almost completely lacked a humus layer in contrast to the Y2 plots, and (2) the OW-n plots had a humus layer and mycoflora which was more comparable to the young than to the other old plots.

Several authors observed or suggested a negative relationship between the presence of humus and the growth or occurrence of mycorrhizas and roots. Alvarez et al. (1979) observed a higher survival rate and better growth of *Abies concolor* (Gord. & Glend.) Lindl. seedlings in mineral soil without than with organic layers, and attributed this to the better mycorrhiza development in mineral soil without organic layers. Bakshi et al. (1972) also attributed the poor root growth and poor mycorrhizal development of *Abies pindrow* Royle to the presence of thick humus layers. Dighton et al. (1986) reported a relation between the succession of mycorrhizal fungi and tree canopy closure, and concluded that this might be causally related to the accumulation

of litter. However, in the present study the young plots had a much higher canopy closure than the old plots (table 9), indicating that the canopy closure is not directly related to the succession of mycorrhizal fungi.

The richer mycorrhizal mycoflora of the young plots compared to the OP and OW-h plots, as well as the presence of several species in the young plots which have shown a decline in the Netherlands, indicate relatively favourable conditions for fructification of mycorrhizal fungi in the young stands. This agrees with observations made in a previous study (Termorshuizen & Schaffers, 1987), where indications were obtained that nitrogen deposition and sulphur dioxide severely affect the occurrence of carpophores of mycorrhizal fungi in old stands, but not in young stands. This apparent difference in sensitivity to air pollution was attributed to differences in the interception and accumulation of air pollutants in the soil and in a different need for nitrogen (Termorshuizen & Schaffers, Ch. 6).

Interestingly, several declining species, which were found to be differential for the young stands in the present study, are also reported to be present in 20 to 100-year-old stands of *P. sylvestris* in Estonia and Finland (table 15) (Hintikka, 1988; Kalamees & Silver, 1988). Hintikka (1988) reported many more carpophores in 20 to 30-year-old stands of *P. sylvestris* than in 5 to 15-year-old stands. The differences between the mycorrhizal mycofloras in Finland and Estonia with the mycorrhizal mycoflora in the Netherlands might be related to differences in nitrogen deposition, which is much higher in the latter country (Buijsman *et al.*, 1987).

The differences in the abundance of carpophores between the young and old plots are also likely to be related to the lower root density in the old plots. The lower root

TABLE 15

Presence (+) or absence (-) of some selected mycorrhizal fungal species in stands of different ages of Pinus sylvestris in Finland (Hintikka, 1988), Estonia (Kalamees & Silver, 1988) and the Netherlands (present research, Termorshuizen).

	Finland				Estonia		Netherlands	
Stand age	5-15	20-30	30-50	>70	25	80-100	5-10	50-80
Boletus edulis	-	+	+	-	-	-	+	-
Chroogomphus rutilans	-	+	-	+	+	+	+	-
Coltricia perennis	-	-	+	-	+	+	+	-
Cortinarius mucosus	-	+	+	+	-	-	+	-
Hygrophorus hypothejus	-	+	+	-	+	+	+	-
Lactarius rufus	+	+	+	+	+	+	+	+
Suillus bovinus	+	+	+	+	+	+	+	+
Suillus luteus	+	+	-	-	+	+	+	+
Suillus variegatus	+	+	+	+	+	+	+	+
Tricholoma portentosum	-	-	+	+	-	+	+	-

density might be caused by silvicultural treatments, which resulted in a lower canopy closure of the old stands (table 9). This is affirmed by the observation that the two old plots of stand type OW-n had a high canopy closure (table 9) and a high root density (table 13).

In addition to the possible influence of the humus layer on the composition of the mycoflora, the negative correlations of the herb layer cover with the abundance of carpophores and the number of fruiting species also suggest a more direct influence of plants on fungi, *e.g.* by competition or allelopathy. However, the most common plant species occurring in the old plots, *Deschampsia flexuosa* (L.) Trin., was quite common in some young plots as well, where many carpophores were found. The rich mycorrhizal mycoflora in Poland, in combination with the presence of a rich herb layer (Rudnicka-Jezierska, 1969), also indicates that inhibition of mycorrhizal mycoflora by competition or allelopathy of higher plants is not a general phenomenon. It is therefore more likely that both the green vegetation and the mycorrhizal mycoflora are influenced by the same soil factors which change during succession.

The mycorrhizal succession seems to progress hand in hand with the succession of the green vegetation (fig. 17). The same was concluded by Arnolds (1981), who studied the coenology of saprophytic macrofungi in grasslands and heathlands. As Arnolds (1981) already concluded, it seems pointless to introduce an independent syntaxonomic system for fungi as was proposed by Darimont (1973). On the other hand, it seems more appropiate to compose an integrated syntaxonomic system for fungi, plants and probably also for other organisms, which is likely to provide more information about the environment.

Dighton & Mason (1985) ascribed 'r' strategies to the 'early-stage' fungi and 'K' strategies to the 'late-stage' fungi. According to them 'r' properties of 'early-stage' fungi are the production of relatively small carpophores, rapid mycelial growth, absence of mycelial strands and use of inorganic material. In contrast, 'late-stage' fungi would produce larger carpophores, show slower mycelial growth, form mycelial strands and use mainly organic material.

The 'r' and 'K' attributes of 'early-stage' and 'late-stage' fungi as formulated by Dighton & Mason (1985), do not agree with the characteristics of the species which were found in the young and old plots in the present study. The carpophores were largest in the young stands (table 10), and many species from the young plots form rhizomorphs abundantly (*e.g. Tricholoma* species, *cf.* Godbout & Fortin, 1985). However, it should be questioned whether carpophore weight, or size, is related to 'r/K' attributes of a fungal species, instead of size and numbers of spores produced. Furthermore, Abuzinadah & Read (1989) showed that the 'early-stage' fungus *Hebeloma crustuliniforme* (Bull.:Fr.) Quél. was much more effective in transferring protein-N than the 'late-stage' fungi *Amanita muscaria* and *Paxillus involutus*.

On the basis of the present research it can be concluded that succession of mycorrhizal fungi is not only limited to the first years after planting, within which the 'early-stage' and 'late-stage' fungi are distinguished (Fox, 1986), but also to the older development stages. It seems that the composition of the mycorrhizal mycoflora is not in the first place related to the ageing of trees, but rather to the ageing of the forest soil. In successive rotations other fungal species are therefore expected to behave as 'early-stage' and 'late-stage' fungi. However, much more research is needed to elucidate the processes underlying mycorrhizal succession in more detail.

5.5 Summary

The composition of the mycorrhizal mycoflora was investigated in 35 stands of *Pinus sylvestris* L. throughout the Netherlands. Three types of young (4-13 years) and old (50-80 years) stands were selected on the basis of number of rotations and soil type. The young stands had either grown spontaneously on drift sands (1st rotation, type Y1-s), were planted and 1st rotation (type Y1-p) or planted and 2nd or 3rd rotation (type Y2). The old stands were distinguished on the basis of their occurrence on soils with or without a pronounced podsol (type OP: old with podsol and two OW types: old without podsol). Stands of type OW-n were characterized by a very thin humus layer and almost complete absence of a herb layer, in contrast to the stands of type OW-h, where a well-developed herb and humus layer were present.

A plot measuring 1050 m^2 within each stand was searched for carpophores of mycorrhizal fungi during the autumns of 1986 and 1987. Ten soil samples per plot were taken in October 1987 in order to assess the mycorrhizal status of the tree roots.

The composition of mycorrhizal mycoflora in the different plots was subjected to a TWINSPAN cluster analysis and a Bray & Curtis ordination. It appeared that the plot groupings generated by these analyses largely parallelled the stand types, indicating that each stand type has its own mycoflora. Differences in mycofloristic composition between stand types were parallelled by differences in the composition of green vegetation.

The young stand types had 3.5-27 times more carpophores and 1.4-6.8 times more species than the OP and OW-h stand types. The OW-n stand type however, was more similar to the Y2 stand type than to the other old stand types. Considerable differences in species composition between the Y1 and Y2 stand types were observed. It is concluded that the succession of mycorrhizal fungi is not primarily influenced by ageing of the trees, but rather by changes in the soil.

The results were compared with data on changes in the occurrence of fruiting species of mycorrhizal fungi in the Netherlands during this century. It appeared that

species which have declined according to these data were more frequent in the young plots than in the OP and OW-h plots. However, these species are reported to be frequent in old stands of *P. sylvestris* in Estonia and Finland. It is argued that this difference is related to the high nitrogen deposition in the Netherlands.

Chapter 6

THE DECLINE OF CARPOPHORES OF MYCORRHIZAL FUNGI IN STANDS OF *PINUS SYLVESTRIS* L. IN THE NETHERLANDS: POSSIBLE CAUSES

6.1 Introduction

The decline of carpophores of mycorrhizal fungi during this century in the Netherlands has been described in detail by Arnolds (1985, 1988). From other countries in Europe such a decline has also been reported (Derbsch & Schmitt, 1987; Fellner, 1990; see also Arnolds, in preparation), and lists of threatened species have been published (Derbsch & Schmitt, 1984; Winterhoff, 1984; Winterhoff & Krieglsteiner, 1984; Wojewoda & Lawrynowicz, 1986; Arnolds, 1989a).

The occurrence of carpophores of mycorrhizal fungi appeared to be closely related to the amount of air pollution and to tree vitality (Schlechte, 1986; Termorshuizen & Schaffers, 1987; Fellner, 1990). This would give the carpophores a bio-indicative value for the amount of air pollution and/or the vitality of a forest (Dörfelt & Braun, 1980; Fellner, 1990). However, Jansen & De Nie (1988) and Termorshuizen & Schaffers (1987) showed that the age of the forest was a complicating factor: younger stands of *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus sylvestris* L. appeared to have a different and richer mycoflora than older stands.

The cause of the decline of carpophores is usually considered to be related to air pollution or to decline of tree vitality, but the exact mechanism is unknown. Generally, two hypotheses have been proposed (Termorshuizen & Schaffers, 1987). The first one states that air pollution affects the photosynthetic apparatus and the transport of photosynthates, decreasing the supply of carbohydrates to mycorrhizal fungi. The second hypothesis states that air pollution affects the soil environment, which may directly or indirectly affect mycorrhizal fungi. Termorshuizen & Schaffers (1987) found indications that both means of action significantly affect mycorrhizal fungi, without indicating the relative importance of the hypotheses.

In our previous article (Termorshuizen & Schaffers, 1987) we found negative effects of air pollution on the occurrence of carpophores in mature stands, but not in young stands of *P. sylvestris*. In this study we report observations in stands of *P. sylvestris* of different age in the Netherlands during 1985-1987. Our aim was to gain a

better insight into the factors which regulate the occurrence of carpophores of mycorrhizal fungi.

6.2 Material & methods

Selection of stands

Stands of *Pinus sylvestris* L. were selected throughout the Netherlands (fig. 18). Selection was confined to homogeneous stands on sandy soils beyond the direct influence of ground water, containing as few other ectomycorrhizal plants as possible. Only stands of a good provenance were selected. It is well known that at the beginning of this century seed of *P. sylvestris* of a bad provenance was regularly used, especially in the southern part of the Netherlands (Huisman, 1983). However, it is not registered. We therefore used a method after Van Goor (pers. comm.) which is based on the assumption that provenances which are well-adapted to local circumstances can be recognized as follows: a) the shape and architecture of the trees within a stand should be homogeneous, b) the stems of the individual trees should be straight, c) annual stem length increment should be more or less regular, especially in the first 15 years, d) reversible chlorosis of the needles during winter should be absent and e) absence of spontaneous death of individual trees. We discarded the last criterion of Van Goor's method, because of all criteria, we suspected this would significantly overlap with our problem.

In 1985, 8 young (5-10 years) and 11 old (50-80 years) stands were selected. Another 6 old stands were selected in 1986. Some of their characteristics are listed in table 16. In 1987, one of the stands under investigation had been accidently clear cut. Because 10 old stands were investigated over three years and 16 old stands over two years, analyses over the three and two years will be presented separately for the old stands.

TABLE 16
Some general characteristics of the young and old plots.

	Age	
	Young	Old
No. Plots	8	11-17[a]
Plot Size (m^2)	1050	1050
Age (yr)	5 - 10	50 - 80
Height (m)	2 - 3.5	13 - 22
Canopy Closure (%)	85 - 98	40 - 75
Generation	2nd-3rd	1st-3rd

[a] In 1985 eleven plots were selected and in 1986 six additional plots; in 1987 one plot had accidently been clear cut.
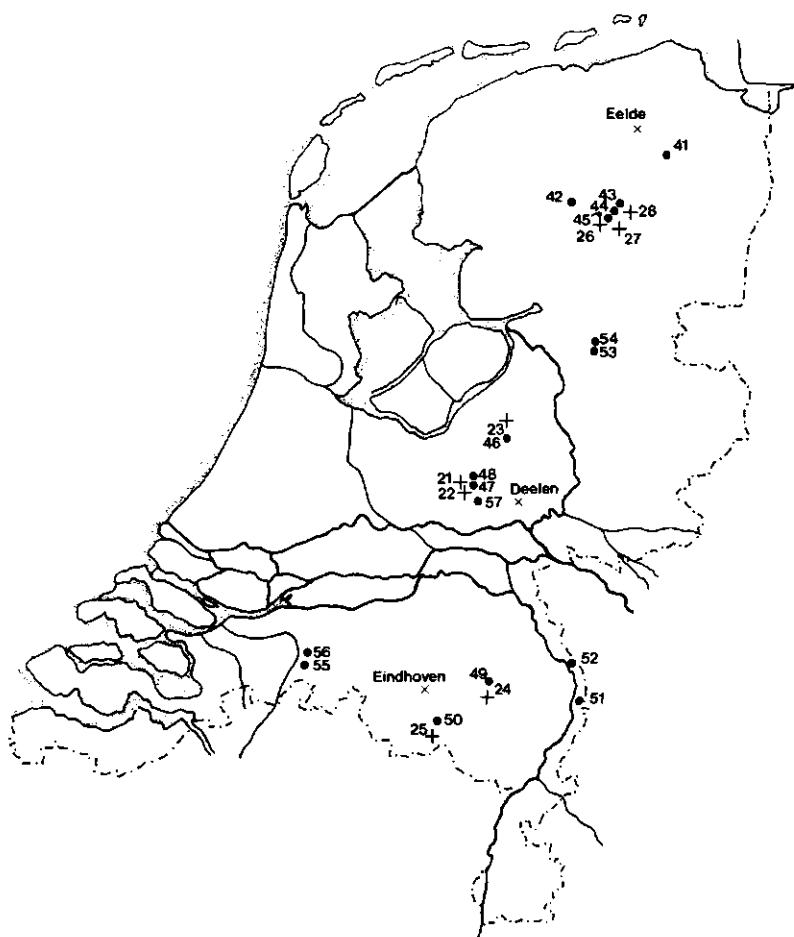
Figure 18. The location of young (+) and old plots (●) and of three meteorological stations (x).

