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Relations between mycorrhizas and fruitbodies of mycorrhizal fungi in Douglas fir plantations in The Netherlands

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SUMMARY

Relations between above-ground fruitbodies of mycorrhizal fungi and mycorrhizas in the soil were studied in 23 Douglas fir stands of various ages in The Netherlands. In the autumn of 1986, fruitbody and mycorrhiza density and diversity were determined.

Quantitative relations were found between above-ground fruitbodies of mycorrhizal fungi and below-ground mycorrhizas. Mycorrhiza density explains fruitbody density better than mycorrhiza frequency, but fruitbody density is an unreliable estimator of mycorrhiza density. Density of root tips does not influence the density of fruitbodies. The above-ground diversity, in number of species of fruitbodies, is reflected by the below-ground diversity, in number of mycorrhiza types, but the below-ground diversity is not explained simply by the number of species fruiting. The strong decline in density and diversity of fruitbodies and mycorrhizas with age of the stands cannot be explained yet.

Key-words: ectomycorrhiza; fruitbody diversity; fungi; mycorrhiza frequency; stand age; succession.

INTRODUCTION

About 25 years ago, J. J. Barkman started studies on the ecology and sociology of macrofungi, as a part of ecosystem studies, in the province of Drenthe (The Netherlands). He and his co-workers carried out research in several vegetation types: Juniper shrub, grass and heathlands, deciduous and coniferous woods (Barkman 1987). From these and other studies in The Netherlands a considerable change in number of species and fruitbodies, and in distribution patterns, has been reported. Mycorrhizal fungi showed a strong decrease, especially those associated with conifer trees on dry, acid, nutrient-poor and humus-poor sandy soils. Patterns of decline or disappearance have been correlated with 'air pollution' (acidification and eutrophication) (Arnolds 1985).

One of the questions that arises is whether the decrease of fruitbody production correlates with a decrease of mycorrhizal infection. The decrease of mycorrhizal infection in turn could be related to the generally observed decline in tree vitality.

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There are few studies on the ecology of macrofungi (Winterhoff 1984) because of methodological problems. Specialized knowledge is necessary because of the high number of mycorrhizal species (more than 650 in The Netherlands, Arnolds 1984). Observation over several years is necessary because of the irregular appearance of fruitbodies depending strongly on weather conditions (Agerer 1985). Myco-ecologists usually study only the above-ground parts of fungi as it is not possible to observe mycelia and mycorrhizas directly. Moreover, it is almost impossible to identify the mycelia and mycorrhizas to species or genus level.

Ecological studies on mycorrhizas are mainly dealing with biomass estimates (Vogt *et al.* 1983) and contribution of mycorrhiza in the nutrient cycle (Fogel 1980; Fogel & Hunt 1979, 1983). In a few studies observations on fruitbodies have only been included. Fogel & Hunt (1979) have determined biomass of mycorrhizas and fruitbodies, but did not distinguish between fruitbodies of mycorrhizal and saprophytic fungi. Mason *et al.*(1984), studying succession of fruitbodies in young stands, have reported a qualitative relation between fungal species and mycorrhiza types. Both studies do not quantify the relation between below-ground mycorrhizas and above-ground fruitbodies of mycorrhizal fungi.

In this article the quantitative relations between the (above-ground) fruitbodies of mycorrhizal fungi and the (below-ground) mycorrhizas will be discussed. Fruitbodies of hypogeous fungi and saprophytic fungi are not included in the study.

METHODS

Twenty-three permanent plots of 500 or 1000 m^2 each were established in Douglas fir plantations of various ages on acid, nutrient-poor, humus-poor, sandy soils in The Netherlands (as in Jansen & de Vries 1987 but without plots 14 and 15 from that list because no data on mycorrhizas were determined). The plots were visited three or four times in September–November 1985 and 1986 to determine density and diversity of fruitbodies of mycorrhizal fungi (Jansen & de Vries 1987). Fruitbodies were removed in order to avoid counting them twice.

Determined were: number of fungal species and genera observed in 1985–1986, total number of fruitbodies counted in 1986 (FT = fruitbodies total), average number of fruitbodies per visit in autumn 1986 (FA = fruitbodies average: FT divided by the number of visits) and the highest number of fruitbodies observed at one visit (FM = fruitbodies maximum). For five plots the FM values of 1985 were much higher than those of 1986 and, therefore, were used. Other data on numbers of fruitbodies from 1985 were not used for this article. All numbers were determined per 1000 m^2, so in fact they represent densities. Number of fruitbodies of the 500-m^2 plots were multiplied by 2 to make comparisons possible.

At the end of November and beginning of December 1986 three root samples were taken from each plot. The samples, which were 5 cm deep and of a volume of 119 cm^3, were taken from the topsoil after removal of litter, thus were from fermentation (F) and humus (H) layers. Samples were taken near three different trees at two-thirds of the crown width. The samples were washed with running tap water over a 2-mm sieve, cleaned by hand of remaining tree needles, twigs, dead roots and roots thicker than 2 mm, and stored in a buffered solution of glutaraldehyde (2·5%) at 4°C until examination.