Two old stands which were included in a former study (Termorshuizen & Schaffers, 1987 and Ch. 5) were omitted in this study due to large differences in soil characteristics and tree growth, compared to the other old plots.

All stands had been planted and a dense herbal vegetation with a high coverage of *Deschampsia flexuosa* (L.) Trin. existed in most plots. Data on the vegetation is presented in more detail in Termorshuizen (Ch. 5).

Within each stand a plot was selected measuring 1050 m^2. Undergrowth possessing ectomycorrhizas was removed in all plots.

Observations on carpophores

The plots were systematically searched each year in October and November three (1985, 1986) or four (1987) times for carpophores of mycorrhizal fungi. In order to prevent re-counting the carpophores, the caps were removed. Taxonomy and nomenclature are according to Moser (1983) and Jülich (1984) except for the taxonomy of *Dermocybe* Fr. (Wünsche) where Høiland (1984) was followed. Of each species the mean dry weight was determined on 10 to 50 carpophores.

We used the number and dry weight production of carpophores and the number of fruiting species as carpophore parameters. The maximum number of carpophores is defined as the cumulative number of carpophores per species of the most productive year. Analogously, the maximum (dry weight) production of carpophores is defined here as the cumulative dry weight production of carpophores per species of the most productive year.

Observations on mycorrhizas

After removing the litter layer, ten root samples were randomly taken from each plot in October 1987 from the upper 13 cm with a 2.5 cm diameter auger (volume 63.8 cm^3). The major part of litter and sand was removed with a 1 mm sieve. Roots were subsequently cleaned in a small glass tray containing water. The dead roots (defined as roots which are desiccated, shrunken and highly fragile) were removed during cleaning. After cleaning, the roots were stored in a glutaraldehyde buffer (Alexander & Bigg, 1981) until further analysis.

In each root sample the root length and the number of non-mycorrhizal and mycorrhizal root tips were determined. It was necessary to develop a working definition for mycorrhizal roots because intermediate types were observed between typical mycorrhizal roots (with a clearly visible mantle and aerial mycelium) and non-mycorrhizal roots (with many, approx. 5 mm long root hairs). Short roots with one or two poorly developed root hairs always possessed a Hartig net between some of the cortical cells. Short roots with three or more poorly developed root hairs often had no Hartig net. Therefore, all short roots possessing less than three root hairs were called mycorrhizal, and those with more than two non-mycorrhizal. Non-mycorrhizal root tips were divided into short (<2 cm) and long (>2 cm) roots.

The mycorrhizas were classified on the basis of their appearance. The first class (so-called well-developed mycorrhizas) possessed a smooth, relatively thick mantle, so that the root cells were not visible under a 12x magnification. The poorly-developed second class mycorrhizas either possessed a dented and more or less wrinkled mantle or no distinct mantle.

Apart from *Cenococcum geophilum* Fr. no attempts were made to identify the mycorrhizas in any way. All root data were recalculated to a volume of 100 cm^3.

Measurement of tree vitality

Each year in July or August parameters of the individual trees were measured: tree girth, tree class, crown width, crown density (estimated percentage of the crown projection), needle occupation, discoloration of needles, recovery shoots, resin flow, fungal diseases, insect pests and death or bending of the leader shoot. Following the method of the Dutch State Forestry Service (Anonymous, 1987), the needle occupation was used as indicator of the tree vitality. The needle occupation is defined as the maximum number of needles occurring on at least two branches, expressed as the cumulated needle occupation percentage per needle-year (*e.g.*, 150% needle occupation means that all needles of one year are present, 50% of a second year and none of the other years, or, alternatively, that 80%, 50% and 20% of the needles are present of three successive years).

Data on air pollution and climate

The data on concentrations of SO_2 and NO_x (x=1, 2) in the air and on total nitrogen deposition were derived from Anonymous (1986a, 1986b, 1988) for the years 1985-1987 separately. Because the NH_3 deposition levels are only rough estimates based on farmyard manure statistics (Buijsman *et al.*, 1985), one deposition level per plot is used, calculated as the mean of the estimated deposition levels of 1986 and 1987 (Anonymous 1986a, 1986b, 1988) (estimated deposition levels of 1985 were not available).

An indication of weather conditions during the research at three meteorological stations of the Royal Dutch Meteorological Institute is presented in figure 19.

6.3 Results[a]

Mycoflora

Each year the average number of carpophores and fruiting species was much higher in young plots than in old plots (fig. 20). A factor 13 more carpophores and a factor 3.0 more fruiting species were found in young compared to old plots. The differences in numbers of carpophores between young and old plots agree with the differences in total dry weights of carpophores.

In the old plots, *Lactarius hepaticus* Plowr. *in* Boud. was by far the most abundant species, accounting for 51%, 82% and 59% of the total number of carpophores in 1985, 1986 and 1987, respectively. *Laccaria proxima* (Boud.) Pat. was the most common species in young plots and accounted for 83%, 50% and 46% of the total number of carpophores in the three respective years.

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[a] The relevant data used in this study are presented in appendices I to III.

Precipitation (mm)

A

Temperature (°C)

B
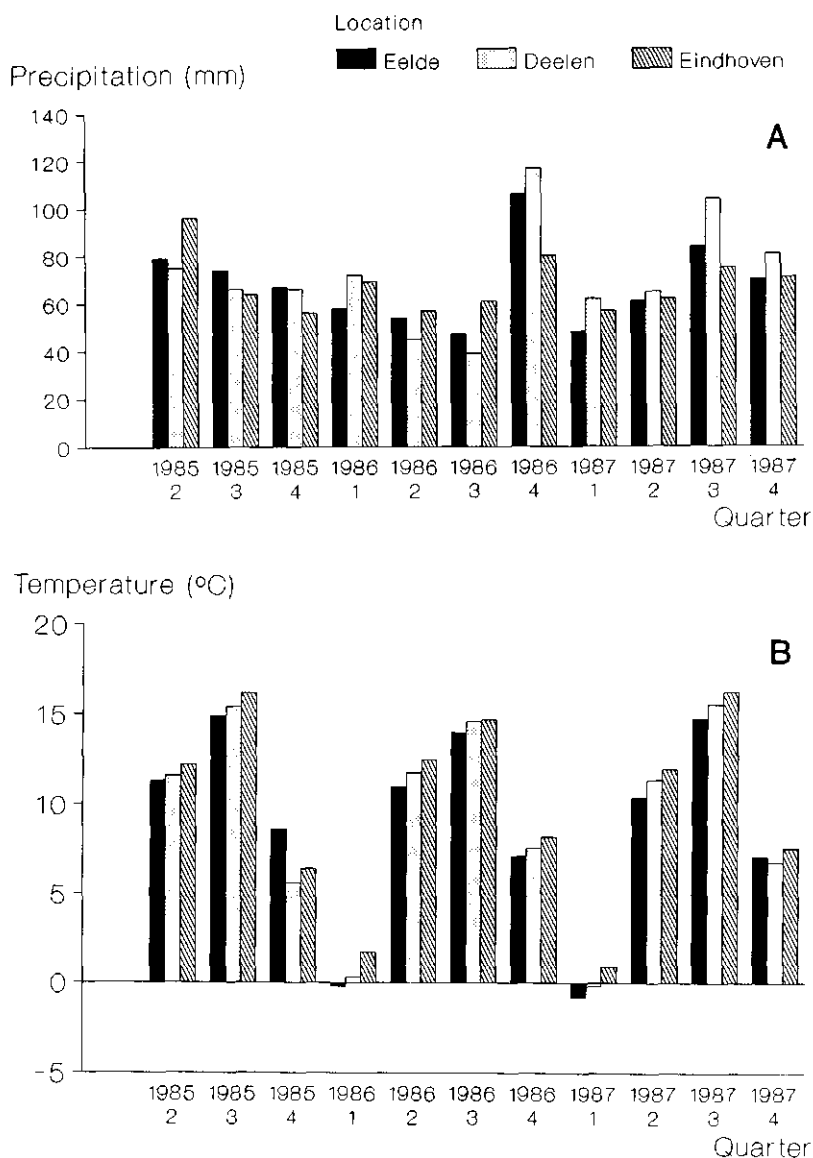
Figure 19. The quarterly precipitation [mm] (A) and average air temperature [°C] (B) reported by the meteorological stations at Eelde, Deelen and Eindhoven for 1985-1987. For the location of the meteorological stations, see figure 18.

Eleven species were found in young plots which appeared to have declined during this century in the Netherlands according to Arnolds (1985a) (table 17). Some of these species occurred abundantly in more than 50% of the young plots, viz. *Dermocybe croceocona* (Fr.) Mos., *D. semisanguinea* (Fr.) Mos., *Lactarius rufus* (Scop.:Fr.) Fr. and *Suillus bovinus* (L.:Fr.) O.Kuntze. Of the decreasing species, only *L. rufus* (in five plots) and *S. bovinus* (in two plots) were found in low quantities in old plots (table 17).
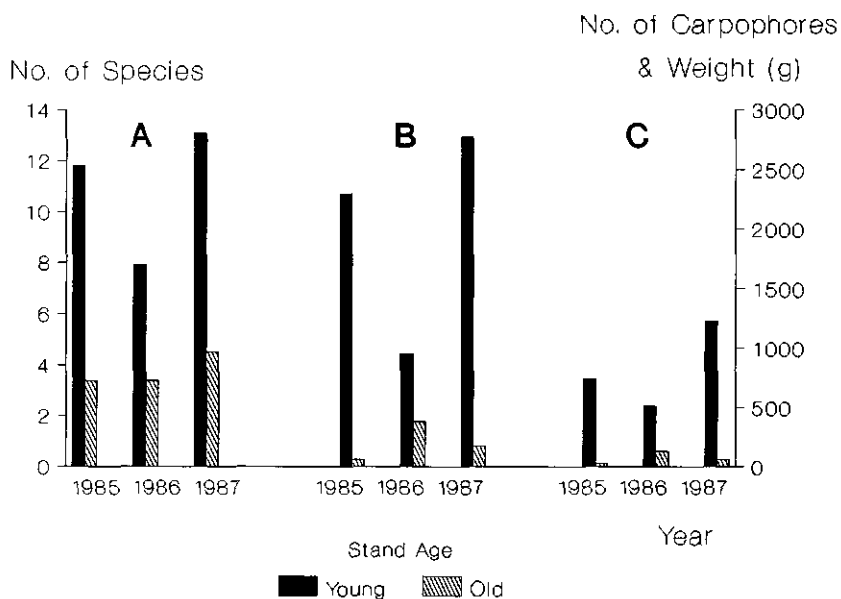
Figure 20. The mean numbers per plot of (A) fruiting species, (B) carpophores and (C) dry weight production of carpophores [g] for the young and old plots during the investigation years.

The number of carpophores and fruiting species differed considerably between the years, and the young and old plots did not show the same pattern (fig. 20). The young plots were poorest in 1986, while an optimum in the old plots for the number and the dry weight production of carpophores was found. For both the young and old plots, the number of species was highest in 1987.

Mycorrhizas

Practically all root tips were mycorrhizal in both the young and old plots (table 18). In only one old plot the mycorrhizal frequency was less than 95%. About two times more roots and mycorrhizas were present in young than in old plots. *Cenococcum geophilum* occurred in all plots and was very common, occupying on average 26% and 12% of the mycorrhizal roots in the young and old plots, respectively. In the young and old plots the proportion of well-developed mycorrhizas was 21% and 15%, respectively.

Tree vitality

The tree vitality, expressed here as needle occupation, increased for both young and old plots during the years. The needle occupation in old plots increased from 195 ± 20% in 1985 to 240 ± 15% in 1987. The bending of leader shoots occurred regularly in the old plots, in up to 77% of the trees in one plot. In the young plots, the increase in

- 81 -

TABLE 17

Presence (%) and average number of carpophores per plot of the most productive year (of the years 1986 and 1987) for each species, classified by their change in occurrence during this century in the Netherlands according to Arnolds (1985a).

	Young Plots		Old Plots	
	Presence (%)	No. Carpophores Plot^{-1}	Presence (%)	No. Carpophores Plot^{-1}
Species with significant decline				
Chroogomphus rutilus	13	1	0	0
Coltricia perennis	25	21	0	0
Dermocybe croceocona	75	240	0	0
Dermocybe semisanguinea	75	424	0	0
Suillus bovinus	75	76	13	2
Suillus variegatus	25	31	0	0
Species with insignificant decline				
Amanita muscaria	38	2	0	0
Gomphidius roseus	38	4	0	0
Hygrophorus hypothejus	25	20	0	0
Lactarius rufus	100	492	31	6
Rhizopogon luteolus	25	18	0	0
Species without change				
Amanita rubescens	50	16	0	0
Hebeloma mesophaeum	13	3	0	0
Inocybe lacera	38	11	0	0
Lactarius helvus	13	12	13	22
Paxillus involutus	100	27	56	7
Species with insignificant increase				
Laccaria laccata	13	7	0	0
Laccaria proxima[a]	100	1276	88	15
Lactarius hepaticus	88	225	94	273
Russula emetica	13	12	13	1
Russula ochroleuca	25	6	56	4
Scleroderma citrinum	63	64	6	2
Xerocomus badius	75	29	50	3
Species with unknown change				
Cortinarius flexipes	25	24	0	0
Cortinarius fusisporus	50	312	0	0
Inocybe brevispora	88	12	0	0
Inocybe boltonii	63	13	0	0
Inocybe lanuginosa	13	3	13	4
Inocybe umbrina	25	5	0	0

[a] Incl. *L. bicolor.*

TABLE 18
Plot average, minimum (min) and maximum (max) of some parameters of the roots of the young and old plots. Figures recalculated to 100 cm^3.

	Young plots			Old plots		
	average	min	max	average	min	max
Root length (cm)	104±34	41	152	52±23	20	88
Total no. mycorrhizas	207±67	72	271	96±47	25	212
Total no. mycorrhizas/cm root length	1.4±0.1	1.2	1.6	1.7±0.4	0.9	2.3
Mycorrhizal frequency	100±0.5	99	100	97±7.1	71	100
Rel. no. well-developed mycorrhizas[a]	21±5.6	13	29	15±7.4	2.6	25
Rel. no. of *C. geophilum* mycorrhizas	26±18	1.5	42	12±6.9	1.0	27

[a] Excl. *C. geophilum.*

the needle occupation ranged from 205 ± 20% in 1985 up to 225 ± 25% in 1987. In part of the young plots the degree of damage caused by *Lophodermium seditiosum* Minter, Staley & Millar was very high.

Tree parameters which did not show a significant variation between the plots (*e.g.* crown width) and those which occurred only rarely (*e.g.* discoloration of the needles, insect pests) are not presented here.

Air pollution

The 98-percentile concentrations of SO_2 (based on diurnal maxima) showed highest mean levels in 1985, 128 ± 12 μg m^{-3}. In 1986 and 1987 the mean levels were 86 ± 22 and 108 ± 43 μg m^{-3}. The 98-percentile concentrations of NO_x (based on diurnal maxima) did not show differences worth mentioning between the years, and were stable, averaging 108-122 μg m^{-3}. The estimated average nitrogen deposition due to NH_3 emission varied between the localities from 115 to 240 kg ha^{-1} yr^{-1}.

Correlations of data from the old plots

The concentration or deposition of all air pollutants under study showed significant negative correlations with the number and dry weight production of carpophores (table 19). NH_3 deposition (fig. 21) and NO_x concentration showed the strongest negative correlations with the number and dry weight production of carpophores (table 19). The SO_2 concentration and the percentage of trees with bending of the leader shoot showed comparable, although in most cases, weaker correlations. The needle occupation had the weakest correlations (positive) with the carpophore parameters. In most cases the number of fruiting species had weaker correlations with the environmental parameters than the number and the dry weight production of carpophores.

Correlations varied more or less between the years, but the same trend usually appeared each year. If only the plots which were investigated during three years are

1985-1987

^{e}log Production of Carpophores (g)

y = -.03x+9.2 (old plots)

Ammonia Deposition (kg N / Ha / Yr)

Stand Age
□ Old
× Young

1986-1987

^{e}log Production of Carpophores

y = -.02x+8.2 (old plots)

Ammonia Deposition (kg N / Ha / Yr)

Stand Age
□ Old
× Young
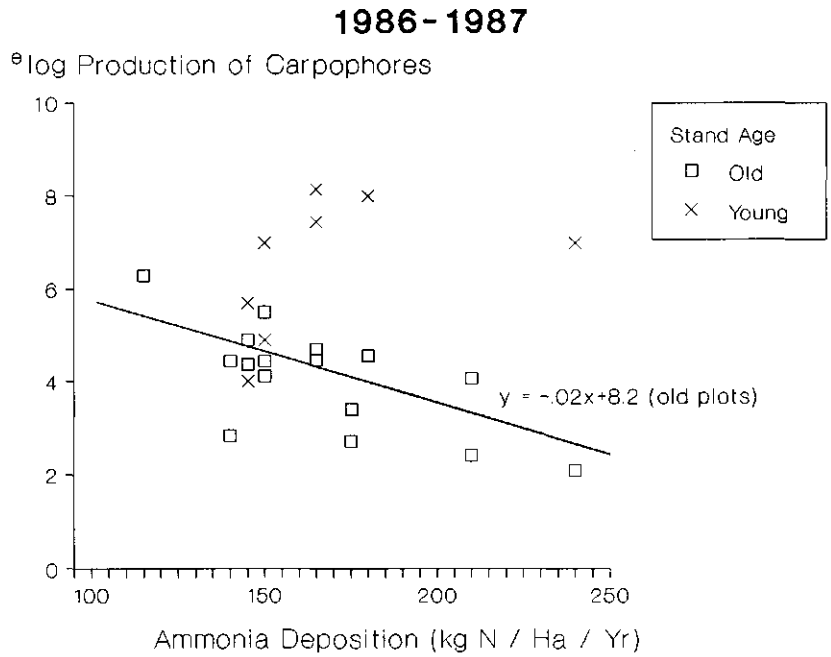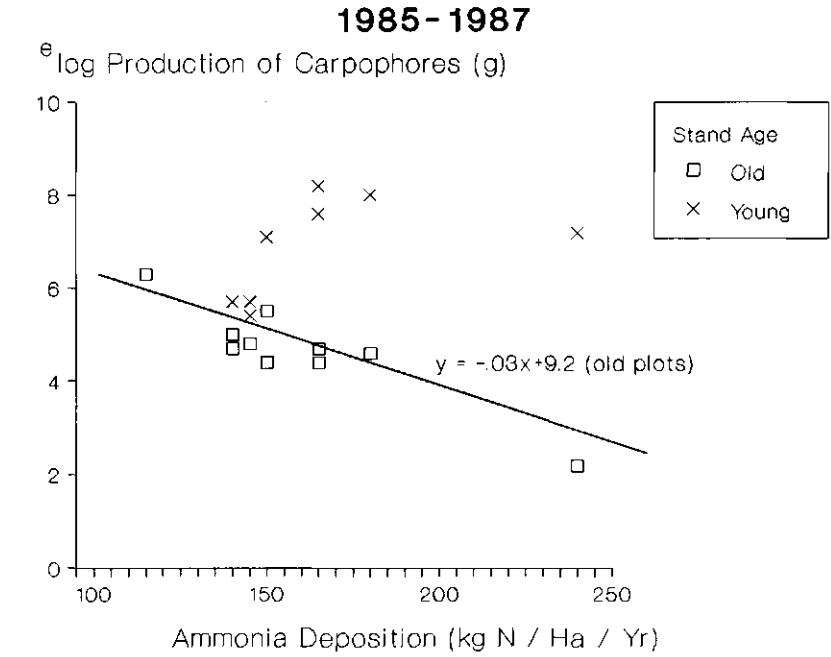
Figure 21. The relation between ammonia deposition and maximum dry weight production of carpophores (^{e}log) [g] per species over the years 1985-1987 and 1986-1987 for the young and old plots.

TABLE 19

Correlation coefficients between carpophore parameters and environmental parameters of the old plots per year and combined over the years 1985-1987 and over the years 1986-1987. SO_2 and NO_x: 98-percentile concentration based on diurnal maxima; NH_3: estimated nitrogen deposition due to NH_3 pollution; Needle: average plot needle occupation; Bent: percentage of trees with bending of the leader shoot; Significance levels: * = P<5%, ** = P<2.5%, *** = P<1%.

		SO_2	NH_3	NO_x	Needle	Bent
No. carpophores[a]	1985	-.78***	-.81**	-.86***	.24	b)
	1986	-.57***	-.79***	-.61***	.42	-.70***
	1987	-.34	-.43*	-.47*	.05	-.24
Dry weight prod.[a]	1985	-.81***	-.82***	-.85***	.26	b)
	1986	-.58***	-.78***	-.58***	.34	-.71***
	1987	-.36	-.57**	-.50**	.16	-.36
No. species	1985	-.63*	-.60*	-.72***	.54	b)
	1986	-.08	-.21	-.04	.03	-.49**
	1987	-.23	-.43*	.00	.23	-.39
Average no. carp.[a]	1985-87	-.66**	-.91***	-.68**	.44	b)
	1986-87	-.40	-.66***	-.58***	.29	-.51**
Average d.w.prod.[a]	1985-87	-.63*	-.96***	-.58*	.48	b)
	1986-87	-.44*	-.74***	-.58***	.32	-.63**
Average no. spec.	1985-87	-.56*	-.54	-.39	.22	b)
	1986-87	-.17	-.38	-.05	.22	-.49**
Max. no. carp.[a]	1985-87	-.64**	-.89***	-.69**	.37	b)
	1986-87	-.40	-.65***	-.62***	.27	-.46*
Max. d.w.prod.[a]	1985-87	-.51	-.94***	-.63*	.44	b)
	1986-87	-.42	-.71***	-.58***	.27	-.55**
Max. no. spec.	1985-87	-.55	-.45	-.18	.39	b)
	1986-87	-.10	-.40	.00	.42	-.46*

[a] ln-transformed.
[b] Not observed.

considered (10 plots), the correlations were stronger than if all the investigated plots are considered over two years (16 plots) (table 19). Nevertheless, the correlations of the two-year investigated plots are still comparable with those of the three-year investigated plots.

The needle occupation and percentage of trees with a bent leader shoot had significant (respectively negative and positive) correlations with NH_3 (table 20 & fig. 22), but the correlations with NO_x and SO_2 were less clear. The crown density, which was expected to be a good tree vitality parameter, had weak correlations with the air pollutants (except in 1985, table 20) as well as with the carpophore parameters (correlations not presented).
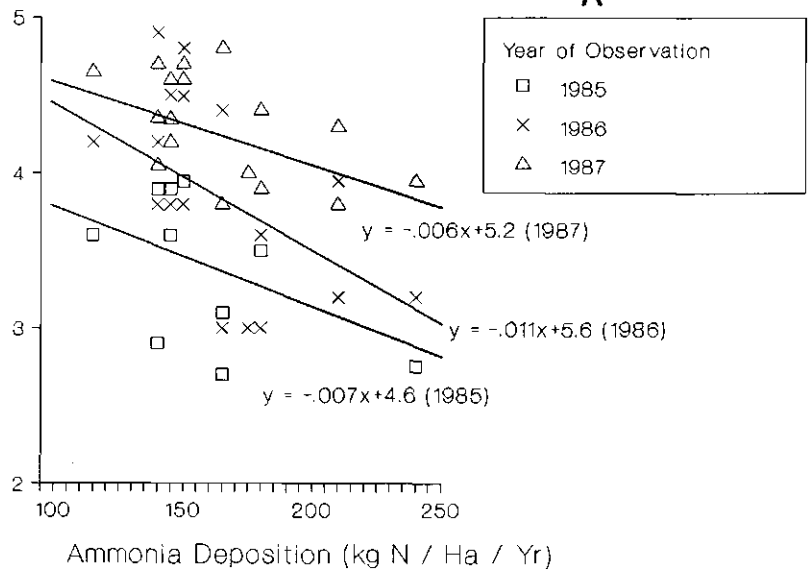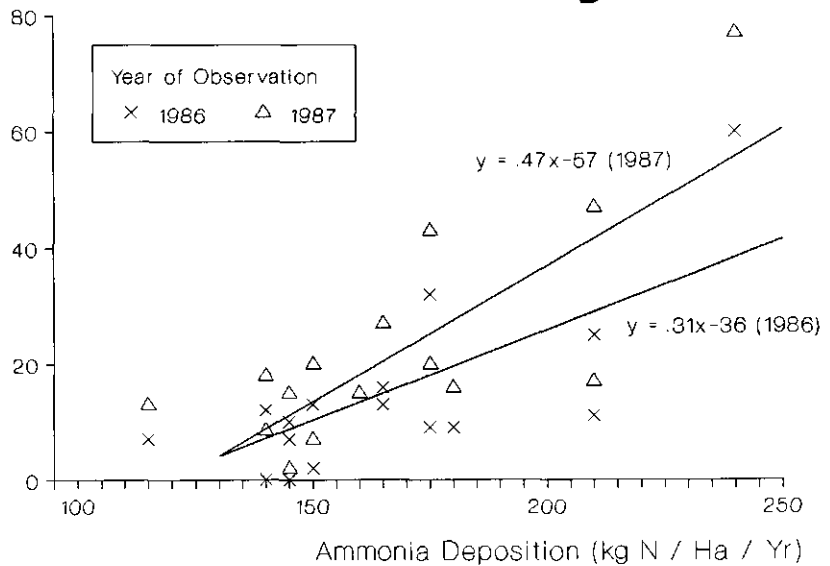
Figure 22. The relations between ammonia deposition and (A) average needle occupation and (B) the percentage of trees with bending of the leader shoot in the old plots.

TABLE 20
Correlation coefficients between air pollution parameters and tree parameters of the old and young plots. For abbreviations and significance levels. see table 19.