For each sample the number of root tips with and without mycorrhizal mantle were counted, sometimes after subsampling, under a dissecting microscope (magnification 6–50 ×). The data from the three samples of one plot were lumped together to give one figure for the determined characteristics per plot.

Table 1. Mycorrhiza and fruitbody characteristics in 23 Douglas-fir plots

Plot no.	Root characteristics				Fruitbody characteristics					Stand age
	No. root tips	No. mycorrhiza			No. species	No. genera	FA	FT	FM	
		Tips	Frequency	Types						
1	237	35	14·8	3	3	3	4	14	47	48
2	1149	850	74·0	5	1	1	1	1	355	23
3	618	512	82·8	4	13	7	146	586	710	18
4	1114	988	88·7	3	6	5	288	1150	1012	12
5	432	262	60·6	3	5	3	12	47	69	22
6	498	45	9·0	3	2	1	1	3	1	41
7	275	8	2·9	2	3	3	21	82	41	36
8	1222	390	31·9	7	7	5	1138	4550	3774	10
9	103	16	15·5	3	2	2	33	130	78	44
10	87	63	72·4	6	6	4	22	68	360	17
11	604	0	0·0	0	5	4	3	13	8	53
12	464	261	56·3	8	18	8	392	1566	1116	12
13	338	55	16·3	2	2	2	1	4	3	47
14	448	395	88·2	5	16	7	418	1254	824	11
15	335	27	8·1	3	7	4	8	23	13	51
16	1002	18	1·8	1	2	2	23	70	41	41
17	353	159	45·0	4	4	4	116	347	261	36
18	866	586	67·7	6	9	8	262	1050	606	8
19	1132	248	21·9	1	2	2	28	112	103	20
20	443	0	0·0	0	1	1	1	1	1	45
21	2080	248	11·9	6	0	0	0	0	0	25
22	304	5	1·6	3	2	2	1	5	3	54
23	886	100	11·3	3	0	0	0	0	0	47

Per plot is given: density of root tips (mycorrhizal and non-mycorrhizal together) and of mycorrhizal root tips; mycorrhiza frequency; number of mycorrhizal types; number of species and genera of mycorrhizal fungi; density of fruitbodies of mycorrhizal fungi in average (FA), total (FT) and maximum numbers of fruitbodies (FM); and the stand age.

Mycorrhiza frequency (the fraction of number of mycorrhizas from number of root tips) was computed for the lumped samples. The mycorrhiza types were identified (or described if identification was not possible) with the key according to Jansen & de Vries (1987). Also, Mycorrhiza types were counted per plot in the lumped sample. Correlations (product moment correlations and F tests) between the below-ground and the above-ground characteristics were computed.

RESULTS

Below- and above-ground characteristics (Table 1) show wide ranges. Densities of root tips, per 357 cm^3, range from 87 to 2080, densities of mycorrhizas from 0 to 988, mycorrhiza frequency from 0 to 89%, and mycorrhiza types from 0 to 8. Number of fungal species range from 0 to 18 and number of genera from 0 to 8. Ranges in average, total and maximum number of fruitbodies, are large: 0–1138, 0–4550 and 0–3774, respectively.

Correlation of density of root tips with fruitbody density is low and not significant (Table 2). Density of mycorrhizas correlates significantly with maximum, average and total density of fruitbodies. Density of mycorrhizas correlates significantly with the

Table 2. Correlation matrix

	Root	mrhs	mfr	mtyp	FA	FT	FM	Species	Genera
mrhs	0·48*								
mfr	0·05	0·78*							
mtyp	0·21	0·41*	0·56*						
FA	0·23	0·37*	0·33	0·57*					
FT	0·25	0·37*	0·31	0·56*	0·99*				
FM	0·26	0·41*	0·34	0·57*	0·98*	0·99*			
Species	−0·19	0·31	0·62*	0·54*	0·51*	0·48*	0·43*		
Genera	−0·20	0·38*	0·64*	0·49*	0·54*	0·52*	0·48*	0·92*	
Age	−0·37*	−0·72*	−0·81*	−0·67*	−0·59*	−0·58*	−0·59*	−0·59*	−0·60*

Root: Density of root tips; mrhs: density of mycorrhizas; mfr: mycorrhiza frequency; mtyp: mycorrhiza types; FA: average density of fruitbodies; FT: total density of fruitbodies; FM: maximal density of fruitbodies.
*Significant correlation: $P < 0{\cdot}05$.