YOUNG PLOTS		SO_2	NH_3	NO_x	*Lophodermium* damage
Needle occupation	1985	.54	.73**	.47	-.74**
	1986	.93***	.75**	.92***	-.81***
	1987	.01	.69*	.90***	-.80***
	1985-87	.51	.77**	.78**	-.96***
Lophodermium	1985	-.30	-.35	-.62*	
damage	1986	-.84***	-.71**	-.79**	
	1987	-.23	-.57	-.49	
	1985-87	-.48	-.71**	-.74**	
OLD PLOTS		SO_2	NH_3	NO_x	
Needle occupation	1985	-.34	-.53	-.30	
	1986	.02	-.57**	-.21	
	1987	.18	-.57**	-.14	
	1985-87	-.39	-.58*	-.29	
	1986-87	.19	-.57**	-.20	
% Trees with bend-	1985	a)	a)	a)	
ing leader	1986	.36	.75***	.40	
shoots	1987	.09	.78***	.45*	
	1985-87	a)	a)	a)	
	1986-87	.23	.75***	.43*	
Crown density (%)	1985	-.45	-.77***	-.25	
	1986	.18	-.15	.06	
	1987	.07	-.06	.46*	
	1985-87	-.41	-.48	.12	
	1986-87	.22	-.12	.25	

a Not observed in 1985.

Correlations of data from the young plots

The number of carpophores and fruiting species showed strongest correlations with the NO_x concentration (positive), the degree of damage caused by *Lophodermium seditiosum* (negative) and the needle occupation (positive) (table 21). Correlations varied more or less between the years but the same trend usually appeared each year.

The degree of damage caused by *L. seditiosum* showed quite understandable high negative correlations with the needle occupation (fig. 23, table 20). *L. seditiosum* occurred most frequently in plots with low air pollution, which may explain the significant positive correlations of the needle occupation with air pollutants.

TABLE 21
Correlation coefficients between carpophore parameters and environmental parameters of the young plots per year and combined over the years 1985-1987. Loph: degree of damage caused by Lophodermium seditiosum. For other abbreviations and significance levels, see table 19.

		SO_2	NH_3	NO_x	Needle	Loph
No. carpophores[a]	1985	-.06	.15	.68*	.31	-.56
	1986	.70*	.45	.83***	.63*	-.82***
	1987	-.33	.42	.89***	.66*	-.11
Dry weight prod.[a]	1985	.24	.39	.93***	.47	-.59
	1986	.69*	.39	.77**	.60	-.81***
	1987	-.34	.46	.92***	.78**	-.32
No. species	1985	.29	.06	.20	.06	.01
	1986	.75**	.44	.78**	.68*	-.76**
	1987	-.04	.46	.74**	.60	-.23
Average no. carpophores[a]		-.01	.35	.83***	.67*	-.66*
Average dry weight prod.[a]		.10	.44	.94***	.72**	-.68*
Average no. species		.37	.40	.67*	.55	-.49
Max. no. carpophores[a]		-.04	.26	.79**	.67*	-.66*
Max. dry weight prod.[a]		.12	.45	.94***	.74**	-.68*
Max. no. species		.41	.20	.36	.32	-.22

[a] ln-transformed.
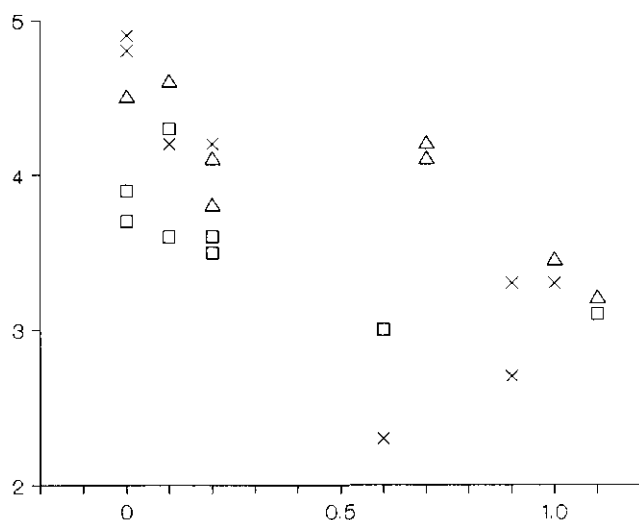
Figure 23. The relation between degree of damage caused by Lophodermium seditiosum in young plots, assessed in an arbitrary scale of 0 (no damage) to 4 (absence of living needles) and the average needle occupation.

Correlations of mycorrhiza parameters

The mycorrhizal frequency was significantly positively correlated with the number and dry weight production of carpophores for the young as well as for the old plots (table 22). The mycorrhizal frequency in the old plots showed weak correlations with air pollution parameters and was weakly significant for NH_3 (negative, P<10%).

In contrast to the mycorrhizal frequency, the total number of mycorrhizas per soil sample was only weakly correlated with the number and dry weight production of carpophores, and showed relatively high positive correlations with NH_3 deposition and NO_x concentration (significantly so for old plots).

The relative number of mycorrhizas of *Cenococcum geophilum* was negatively correlated with the number and dry weight production of carpophores in old plots, but positively in young plots. In both the young and old plots the absolute and relative number of mycorrhizas of *C. geophilum* was positively correlated with NH_3 and NO_x. In the young plots these correlations were highly significant (P<1%).

TABLE 22

Correlations of the root and mycorrhiza parameters with carpophore, air pollution and tree parameters for 1987. For abbreviations and significance levels, see table 19.

YOUNG PLOTS	Dry weight prod.	No. carp.	SO_2	NH_3	NO_x	Needle
Root length	-.27	-.29	.07	.00	-.03	.25
Tot.no. mycorrhizas	.31	.22	-.08	.50	.50	.71*
Tot.no. mycorrh. excl. *C. geophilum*	-.41	-.45	.19	-.13	-.20	.11
Mycorrhizal frequency	.96***	.88***	-.47	.69	.84**	.67
Tot.no. mycorrh. / unit root length	.87***	.76**	-.23	.80**	.85***	.82**
Rel.no. well-developed mycorrhizas	.71*	.72*	-.72*	.55	.79**	.59
Tot.no. mycorrhizas of *C. geophilum*	.97***	.89***	-.36	.87***	.96***	.88***
Rel.no. mycorrhizas of *C. geophilum*	.98***	.92***	-.43	.78**	.90***	.74*
OLD PLOTS						
Root length	.17	.27	-.29	.26	.37	-.13
Tot.no. mycorrhizas	.02	.22	-.21	.43*	.43*	-.14
Tot.no. mycorrh. excl. *C. geophilum*	.07	.26	-.23	.42	.35	-.11
Mycorrhizal frequency	.52**	.54**	.05	-.36	.06	-.01
Tot.no. mycorrh. / unit root length	-.08	.08	.07	.09	.09	-.40
Rel.no. well-developed mycorrhizas	.17	.27	-.45*	-.10	-.06	.05
Tot.no. mycorrhizas of *C. geophilum*	-.27	-.14	.07	.18	.56**	-.21
Rel.no. mycorrhizas of *C. geophilum*	-.44*	-.45*	.28	.10	.40	-.07

6.4 Discussion

Large differences in the number of carpophores and fruiting species between young and old plots were observed. The old plots produced less carpophores and fruiting species compared to mature *P. sylvestris* forests in other countries (table 23). The reduction is most prominent in plots exposed to relatively high concentrations of air pollutants, notably nitrogen pollutants. In the young plots, however, the numbers of carpophores and fruiting species are comparable with, or even higher than those of *P. sylvestris* of different ages in other countries (table 23), and are not negatively correlated with air pollution parameters. Several species which have shown a decline during this century in the Netherlands (Arnolds, 1985a) are abundant in young plots. In old plots however, only two declining species (viz. *Lactarius rufus* and *Suillus bovinus*) were found (table 17). Apparently, the decline of carpophores of mycorrhizal fungi has occurred more prominently in old stands.

The reduction of carpophores in the old plots may be explained by the fact that leaf damage by NH_3 (Van der Eerden, 1982; Kaupenjohann *et al.*, 1989) or by other air pollutants (Kozlowski & Constantinidou, 1986) can decrease the photosynthesis and in addition, a decrease in the transport of photosynthates (Lorenc-Plucinska, 1984; McLaughlin *et al.*, 1982). Nitrogen pollution also leads to an increased uptake of nitrogen by the trees, increasing the conversion of nitrogen into amino acids. This conversion requires carbohydrates, and thus decreases the supply of carbohydrates to the mycorrhizas (Björkman, 1942). In addition, increased nitrogen uptake may lead to deficiency of other nutrients, especially on poor and acid soils (Paavilainen & Pietiläinen, 1983; Rehfuss *et al.*, 1983). This may explain the fact that the decline of carpophores of mycorrhizal fungi is most pronounced on poor and acid soils (Arnolds, 1985a).

Most old stands which were investigated belonged to the most vital class, according to the Dutch State Forestry Service (Anonymous, 1987), and a few stands belonged to the second most vital class. The absence of low vitality classes can be ascribed to the selection criteria we used, especially the criterion of good provenance. Apparently, a decline of carpophores of mycorrhizal fungi may occur in stands before macroscopical decline of tree vitality becomes visible (Fellner, 1990). This may be explained by the fact that in the case of stressed carbohydrate economy, plants invest relatively more energy in the shoot than in the root in order to repair or replace injured tissue.

The decline of carpophores seems to precede a decline in the mycorrhizas, since the mycorrhizal frequency reached very high levels in all plots except one (>95%). In some poor stands of *Pseudotsuga menziesii* in the Netherlands, mycorrhizas have

TABLE 23

Comparison of some studies on the mycoflora of stands of P. sylvestris at different locations.

Location	Stand age (yr)	No. plots	Plot size (m²)	Period of investigation	Carpophore production[a]	No. Carpophores ha⁻¹ yr⁻¹	No. species[b]	Reference
The Netherlands	5-10	8	1050	1985-1987	7.8	19102	16	This study
"	50-80	16	"	"	0.5	1460	5.4	"
Estonia	25	1	1000	1978-1981	50.4[c,d]	?	11	Kalamees & Silver (1988)
"	80	1	"	"	15.3[c,d]	?	22	"
"	100	1	"	"	19.7[c,d]	?	24	"
Finland	5-15	7	750	1975-1977	?	791[f]	9.9	Hintikka (1988)
"	20-30	8	"	"	?	2178[f]	20	"
"	30-50	4	"	"	?	1726[f]	19	"
"	>70	6	"	"	?	855[f]	12	"
Kareli (USSR)	27-33	1	?	1979-1985	15.3[c,e]	?	?	Shubin (1988)
Poland	?[g]	4	200	1960-1964	?	?	7-11	Rudnicka-Jezierska (1969)

a kg ha⁻¹ yr⁻¹ dry weight.
b Average total number of species per plot over the period of investigation.
c Fresh weight data recalculated to dry weight data assuming a dry weight content of 10%.
d Inclusive relatively few saprophytic species, but exclusive non-edible mycorrhizal species.
e Inclusive some species which are associated with *Betula spp.*, but exclusive non-edible mycorrhizal species.
f Data corrected for differences in number of surveys per plot. The data are not directly comparable with the other studies because of limited number of observations.
g Pine forest plantation lacking other ectomycorrhizal tree species (tables 10 & 11 in Rudnicka-Jezierska, 1969).

already been reported to be completely absent (Jansen & De Nie, 1987). The situation of mycorrhizas in less vital old stands of *P. sylvestris* therefore needs additional research. It seems logical that the formation of carpophores is hampered before the formation of mycorrhizas decreases, because successful colonisation of the soil by mycorrhizal fungi depends basically on the availability of carbohydrates from a host. Therefore, under circumstances of a decreased carbohydrate availability, the mycorrhizal fungus is likely to invest relatively more energy in the maintenance of the mycorrhizal infection than in the formation of carpophores.

Our correlations indicate that nitrogen pollution affects the mycorrhizal fungi in old stands more than SO_2 pollution. Nitrogen fertilization experiments normally have a negative effect on the number of both carpophores and fruiting species (Ritter & Tölle, 1978; Menge & Grand, 1978; Wästerlund, 1982; Ohenoja, 1988; Shubin, 1988), which is usually more clear than the effect on the mycorrhizas.

Nitrogen deposition has increased tremendously in the Netherlands since the sixties. This is caused predominantly by the enormous growth in cattle population, increasing the production of manure from 24.10^9 kg in 1950 up to 85.10^9 kg in 1988 (Anonymous, 1980, 1989). Besides, the NH_3 losses from manure have increased due to the change from solid to liquid manure storage (Buijsman *et al.*, 1987). Furthermore the NO_x concentrations in the air have also increased considerably (Kozlowski & Constantinidou, 1986). Local extremes in nitrogen deposition in forests can be largely ascribed to ammonia because of its relatively high dry deposition rate (Asman & Maas, 1987) and because its sources are often close to the forests. The increase in nitrogen deposition of the last decades coincides with the observed decline in carpophores.

In most forests without human influence, nitrogen is the limiting factor for tree growth (Carlyle, 1986). On a short-term basis, or at low concentrations, extra nitrogen may therefore stimulate growth (Termorshuizen & Ket, Ch. 3) and photosynthesis (Pérez-Soba *et al.*, 1990). However, as trees age, this growth stimulating effect may lead to deficiency of other nutrients as explained above. In addition, the need for external nitrogen by younger trees is larger than for older trees, where internal recycling becomes more important (Carlyle, 1986). Younger trees and their mycorrhizas may therefore benefit from nitrogen at levels where older trees may suffer. Other important factors creating more favourable circumstances for mycorrhizal fungi in young stands may be:

(1) The smaller size of young trees and protection under the lee of old stands might contribute substantially to a lower interception of air pollutants compared to that of old stands. Quantitative data on this subject are, unfortunately, not available.

(2) Clear-cutting and disturbance of the soil before and at the time of planting result in increased leaching of nitrate (Vitousek, 1981; Tamm, 1982). Consequently, young stands start to grow on soil relatively free from nitrogen pollution, also

because the polluted upper soil-layer is mixed with the underlying relatively unpolluted soil. The importance of the humus layer is stressed by De Vries *et al.* (1985), who found significant negative correlations between the thickness of the humus layer and the number of carpophores. Removal of the upper soil-layer (litter and humus) in mature stands of *P. sylvestris* had a positive effect on the occurrence of carpophores of mycorrhizal fungi (Jansen & Van Dobben, 1987; Kuyper, 1988). The same was observed in this study in plot no. 49 (the poorest plot with respect to carpophores, with the highest NH_3 pollution) where approx. 30 m^2 of the litter and humus layer (including the vegetation) was removed illegally by unknowns in 1987. In the autumn of 1987, 88 carpophores of mycorrhizal fungi were counted on this spot, which was 94 times more per unit area than on the remaining 1020 m^2 of the plot.

Instead of air pollution, the variation in the occurrence of carpophores in the young plots was primarily influenced by the incidence of *L. seditiosum*. Interestingly, the incidence of *Lophodermium spp.* is likely to be negatively affected by air pollution, as was reported for SO_2 by Scheffer & Hedgcock (in Horn, 1985) and Grzywacz & Wazny (1973). The positive correlations between the number of carpophores and air pollution in young plots might therefore be well explained by the sensitivity of *L. seditiosum* to air pollution.

In the course of stand development, a decline in the occurrence of carpophores can be expected to start when nitrogen uptake (as a result of nitrogen pollution) inhibits the transport of carbohydrates to the mycorrhizal fungi. Decline in occurrence of carpophores is influenced by many factors (*e.g.* soil characteristics, silvicultural measures, stress factors) and consequently depends on the local situation. Observations in closed stands of *P. sylvestris* in the Netherlands (Termorshuizen, unpublished) suggest that this may begin between the 15th and 25th year. Jansen & De Nie (1988), who studied the occurrence of carpophores of mycorrhizal fungi in stands of *Pseudotsuga menziesii* in the Netherlands, also reported a sudden reduction in number of carpophores of mycorrhizal fungi in stands older than 20 years. Termorshuizen (Ch. 4) observed negative effects on the number of carpophores in two young stands of *P. sylvestris* by fertilization with $(NH_4)_2SO_4$ or $NaNO_3$ at 60 kg N ha^{-1}. Apparently decline will commence earlier if air pollution increases.

Of the mycorrhiza parameters we estimated, only the mycorrhizal frequency was significantly correlated with the number and dry weight production of carpophores in both young and old plots. However, the total number of mycorrhizas did not show such correlations. In young plots, the positive correlations of total number of mycorrhizas with the nitrogen air pollutants can be ascribed largely to *Cenococcum geophilum* (table 22). However, we are not able to explain the significantly positive correlations of the total number of mycorrhizas with NH_3 deposition and NO_x

concentration (table 22). Clearly more research is needed in order to evaluate the ecological significance of, and the relation between different mycorrhiza parameters.

Cenococcum geophilum occupied large parts of the roots (table 18), and was positively correlated with nitrogen pollution. The correlations of this non-fruiting species were significantly negative with the number and dry weight production of carpophores in old plots, but highly positive in young plots. We are not able to explain this phenomenon, and more research is needed on this species.

It seems plausible that the nitrogen enrichment of the forests is responsible in the first place for the decline of carpophores in the Netherlands. In addition, other pollutants which affect the trees worsen effects on mycorrhizal fungi. The high mycorrhizal frequencies observed in all plots, and the change towards a richer mycoflora after replanting the site indicate that the mycoflora may recover to some extent after replanting, if the air pollution, especially the NH_3 pollution, is drastically diminished.

6.5 Summary

This study was set up after a drastic decline in the mycorrhizal mycoflora in the Netherlands had been reported. A number of 8 young (5-10 years) and 17 old (50-80 years) stands of *Pinus sylvestris* L. were selected throughout the Netherlands. In each stand, a plot measuring 1050 m^2 was selected and during each autumn of the years 1985, 1986 and 1987 was searched for carpophores of mycorrhizal fungi. The vitality of the stands was annually determined by quantifying the needle occupation of the trees. Ten soil samples per plot were taken in October 1987 in order to assess the mycorrhizal status of the roots.

Each year the young plots had a higher number of carpophores and more fruiting species than the old plots. A highly significant negative correlation was found between the estimated pollution by nitrogen compounds and both the number of carpophores and the number of fruiting species in the old plots. The correlations with the 98-percentile concentrations of SO_2 were also negative but less significant than the correlations with the deposition and pollution levels of the nitrogen compounds, or not significant at all. Compared with recent data on the mycorrhizal mycoflora of old stands of *P. sylvestris* in countries less polluted with nitrogen, the mycoflora of the Dutch stands was extremely poor. However, the mycorrhizal frequency was higher than 95% in all plots except one, indicating that decline was mainly restricted to the production of carpophores. Possible causes are discussed, and the possible role of nitrogen pollution is stressed.

The young plots, however, did not show negative correlations with the air pollutants, and their mycofloras were comparable with those of stands of *P. sylvestris* in less polluted countries. It is concluded that no decline of mycorrhizal fungi has occurred in the young stands, or at least not to the same extent as in the old stands. It is suggested that the most important cause for this difference is clear-cutting and disturbance of the soil before and at the time of replanting, which results in a relatively unpolluted upper-layer of the soil.

Appendix I: Mycoflora.

(A) Old plots: Number of carpophores, dry weight production of carpophores [g] and number of species per plot per year and total number of species over the years 1986-1987.

Plot number		47	48	57	46	49	50	42	45	44	43	41	55	56	51	52	54	53	Average
No. carpophores	1985	7	6	7	4	0	0	200	45	81	45	202	-	-	-	-	-	-	54± 77
	1986	268	49	109	4	4	109	696	330	257	209	1680	12	41	41	103	87	8	245±407
	1987	258	171	-	135	31	1	502	130	171	120	192	1	34	21	32	191	1	124±131
Dry weight prod.	1985	5	12	1	3	0	0	42	25	48	46	77	-	-	-	-	-	-	24± 26
	1986	87	47	32	63	1	96	220	135	77	71	504	30	11	14	42	26	11	86±121
	1987	93	67	-	66	5	1	149	67	56	46	71	3	7	8	35	58	1	46± 41
No. species	1985	2	2	3	2	0	0	3	5	9	7	4	-	-	-	-	-	-	3.4±2.8
	1986	3	5	2	3	1	5	5	7	1	3	1	2	2	3	2	1	3	2.9±1.7
	1987	5	7	-	7	3	1	4	5	4	7	2	1	3	4	5	2	1	3.8±2.1
Tot. no. species	'85-'87	5	6	-	7	3	4	4	7	8	6	4	-	-	-	-	-	-	5.4±1.6
Tot. no. species	'86-'87	5	6	-	7	3	4	4	7	4	6	2	2	3	4	6	2	3	4.3±1.7

(B) Young plots: Number of carpophores, dry weight production of carpophores [g] and number of species per plot per year and total number of species over the years 1985-1987.

Plot number		21	22	23	24	25	26	27	28	Average
No. carpophores	1985	8897	4747	1091	1152	713	396	503	811	2289±3021
	1986	1999	2273	322	752	2000	69	206	5	953± 972
	1987	8589	7313	524	1326	3964	82	356	49	2775±3454
Dry weight prod.	1985	1646	1668	792	705	606	192	99	203	739± 621
	1986	778	778	182	328	1673	108	205	11	508± 553
	1987	1712	3349	952	960	2550	42	186	49	1225±1225
No. species	1985	8	15	12	11	15	13	8	12	12± 2.7
	1986	7	13	7	9	14	4	7	2	7.9± 4.1
	1987	12	16	15	15	18	10	13	6	13± 3.8
Tot. no. species	'85-'87	12	19	15	15	21	16	14	12	16± 3.2

Appendix II: Tree vitality.

(A) Old plots: Number of trees, tree girth (cm), needle occupation, percentage of trees with bending of the leader shoot and crown density per plot per year.

Plot number	47	48	57	46	49	50	42	45	44	43	41
Number of trees	75	62	51	57	108	68	43	35	48	40	104
Tree girth (cm)	20±3.5	19±2.8	23±3.7	23±4.1	16±3.0	23±4.3	24±4.7	24±3.4	22±4.5	26±4.7	16±3.6
Needle occupation 1985	180±30	160±25	200±30	225±25	165±30	200±30	170±35	205±40	215±30	215±35	205±35
1986	245±40	175±25	200±30	245±40	185±40	205±30	215±40	215±35	215±35	250±55	235±35
1987	265±25	215±30	-	260±25	220±20	245±30	230±25	235±30	245±30	245±30	255±30
Bending 1985	-	-	-	-	-	-	-	-	-	-	-
1986	13	16	8	2	57	9	0	0	10	10	7
1987	27	15	-	7	77	16	7	0	9	10	13
Crown density (%) 1985	32±5.4	29±4.2	36±11	38±9.1	24±8.6	33±6.2	33±11	31±8.6	29±11	34±11	43±13
1986	37±13	26±7.4	31±9.8	38±10	24±9.5	34±6.8	32±12	25±7.2	24±7.8	27±8.2	40±14
1987	33±8.8	34±10	-	36±8.7	24±6.6	34±8.0	30±11	23±7.3	24±9.9	22±9.2	34±14

Plot number	55	56	51	52	54	53	Average
Number of trees	62	44	60	70	59	47	61± 21
Tree girth (cm)	18±3.4	21±3.3	22±4.7	20±3.5	26±5.2	25±4.6	22±3.1
Needle occupation 1985	175±10	175±30	255±45	265±45	185±45	-	195± 20
1986	225±20	220±35	260±35	255±35	215±30	225±15	215± 30
1987	-	-	-	-	240±25	240±25	240± 15
Bending 1985	-	-	-	-	-	-	-
1986	32	9	12	14	27	11	14± 14
1987	43	18	18	20	47	17	22± 19
Crown density (%) 1985	34±8.0	25±9.4	40±13	42±14	35±13	42±14	33± 5
1986	32±6.3	37±12	36±9.1	38±13	32±9.5	33±6.9	33± 7
1987	-	-	-	-	-	-	31± 5

Appendix II: Tree vitality (contd.)

(B) Young plots: Number of trees, tree girth (cm), needle occupation and degree of damage caused by *Lophodermium seditiosum* per plot per year.

Plot number	21	22	23	24	25	26	27	28	Average
Number of trees	320	372	304	200	204	256	340	364	295± 68
Tree girth (cm)	2.1±.7	1.9±.8	4.0±.8	2.8±.9	5.9±1.3	3.5±.9	4.5±1.1	3.8±.9	3.6±1.3
Needle occupation 1985	210±30	200±35	205±40	240±50	205±35	180±25	175±20	220±30	205±20
1986	235±40	235±35	190±45	265±40	270±35	160±50	140±55	190±40	210±50
1987	230±40	235±45	230±40	250±30	255±35	190±35	185±40	215±20	225±25
Lophodermium[a] 1985	.03	.15	.14	.10	.12	1.08	.60	.00	0.3±0.4
1986	.14	.20	.97	.05	.02	.85	.59	.98	0.5±0.4
1987	.65	.66	.22	.04	.06	1.03	1.08	.20	0.5±0.4

[a] Degree of damage caused by *L. seditiosum* estimated in an arbitrary range from 0 to 4, where 0 means no damage and 4 absence of living needles.

Appendix III: Air pollution.

98-Percentile concentrations of SO_2 (μg m^{-3}, based on hourly maxima) and of NO_x (μg m^{-3}, based on diurnal maxima) and NH_3 deposition (kg N ha^{-1} yr^{-1}) per plot per year.

(A) Old plots

Plot no.		47	48	57	46	49	50	42	45	44	43	41	55	56	51	52	54	53	Average
SO_2	1985	126	126	126	119	149	150	120	122	122	122	122	-	103	-	-	-	-	128±11
	1986	85	85	87	77	109	110	65	65	65	65	50	103	103	140	124	73	73	87±24
	1987	55	55	-	48	137	143	114	114	114	114	105	114	114	213	197	90	90	114±45
NO_x	1985	129	129	129	131	124	123	87	87	87	87	80	-	-	-	-	-	-	108±22
	1986	119	119	119	115	125	125	103	97	97	97	88	125	125	125	125	104	104	112±13
	1987	136	136	-	120	140	140	105	100	100	100	83	140	140	136	136	110	110	121±19
NH_3		165	165	150	150	240	180	145	145	145	145	115	178	178	143	150	210	210	163±30

(B) Young plots

Plot no.		21	22	23	24	25	26	27	28	Average
SO_2	1985	123	123	119	149	150	122	122	122	129±13
	1986	85	85	77	109	110	65	65	65	83±19
	1987	55	55	48	137	143	114	114	114	98±39
NO_x	1985	129	129	131	124	123	87	87	87	112±21
	1986	119	119	115	125	125	97	97	97	112±19
	1987	136	136	120	140	140	100	100	100	122±19
NH_3		165	165	150	240	180	145	145	145	167±32

Chapter 7

GENERAL DISCUSSION

In the previous chapters, different aspects of the decline of mycorrhizal mycoflora
have been treated. An attempt is made to integrate these various aspects in section
7.1. The hypothesis formulated in the general introduction (sect. 1.3) is evaluated in
section 7.2. The implications of this research for the conservation of the mycorrhizal
mycoflora are examined in section 7.3. Finally, new questions raised by the present
research are discussed and the need for further research is indicated in section 7.4.

7.1 Synthesis of factors causing decline of carpophores of mycorrhizal fungi

The principal factor underlying the differences in mycorrhizal mycoflora between old
plots is the influence of nitrogen pollution on the nitrogen and carbon cycle of the
plant (fig. 24). Together these cycles determine the availability of carbohydrates for
mycorrhizal fungi. Also, pollution by sulphur dioxide (and probably photo-oxidants) is
likely to affect mycorrhizal mycoflora.

    According to the literature, N uptake by the plant increases amino acid synthesis,
decreasing the supply of carbohydrates to mycorrhizas. Effects on the mycorrhizal
fungi consequently depend on the amount of N uptake in proportion to the amount of
photosynthesis by the plant. An increased uptake of nitrogen might initially increase
the photosynthesis and growth, but a decrease can be expected if other nutrients
become deficient (Ch. 3).

    The supply of carbohydrates to mycorrhizas may also be decreased if photosyn-
thesis is inhibited by NH_3, NO_x or SO_2 (Ch. 2). In the Netherlands, these air pollutants
appear to have a significant influence on the frequency of carpophores of mycorrhizal
fungi (Ch. 6). Of these pollutants, nitrogen pollutants reduce the frequency of
carpophores most significantly, which is probably caused by the relatively high dry
deposition rate of ammonia and because the sources of ammonia are often close to the
forests (Ch. 6).

    N uptake will be increased if N pollution increases, if N interception increases (as
a result of increased aerodynamical roughness of the tree canopy) and if nitrification
decreases, and consequently, leaching decreases. In addition, the effects of N uptake
will be worse for mycorrhiza in older trees, where redistribution of N compounds
becomes more important due to decreased N demands (Carlyle, 1986). Quantification of
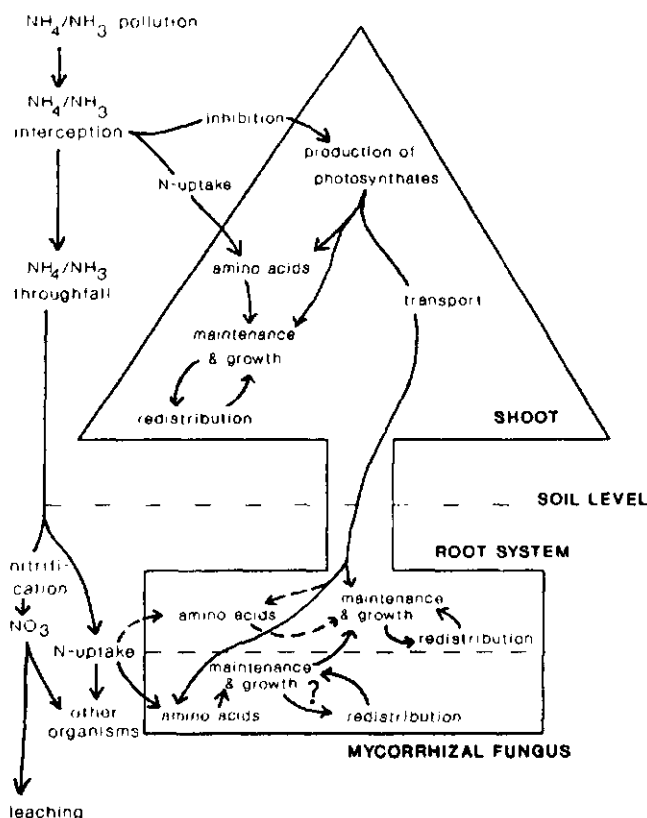
NH$_4$/NH$_3$ pollution

NH$_4$/NH$_3$ interception

inhibition

N-uptake

production of photosynthates

NH$_4$/NH$_3$ throughfall

amino acids

transport

maintenance & growth

redistribution

SHOOT

SOIL LEVEL

ROOT SYSTEM

nitrifi- cation

NO$_3$

amino acids

maintenance & growth

redistribution

N-uptake

maintenance & growth ?

other organisms

amino acids

redistribution

MYCORRHIZAL FUNGUS

leaching

Figure 24. Schematic representation of the carbon and nitrogen cycles of an ectomy-corrhizal tree under ammonia pollution. Dotted arrows represent less important processes.

these effects under field-conditions is quite difficult because of the many factors which influence photosynthesis and N uptake and deserves much more research. Special attention should be paid to the effects of nitrogen pollution on the properties of the litter and humus layer, and to effects of the presence or absence of such a polluted layer on the mycorrhizal fungi (Ch. 5,6).

In young stands, N uptake is likely to be less than in old stands, because of the relatively low interception of air pollutants and the increased nitrification and leaching of nitrates due to the recent clear-cutting and soil disturbance. The absence of *Lophodermium seditiosum* in heavily polluted areas (including SO$_2$) offered better conditions for root growth and mycorrhizal development in these areas.

In conclusion, the decline of mycorrhizal mycoflora can be explained in terms of carbohydrate deficiency for mycorrhizal fungi. Factors which increase N uptake by the plant increase the effects of decreased photosynthesis and transport of photosynthates on mycorrhizas. Carbohydrate deficiency does not initially affect the formation of

mycorrhizas, but rather inhibits carpophore fructification (Ch. 4,6). In a later stage, mycorrhizal formation starts to decrease. The formation of carpophores and mycorrhizas both seem to be hampered before the aboveground vitality of the tree is reduced significantly (Ch. 2-4,6).

Photosynthesis can be affected by air pollutants or by many other factors, for instance by pests or diseases. In the present study, it seems very plausible that infection by *Lophodermium seditiosum* significantly influenced the occurrence of carpophores by affecting production of photosynthates (Ch. 6).

In spite of the drastic decrease in fruiting species and abundance of carpophores in the old plots, mycorrhizal frequency was still nearly 100% everywhere (Ch. 5,6). This means that the species concerned were only affected in their ability to fructify or that their places on the roots have been taken over by less sensitive species. The latter possibility, the taking over by less sensitive species, is supported by several observations: (1) Several species showed an increase in frequency of carpophores during this century in the Netherlands (*e.g.*, *Laccaria proxima* and *Russula ochroleuca*; Arnolds, 1985a), (2) *Laccaria proxima* may have taken over the places of *Lactarius rufus* in the N fertilized plots at Deurne (Ch. 4), and (3) in the laboratory experiments (Ch. 2,3), the mycorrhizal frequency was generally negatively affected by the treatments, but there were considerable differences between the fungal species. *Laccaria proxima* appeared to be insensitive to SO_2, whereas *Paxillus involutus* appeared to be sensitive to SO_2 fumigation.

If the decline of mycorrhizal mycoflora can be explained in terms of carbohydrate deficiency for mycorrhizal fungi, apparently great intraspecific differences exist in tolerance to carbohydrate deficiency. In the experiments (Ch. 2-4), clear differences appeared between species in sensitivity to SO_2 and N fertilization. Species which survive under circumstances of carbohydrate deficiency must have either low carbohydrate demands, high sink capacities or saprophytic capabilities. It was suggested that *Laccaria proxima* was a relatively insensitive species to reduction in the supply of carbohydrates (Ch. 2,4).

Little is known about the minimum carbohydrate demands of mycorrhizal fungi. High carbohydrate sink capacities have been shown to exist for several fungal species (Dighton *et al.*, 1987; Stenström & Nyland, 1987; Gagnon *et al.*, 1988) and saprophytic capabilities of mycorrhizal fungi have been demonstrated by Trojanowski *et al.* (1984), Haselwandter *et al.* (1987) *in vivo* and by Dighton *et al.* (1987) *in vitro*. However, it seems unlikely that the insensitivity of *Laccaria proxima* in the SO_2 fumigation experiment (Ch. 2) can be explained in terms of a change towards saprotrophic nutrition by the fungus, because of the almost complete absence of organic matter in the soil used.

The mechanisms underlying the succession of mycoflora in first and second generation stands remain hypothetical. It was concluded (Ch. 5) that the explanation must be found in differences in the biotic or abiotic characteristics of the soil. Because of differences of the present data with data from abroad, an interaction between air pollution and this succession can however not be excluded.

7.2 Evaluation of the hypothesis on the decline of carpophores of mycorrhizal fungi

The working hypothesis proposed in section 1.3 stated that the decline of mycorrhizal mycoflora was caused by air pollution, affecting the fungus either via decreased tree vitality or via changes in soil chemistry. From the present research it appeared that air pollution does not affect mycorrhizal mycoflora everywhere to the same extent (Ch. 6). Stand age strongly influences the effects of air pollution on mycoflora. The hypothesis that air pollution is the most important factor seems to be true for mature stands of *P. sylvestris*, but not for young stands. However, more severe levels of air pollution are likely to affect the mycorrhizas in young stands as well (Ch. 2-4,6). Air pollution affects mycorrhizas via injury of trees aboveground (Ch. 2) as well as by changes in soil chemistry (Ch. 3,4). It is very difficult to judge which is the more important process. However, both act via the tree and both influence the carbohydrate availability for mycorrhizal fungi (Ch. 2,3). It seems that nitrogen pollution in the Netherlands affects mycorrhizal fungi stronger than other forms of pollution (Ch. 6).

Decreased frequency of carpophores of mycorrhizal fungi is not parallelled by decreased mycorrhizal frequency (Ch. 6). The decline of carpophores of mycorrhizal fungi probably indicates a decrease in mycorrhizal frequency in the near future (Ch. 6). This is supported by laboratory experiments, where pollution (Ch. 2) and nitrogen fertilization (Ch. 3) negatively affected mycorrhizal frequency.

In addition to air pollution, succession of Dutch forests also explains part of the change in mycorrhizal mycoflora (Ch. 5). Young stands of first rotation, nowadays rare in the Netherlands, appeared to have several fungal species which did not occur in stands of second or third rotation or in old stands of first rotation. However, data of old pine forests from abroad indicate that air pollution might influence this succession (Ch. 5).

7.3 Implications

The present-day situation of fructification of mycorrhizal fungi in the Netherlands is alarming. Many species have declined or even disappeared, and it seems that this decline still continues.

From the mycorrhizal point of view, the situation is less precarious. Mycorrhizal frequency in all plots but one was higher than 95%. This, and the rapid change into a rich mycoflora after replanting indicate that the soil is not polluted to such an extent that the growth of all mycorrhizal fungal species is structurally hampered. However, since mycorrhizal frequency was also negatively correlated with nitrogen pollution (Ch. 6, significant at $P<10\%$), the decrease of mycorrhizal frequency might just have been started recently. In addition, it is likely that high mycorrhizal frequencies are caused by few insensitive species (sect. 7.1). Bearing in mind the results of two experiments given in chapters 2 and 3 where negative effects of SO_2 and nitrogen compounds on the mycorrhizal frequency were reported, a continuation of this decrease can be expected.

In addition, the proportion of well-developed mycorrhizas may have decreased this century. Although data on this mycorrhiza parameter are not known from localities abroad, it was found to be rather low in the stands under study and lower in old stands than in young stands (sect. 6.3). As indicated in section 7.4, this subject requires more research.

With respect to the level of air pollution in the Netherlands, the decrease of mycorrhizas and of mycorrhizal mycoflora under the present conditions is likely to continue. The observations of Fellner (1988) in the Giant Mountains of Czechoslovakia show that the complete disappearance of the mycorrhizal mycoflora is possible.

The decrease of species diversity and in a later stage, the possible decrease of mycorrhizal frequency, will probably negatively affect tree vitality with respect to nutrient and water uptake, and sensitivity to root pathogens. Assuming that each fungal species has its own (unique) role in an ecosystem, an unnatural decrease in species diversity is likely to hamper the functioning of the present ecosystem.

On the other hand, the present status of mycorrhizal mycoflora of young plots appears to be relatively good compared to mycoflora of the old plots or to mycological data from abroad. The relative insensitivity of young stands to air pollution decreases with age, as explained in chapter 6 and section 7.1. A collapse of the mycoflora seems to occur in ca. 15 to 25-year-old stands of *P. sylvestris* (unpublished observations) (Ch. 6).

In general, decline will be delayed if N uptake decreases, or if photosynthesis and transport of photosynthates increase. In the absence of nitrogen pollution, the decline of mycorrhizas and their carpophores during stand development is not expected. If

nitrogen pollution is decreased but still present, mycorrhizal mycoflora in stands of *P. sylvestris* will probably show a less and more gradual decline, occurring in a later stage of stand development.

The rate of change of mycorrhizal mycoflora in old stands after a decrease of nitrogen pollution is more difficult to predict. A richer development of the mycoflora can be expected to start when (1) the plant has recovered from nitrogen pollution stress, (2) the nitrogen uptake by the plant has decreased and (3) mycorrhizal fungi have recovered from their carbohydrate deficiency. The change is likely to be much slower in old stands than in recently replanted stands. Nitrogen losses due to leaching will be much lower in old stands than in young stands where considerable nitrification occurs after cutting the stand. In addition, restoration of mycorrhizal mycoflora depends on the occurrence and effects of other air pollutants as well.

The most needed action for the protection and restoration of mycorrhizal mycoflora is, of cause, to decrease air pollution, especially nitrogen. Of the two compounds causing nitrogen pollution, ammonia is likely to have the strongest impact. In the Netherlands, sources of ammonia (bio-industries) are often close to forests on sandy soils and the dry deposition rate of ammonia is relatively high compared to NO_x compounds (Asman & Maas, 1987).

Without a change in air pollution policy, other measures in favour of mycorrhizal fungi will not be very useful. However, there may be some possibilities, which are discussed below: (1) removal of the litter and humus layer, (2) accelerated clear-cut of forests, (3) ploughing the soil preceeding replanting, and (4) fertilization.

Ad (1). Removal of the humus and litter layer has a drastic positive effect on mycorrhizal mycoflora (Ch. 6), but the solution is an academic one. Costs are extremely high, and an enormous waste problem arises. However, if it is carried out on a small scale, it may be useful for conservation of forest ecosystems with their characteristic vegetations. In the Netherlands this measure is already being practiced for conservation of heathlands.

Ad (2). Clear-cutting and replanting have a positive effect on mycorrhizal mycoflora (Ch. 6). However, this should be considered only if it is justified from a silvicultural point of view. If the aim is to clear-cut and replant a stand only to restore the mycoflora, this should not be done before the nitrogen pollution has been decreased, because of its temporary positive effect. Furthermore, fungal species which only occur in old forests will not be made to appear by this measure.

Ad (3). Ploughing the soil preceeding replanting increases nitrification and consequently leaching of nitrate. Deep-ploughing will have a greater positive effect on mycoflora than superficial row-ploughing. Deep-ploughing on the other hand, is more expensive and it should only be seriously considered after the nitrogen pollution has decreased.

Ad (4). From a plant nutritionist's view, the effects of nitrogen pollution on forests is a deficiency problem of the plant with respect to for example K, Mg and P. Fertilization with these elements would diminish this imbalance of nutrition. The relative surplus of nitrogen uptake compared to photosynthesis would disappear due to increased photosynthesis, and therefore it can be expected that mycorrhizas are affected positively. The latter may be true, but we have to bear in mind that many forest fertilization experiments show negative effects on mycorrhizas (reviewed by Kuyper, 1989). The Dutch situation however, is not comparable with most of these experiments, because of the surplus of available nitrogen in forest soil, making the effects of additional fertilization on mycorrhizas unpredictable.

Liming forests is practiced in some areas (*e.g.* Germany and Sweden) and is being considered in the Netherlands in order to decrease the soil acidity. Liming in the Netherlands would result in an enormous nitrification and this is likely to be detrimental to the trees and the mycorrhizas. However, in combination with clear-cutting and ploughing, liming before replanting might be considered as a measure to increase nitrification and decrease acidity.

Increased tree growth due to fertilization is likely to cause problems with the water supply, especially if mycorrhizas react negatively to fertilization. Besides, increased sensitivity to obligate pathogens and to frost is likely to occur. Before applying forest fertilization in practice, more research is needed in order to evaluate the effects on mycorrhizas. If the effects appear to be negative, forest fertilization should therefore be cancelled. Forest fertilization should also be avoided if nitrogen pollution will decrease in the near-future, because (1) the favorable development of mycorrhizal mycoflora after replanting indicates that the negative effects of nitrogen pollution are diminished in young stands, removing the reason for fertilization and (2) the long-term effects of fertilization on mycorrhizas, and also on many other components of the forest ecosystem, are unknown.

In conclusion, there are hardly any measures which can be taken to improve mycorrhizal fungi if the amount of air pollution is not reduced. On the other hand, it seems likely that a decrease in nitrogen pollution will have positive effects on the mycoflora after replanting a site. Possibly ploughing before replanting increases this positive effect.

7.4 Suggestions for further research

In this study attention was mainly focused on three variables of the forest ecosystem: tree, mycorrhizal fungus and air pollution. Naturally, an ecosystem is composed of many more factors. Nitrogen deposition has many more effects on the soil ecosystem, on the

vegetation, the microflora and -fauna and abiotic factors. All these changes in turn affect other abiotic factors and organisms, and certainly also the mycorrhizal fungi. Special attention should be paid to the nitrogen cycle in forest ecosystems under increased nitrogen pollution.

However, the causes of decline of the mycorrhizal mycoflora have become more clear through the results of this study and measures can be taken to stop the decline. Based on the results and experiences in this study, the following subjects are recommended for further research: (1) soil chemistry, (2) intra- and interspecific variation in ectomycorrhizal fungi, (3) experimental evidence for the relation between air pollution and mycoflora- and mycorrhiza parameters in mature stands, (4) the functioning of "poorly-developed" mycorrhizas (chapters 1-3,6), (5) the ecology of *Cenococcum geophilum* and (6) the generalization of the conclusions of this study to other tree species and to other countries.

Ad (1). In a previous publication (Termorshuizen & Schaffers, 1987), results of chemical analysis of the upper 0-5 cm in 21 stands were presented. There appeared to be no significant differences between the plots, which were situated in different parts of the Netherlands. One reason may be that too few samples have been taken. Alternatively, nitrogen was likely taken up by the vegetation. Part of the nitrogen was possibly volatilized during drying of the soil samples. This was not further investigated because the subject seemed to be very complicated and time consuming.

Ad (2). The experiments (Ch. 2-3) showed differences in effects of treatments between the various species. However, one cannot draw general conclusions on the results of separate species, because only one isolate was used for each species. Therefore, experiments should be repeated with many more isolates of one species and with many more species. Intraspecific variability in nitrogen nutrition of numerous species *in vitro* has been shown by Lundeberg (1970) but the characteristics might be quite different when functioning as a symbiont. It is a well-known fundamental problem to work with different isolates because the amount of work soon increases to an unacceptably high level. Studies of this kind however, are essential to make further progress in ecological mycorrhiza research.

Special attention should also be given to the possible causes of differences between declined and increased fungal species. It would be very interesting to test the hypothesis that species either differ in saprotrophic capabilities, in carbohydrate demands or in sink capacities for carbohydrates.

Ad (3). The difficulties in performing experiments under controlled conditions with mature trees is also a fundamental problem in ectomycorrhiza research. The best solution seems to perform experiments in the field with a given mycorrhizal population, instead of an (theoretically preferable) inoculated population of one or more known

fungal species. The disadvantage to this approach is however, that one must accept the starting-point, which makes this kind of experiments difficult to reproduce.

As a result of the field observations in the present study, special attention should be paid to the possible influence of the litter and humus layer on the mycorrhizas in the presence or absence of nitrogen air pollution.

Ad (4). As described in section 6.2, it appeared to be difficult to make a clear distinction between mycorrhizal and non-mycorrhizal roots. Very often short roots were found without a mantle, without root hairs and with a Hartig net, present in only a few cells. Intracellular growth did not occur. These roots are not entirely non-mycorrhizal, neither are they typically mycorrhizal. It might be supposed that these roots are young, newly developing mycorrhizas. However, the mycorrhizas looked old, with usually a shrunken surface and very little or no extramatrical mycelium. Young mycorrhizas always had a mantle and a well-developed Hartig net.

The poor-looking mycorrhizas with a poorly developed Hartig net seem to be the same atypical mycorrhizas as described concisely by Harvey *et al.* (1976) and Blasius *et al.* (1985), who omitted these roots from the countings. However, this might essentially influence mycorrhizal frequency, which is usually based on the total number of root tips. Therefore, two classes of mycorrhizas were distinguished in this study, one with a typical morphology (the 'well-developed' mycorrhizas) and the other with the limited development (the 'poorly-developed' mycorrhizas).

Poorly-developed mycorrhizas were not only observed in the field, but in both pot experiments as well (Ch. 1,2). Because infection by other mycorrhizal fungi only occurred sporadically in these experiments, it seems that one fungal species can cause both well- and poorly-developed mycorrhizas.

Ad (5). During soil analysis, mycorrhizas (with mycorrhizal fungi other than *C. geophilum*) covered with patches of *C. geophilum* were often observed, which suggests that *C. geophilum* overgrew existing mycorrhizas. There are two explanations for this apparent aggression of *C. geophilum*: (1) *C. geophilum* may decompose older mycorrhizas by possessing weak saprotrophic and/or pathogenic capabilities and (2) *C. geophilum* may take over the places of other, weakened mycorrhizal fungi. An interesting question is whether the *C. geophilum* which overgrows existing mycorrhizas and causes the "patchy" appearance is the same species as the *C. geophilum* which causes the typical, uniformly black mycorrhizas.

Because of the high frequency of *C. geophilum* in all stands investigated in the present study, and because the variation in the occurrence of *C. geophilum* between plots could not be explained (Ch. 6), more research is urgently needed to elucidate the functioning of this fungal species in the forest ecosystem.

Ad (6). Tree species may have different mycorrhizal fungi, differ in sensitivity to air pollutants and nutrition physiology and in many other aspects. However, the

essential parts of the theory (section 7.1) are likely to occur in other tree species as well. Air pollutants always reduce photosynthesis to a certain extent, and the supply of carbohydrates to ectomycorrhizal fungi always depends on photosynthesis. However, levels of tolerance to air pollutants may differ. In the present study only vital stands of *P. sylvestris* were studied, which all had high mycorrhizal frequencies (Ch. 6). Therefore, the mycorrhizal status of less vital stands should be investigated in near future. The results of such a study are likely to provide insight into future development of mycorrhizal fungi if the decrease of the forest vitality in the Netherlands continues.

In this study, it was concluded that nitrogen pollution is the main cause of the decline of mycorrhizal mycoflora in the Netherlands. Because the nitrogen pollution in many parts of Europe is much lower (Buijsman *et al.*, 1987), other factors are likely to be important as well. These factors are probably closely related to those which explain the decrease of tree vitality in Europe (*cf.* Schütt & Cowling, 1985). In other countries, more emphasis should be placed on the effects of SO_2, O_3, acid mist and soil acidification. However, the decline of mycorrhizal mycoflora can probably still be explained in terms of a decreased supply of carbohydrates to the mycorrhizal fungus.

GENERAL SUMMARY

The carpophores of mycorrhizal fungi have declined drastically during this century in the Netherlands and in other European countries. In contrast, saprophytic and pathogenic fungi did not show a significant change. In this thesis, the possible causes of the decline of mycorrhizal mycoflora have been examined. The hypothesis was put forward that the functioning of mycorrhiza was hampered, either through a decrease of tree vitality or by changes in soil chemistry, both resulting from air pollution. *Pinus sylvestris* was chosen as study object, because in the Netherlands (1) its vitality has decreased considerably, (2) mycorrhizal mycoflora of coniferous tree species decreased more strongly than that of deciduous species, (3) it is the only native conifer which possesses ectomycorrhizas and (4) plantations of *P. sylvestris* of the same age and on the same soil type can be found throughout the country.

In a pot experiment, mycorrhizas of *Paxillus involutus* appeared to be sensitive to SO_2 fumigation alone, or in combination with NH_3 pollution, in contrast to mycorrhizas of *Laccaria proxima*. Photosynthesis, measured on *P. involutus*-inoculated seedlings, was inhibited by SO_2 fumigation. However, effects on plant growth were negligible (Chapter 2).

Nitrate and ammonium salts in a pot experiment had a significant negative effect on the mycorrhizas (*Paxillus involutus* and *Suillus bovinus*), and a significant positive effect on plant growth. Ammonium treatments affected the seedlings more positively and the mycorrhizas more negatively than nitrate. The N content of seedling needles fertilized with ammonium was higher than those treated with nitrate. It was suggested that a high N uptake by the plant decreased the carbohydrate availability for the mycorrhizal fungi (Chapter 3).

Ammonium and nitrate fertilization at rates of 0, 30 and 60 kg N ha^{-1} yr^{-1} in two young stands of *P. sylvestris* during three years had a similar, significantly negative effect on the number and total dry weight of carpophores and on the number of fruiting species. However, the number of carpophores of *Laccaria proxima* increased due to the fertilization treatments in one stand. Mycorrhizal frequency and number of mycorrhizas were not affected (Chapter 4).

Field observations revealed that the mycorrhizal mycoflora of young stands of *P. sylvestris* included species which have become rare during this century in the Netherlands (Chapter 5). Especially in first rotation young stands on drift sands many of these species were found, several of them in large numbers. This seems to be related to the fact that first rotation stands have become rare in the Netherlands. There was a considerable difference in the mycorrhizal mycoflora of first rotation young stands compared to that of second rotation young stands and of old stands. However, a

literature survey showed that the fungal species which appeared to occur specifically in the first rotation young stands were also common in humus-rich and mature stands in Poland, Finland and Russia. Possible explanations for this difference are discussed.

In 50 to 80-year-old stands of *P. sylvestris*, the number of mycorrhizal fruiting species as well as the number and total dry weight of their carpophores had highly negative correlations with the NH_3 deposition and ambient NO_x concentration, and to a lesser extent with ambient SO_2 concentration (Chapter 6). The mycorrhizal mycoflora showed insignificant positive correlations with tree vitality, expressed as the needle occupation of the trees. The mycorrhizal mycoflora was very poor in most old plots.

Young stands had a much richer mycoflora than old stands. Over the three years of field observations, more species (factor 3) and more carpophores (factor 13) were found in the young plots.

The mycorrhizal mycoflora in 5 to 10-year-old stands was negatively influenced by infection of trees by *Lophodermium seditiosum*. High positive correlations were found with ambient NO_x concentrations and could be partially ascribed to infection by *L. seditiosum*, which occurred in the less polluted areas.

The mycorrhizal frequency exceeded 95% in all but one of the old plots and in all young plots, indicating that the decrease of carpophores preceedes that of mycorrhizas.

The following conclusions were drawn:

(1) No decline of carpophores of mycorrhizal fungi could be detected in young stands of *P. sylvestris*, this in contrast to the situation in old stands.

(2) The carpophores of mycorrhizal fungi in young stands are negatively affected by (artificial) nitrogen fertilization. The mycorrhizas of seedlings can be negatively affected by nitrogen fertilization and by SO_2 fumigation.

(3) The nitrogen effect on the mycorrhizas is likely to be a result of decreased supply of carbohydrates by the plant, caused by the increased uptake of nitrogen compounds.

(4) The effects of nitrogen deposition and SO_2 pollution on *P. sylvestris* and mycorrhiza depend on the fungal species involved.

(5) The age of forest soils determines to a great extent the mycoflora. The ageing of Dutch forests contributes to the decrease of mycorrhizal mycoflora. It is not clear to what extent air pollution influences the succession of mycorrhizal fungi.

(6) Nitrogen pollution is the major factor explaining the decrease of mycorrhizal mycoflora. The absence of effects of nitrogen pollution in young plots is explained by the high nitrogen losses due to clear-cutting and soil ploughing, decreased interception of air pollutants by smaller trees and the higher need for external nitrogen of young trees.

(7) Carpophores of mycorrhizal fungi are more sensitive to nitrogen pollution than mycorrhizas.

(8) It is proposed that the decline in mycorrhizal mycoflora during stand development might not occur if air pollution, particularly nitrogen pollution, is drastically diminished, and if the excess nitrogen in the ecosystem is removed.

ACHTERUITGANG VAN PADDESTOELEN VAN MYCORRHIZAVORMENDE SCHIMMELS IN OPSTANDEN VAN GROVE DEN

Samenvatting

Uit historisch onderzoek is gebleken dat in deze eeuw een grote verarming opgetreden is in de soortenrijkdom van paddestoelen in Nederland en andere Europese landen. Deze verarming heeft zich bijna uitsluitend voorgedaan bij de groep van schimmels die samenleeft met houtige gewassen, de zogenaamde mycorrhizavormende schimmels. In deze samenlevingsvorm gaan schimmel en plant een relatie aan waarvan beide profiteren door uitwisseling van voedingsstoffen.

In dit proefschrift wordt de verarming in de soortenrijkdom van paddestoelen van mycorrhizavormende schimmels nader onderzocht aan de mycorrhiza's van grove den en wordt getracht de oorzaak ervoor te vinden.

Aangezien de verarming niet is opgetreden bij andere paddestoelen dan die van mycorrhizavormende schimmels lijkt een verband met de algemene daling in vitaliteit van de Europese bossen voor de hand te liggen.

Een indicatie voor de vitaliteit van de bossen wordt in het algemeen verkregen door bepaling van de naaldbezetting van de bomen. Onder normale omstandigheden blijven de naalden van grove den ongeveer drie jaar aan een boom zitten voordat ze eraf vallen. Bomen met een lagere vitaliteit laten hun naalden eerder vallen en kunnen daardoor minder goed in de suikerbehoefte van de mycorrhizavormende schimmel voorzien. Hierdoor kan de groei van de schimmel en daarmee de vorming van paddestoelen geremd worden, aangezien mycorrhizavormende schimmels weinig of geen andere bronnen voor bevrediging van hun suikerbehoefte hebben.

De daling van de vitaliteit van de Europese bossen wordt in het algemeen toegeschreven aan luchtverontreiniging. Luchtverontreiniging kan niet alleen beschadiging van de bovengrondse delen van de plant veroorzaken, het kan ook allerlei bodemchemische veranderingen tot gevolg hebben, waarvan verzuring een bekend voorbeeld is. Naast het feit dat luchtverontreiniging via de boom een effect kan hebben op de schimmel, kan dus ook luchtverontreiniging de schimmel door veranderingen in het bodemmilieu aantasten.

Het onderzoek is toegespitst op de effecten die luchtverontreiniging kan hebben op de mycorrhiza's, mycorrhizavormende schimmels en hun paddestoelen. Het onderzoek is beperkt tot de mycorrhiza's van grove den. Deze boomsoort heeft recentelijk in Nederland een achteruitgang in vitaliteit vertoond. Verder was gebleken dat de verarming in de rijkdom aan paddestoelen duidelijker was voor de paddestoelen die bij coniferen groeien dan voor paddestoelen die aan loofbomen of aan beide gebonden zijn.

Bovendien is grove den vanuit bosbouwkundig oogpunt voor Nederland interessant, omdat het de meest algemeen aangeplante boomsoort is.

In het laboratorium werden twee proeven verricht waarin de effecten van begassing met zwaveldioxide (al dan niet in combinatie met begassing met ammoniak) en van stikstofbemesting onderzocht werden op kiemplanten van grove den. Deze kiemplanten werden voorafgaand aan de behandelingen geïnfecteerd met bepaalde soorten mycorrhizavormende schimmels. Zwaveldioxide bleek een negatief effect te hebben op de infectie van de kiemplantjes door de mycorrhizavormende schimmel Krulzoom (*Paxillus involutus*), maar de Fopzwam (*Laccaria proxima*) bleek niet beïnvloed te worden. Additionele begassing met ammoniak bij twee niveaus van begassing met zwaveldioxide bleek alleen bij Krulzoom een negatief effect te hebben vergeleken met hetzelfde niveau zwaveldioxide zonder ammoniak.

Het effect van stikstofbemesting op mycorrhiza's werd onderzocht omdat de luchtverontreiniging met ammoniak (van de bio-industrie) en stikstofoxiden (van verkeer en industrie) een zo grote vlucht heeft genomen dat momenteel grote hoeveelheden stikstof in de bossen voorkomen, hetgeen een onnatuurlijke situatie is. Bemesting met ammonium- en nitraat-stikstof in een potproef bleek een positief effect te hebben op de plantegroei tot een niveau, waarboven de groei niet meer veranderde, maar er bleek een sterk negatief effect op te treden op de mycorrhiza's. Dit negatieve effect is ook beschreven in de literatuur en wordt verklaard door de toegenomen productie van aminozuren door de plant, waarvoor zowel stikstof als suikers nodig zijn. Hierdoor komen minder suikers beschikbaar voor de mycorrhizavormende schimmels, die hierdoor dus geremd worden in de groei.

In een veldexperiment in een jonge opstand van grove den waarin bemest werd met ammonium- en nitraatzouten bleek de bemesting een duidelijk negatief effect te hebben op de paddestoelen van mycorrhizavormende schimmels. Er bleek echter nauwelijks een effect te zijn op de mycorrhiza's zelf. Geconcludeerd werd dat de paddestoelen waarschijnlijk gevoeliger reageren op stikstofbemesting dan mycorrhiza's.

Door geheel Nederland werden opstanden van grove den geselecteerd met het doel de aantallen en soorten paddestoelen van mycorrhizavormde schimmels van deze opstanden met elkaar te vergelijken en te onderzoeken welke milieufactoren gecorreleerd zouden zijn met waargenomen verschillen in aantallen en soorten paddestoelen tussen de terreinen. De terreinen werden uitgezet op zandgronden in Noord-Holland, Drenthe, Overijssel, Gelderland, Noord-Brabant en Limburg. Aangezien uit de literatuur bleek dat jonge bossen andere soorten paddestoelen hebben dan oude bossen werden terreinen uitgezet zowel in de leeftijdsklasse van 5-10 jaar als in die van 50-80 jaar. In 1985 zijn 8 jonge en 13 oude terreinen uitgezet. Dit aantal is in 1986 uitgebreid met 6 oude terreinen.

In de terreinen (oppervlakte 1050 m^2) werden in de jaren 1985, 1986 en 1987 de

paddestoelen van mycorrhizavormende schimmels geïnventariseerd. Verder werd ieder jaar de boomvitaliteit bepaald. Tevens werd de vegetatie beschreven. Gegevens over de luchtverontreiniging werden verkregen uit de literatuur.

In de oude terreinen bleek een duidelijk verband te bestaan tussen de hoeveelheid luchtverontreiniging door stikstof en het aantal paddestoelen van mycorrhizavormende schimmels. In de terreinen met de hoogste luchtverontreiniging door stikstof bleken de geringste aantallen paddestoelen voor te komen. Ook de correlatie tussen aantal paddestoelen en zwaveldioxide luchtverontreiniging was negatief, maar zwakker dan de correlaties met stikstof luchtverontreiniging. De soortenrijkdom aan paddestoelen was zwakker, maar op dezelfde manier gecorreleerd met de luchtverontreiniging als het aantal paddestoelen.

De infectiegraad van de mycorrhiza's (dit is het aantal mycorrhiza-worteltopjes ten opzichte van het totaal aantal worteltopjes) gaf dezelfde correlaties met luchtverontreiniging te zien als het aantal paddestoelen, en was in alle terreinen op één na hoger dan 95%. Blijkbaar reageren de paddestoelen van mycorrhizavormende schimmels veel gevoeliger dan de mycorrhiza's zelf. Deze conclusie werd ook reeds getrokken uit de resultaten van de stikstof veldbemestingsproef (zie boven). Dit verschijnsel is goed verklaarbaar: de belangrijkste eis voor een mycorrhizavormende schimmel is een goed functionerende symbiose; zonder symbiose valt, onder natuurlijke omstandigheden, niet te leven voor de mycorrhizavormende schimmel.

Bovengenoemde correlaties van de oude terreinen werden niet aangetroffen in de jonge terreinen. Hier bleek het voorkomen van een schimmelziekte die naaldval veroorzaakt (naaldschot, veroorzaakt door *Lophodermium seditiosum*) negatief gecorreleerd te zijn met de rijkdom aan paddestoelen en soorten paddestoelen. Deze pathogene schimmel kwam voornamelijk in hevige mate voor in gebieden met relatief weinig luchtverontreniging. In de literatuur werden aanwijzingen gevonden dat dit verband tussen het voorkomen van *Lophodermium seditiosum* en luchtverontreiniging causaal kan zijn.

Ondanks het plaatselijk voorkomen van *Lophodermium seditiosum* waren alle jonge terreinen gemiddeld rijker aan paddestoelen en veel rijker aan soorten paddestoelen dan de oude terreinen. Bovendien bleken verscheidene soorten die gedurende deze eeuw in Nederland een significante achteruitgang vertoond hebben, in de jonge terreinen algemeen voor te komen.

Geconcludeerd werd dat de verarming in de soortenrijkdom aan paddestoelen niet of nauwelijks opgetreden is in de jonge terreinen, terwijl deze in de oude terreinen juist zeer duidelijk is.

Vanaf 1986 werden de jonge terreinen aan een verder onderzoek onderworpen. Naast de reeds uitgezette tweede generatie terreinen (dit zijn terreinen waar vóór de huidige opstand ook bos is geweest) werden acht jonge terreinen geselecteerd op

plaatsen zonder recente bosgeschiedenis: vier op stuifzand, twee op voormalige landbouwgrond en twee op ontwaterd hoogveen.

Het bleek dat de soortensamenstelling van de paddestoelen van de eerste generatie terreinen sterk verschilde met die van de tweede generatie terreinen. Opvallend was de aanwezigheid van relatief veel soorten die een significante achteruitgang vertoond hebben gedurende deze eeuw in Nederland.

Gesuggereerd werd dat de aanwezigheid van relatief veel achteruitgegane soorten in de jonge terreinen samenhangt met de algemeenheid van dit type terreinen in Nederland. Gezien de ongelijke leeftijdsopbouw van het Nederlandse bos zijn jonge terreinen van grove den zeldzamer dan oude, en eerste generatie jong bos is zelfs erg zeldzaam.

Het bleek echter dat soorten paddestoelen die in dit onderzoek alleen in jonge terreinen gevonden werden, in het buitenland (Finland, Polen en Rusland) ook in oude bossen gevonden worden. Op basis hiervan, en op basis van het gevonden verband tussen de mate van luchtverontreiniging en het voorkomen van paddestoelen in de oude terreinen, werd geconcludeerd dat in oude terreinen een factor aanwezig is die de fructificatie van mycorrhizavormende schimmels remt, en die afwezig of minder prominent aanwezig is in de jonge terreinen.

Op basis van deze conclusie en gegevens uit de literatuur werd een theorie opgesteld die bovenvermelde waarnemingen uit experimenten en veldwaarnemingen verklaart.

De hoofdcomponent die de verarming in de paddestoelenflora van mycorrhizavormende schimmels in Nederland verklaart, is volgens die theorie luchtverontreiniging met ammoniak en stikstofoxiden en, in geringere mate, zwaveldioxide. Deze veroorzaakt een tekort aan suikers voor de mycorrhizavormende schimmels. In jonge terreinen is de stikstofopname door de planten echter geringer doordat (1) de stikstofbehoefte van jonge bomen groter is dan die van oude bomen, (2) de planten minder stikstofverbindingen onderscheppen omdat ze lager zijn dan oude bomen en omdat ze meestal relatief beschut tussen oudere opstanden gesitueerd zijn en (3) er grote stikstofverliezen zijn opgetreden na het vellen van het oude bos tot en met het inplanten van jong bos.

Met het ouder worden van jong bos is de verwachting dat bij gelijkblijvende luchtverontreiniging door stikstof de paddestoelenflora achteruit zal gaan. Deze achteruitgang zal trager verlopen bij geringere luchtverontreiniging door stikstof. Een verlaging van de luchtverontreiniging door zwaveldioxide zal eveneens een positieve invloed hebben op de paddestoelenflora. Verwacht wordt echter dat de invloed van stikstof groter is dan de invloed van zwaveldioxide, aangezien ammoniakemissies in Nederland veelal dicht in de buurt van bossen gelocaliseerd zijn. Gezien de relatief hoge depositiesnelheid van ammoniak is de invloed vooral dicht bij de emissiebron groot. Het heeft derhalve meer zin de ammoniakemissies te bestrijden dan de stikstofoxideëmissies.

Om een verdere achteruitgang van de paddestoelenflora en een daling in de infectiegraad van mycorrhiza's tegen te gaan dient de stikstof luchtverontreiniging, met name de ammoniak luchtverontreiniging, drastisch beperkt te worden.

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