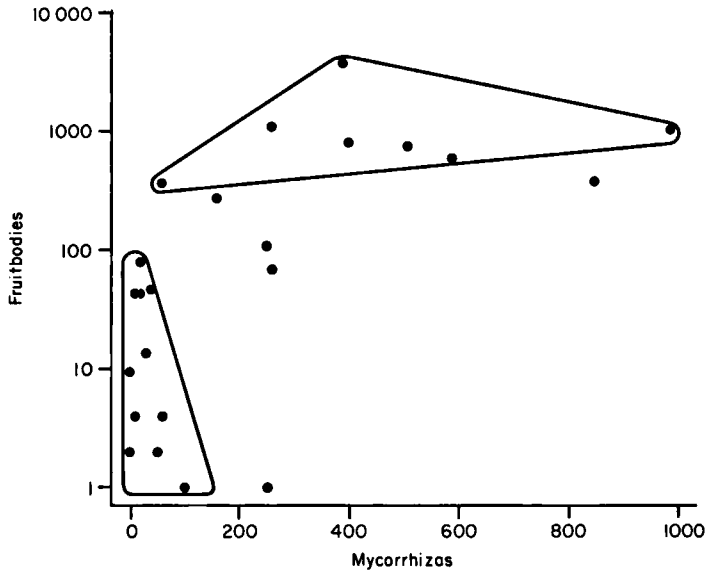

Fig. 1. Relation between density of mycorrhizas and maximum density of fruitbodies (log scale). The young stands (< 20 years) are all found in the upper outlined part, the old stands (> 40 years) in the outlined part at the bottom left, the medium aged stands (21–40 years) have an intermediate position.

number of genera but not with the number of species. Mycorrhiza frequency correlates only weakly, and not significantly, with total, average and maximum density of fruitbodies, but significantly with number of species and genera.

The correlation of number of mycorrhiza types is significant with the number of species and genera. Number of mycorrhiza types also correlates significantly with average, total and maximum density of fruitbodies.

The relation of number of mycorrhizas and maximum density of fruitbodies (Fig. 1) shows a strong influence of the age of the stands. In young stands (< 20 years), before

canopy closure, fruitbody density is high, irrespective of the density of mycorrhizas. After canopy closure the fruitbody density drops drastically. Mycorrhiza density drops only very little. In the old stands (>40 years), after thinning, the mycorrhiza density drops and the fruitbody density remains the same as in the medium aged stands.

DISCUSSION

Studying the mycorrhizas occurring in the soil under fruitbodies, it turned out to be possible to identify the mycorrhizas as belonging to a certain fungal species or genus. The same has been found by other authors (Agerer 1985; Mason *et al.* 1984). Beside a qualitative relation, we also found a quantitative correlation. The correlation with the number of species is better than with the number of genera (Table 2), but both correlation coefficients are not very high. This may have been due to several factors.

(1) We were able to identify the mycorrhizas in some cases to genus level (e.g. *Lactarius, Laccaria*), and in other cases to species level (e.g. *Russula ochroleuca, Scleroderma citrinum*).

(2) In some plots mycorrhiza types were found of fungal species that never form fruitbodies (*Cenococcum graniforme*).

(3) Mycorrhiza types were found of species of which no fruitbodies were found (e.g. *Hebeloma* sp., *Rhizopogon* sp.). Prolonged observation is needed to see if they will not fruit at all in the plots or in Douglas fir stands in The Netherlands.

As a result of these arguments, the number of mycorrhiza types cannot be simply explained by means of counting the species or genera of fruitbodies above-ground.

(4) The sampling method of three relatively small samples per plot may be insufficient for determining the mycorrhiza types present in the whole plot. This method may well be inadequate especially in plots with a low density of fine roots.

A relation between density of mycorrhizas and density of fruitbodies is expected. At the start of this study it was not clear which parameters should be used for these characteristics. Therefore mycorrhiza quantity is expressed by two and fruitbody quantity by three parameters. Density of mycorrhizas explained the three parameters for fruitbody density better than the mycorrhiza frequency. The maximum density of fruitbodies correlates slightly better with mycorrhiza density than the average or total number of fruitbodies. Apparently the density of mycorrhizas is of greater influence on the amount of fruiting of mycorrhizal fungi than the mycorrhiza frequency. So, the amount of mycelium, reflected by mycorrhiza density, explains fruitbody production better than the amount of mycorrrhization of the trees, reflected by mycorrhiza frequency.

Because we counted the fruitbodies with intervals of 3 weeks, a part of the fruitbody production might be missed, especially of species with a fruitbody lifetime shorter than 3 weeks. The most common species, *Lactarius hepaticus* (62·5% of the total observed number of fruitbodies), *Lactarius theiogalus* (10·7%), *Laccaria proxima* (5·2%), *Lactarius necator* (3·7%) and *Paxillus involutus* (3·3%), however, have a lifetime of 2–4 weeks and not many of them are thought to have been missed. Short-living fruitbodies, e.g. of *Amanita gemmata* and *Russula emetica,* contributed very little to the total number of fruitbodies (0·06 and 0·04%, respectively), so, there might be an underestimation of the total fruitbody production. However, an underestimation of more than 40–60%, as suggested by Richardson (1970), is very unlikely.

The maximum density of fruitbodies, which is thought to approximate the potential production, is obviously the best parameter to estimate the below-ground mycorrhiza

density. Prolonged observation will yield better estimates of maximum fruitbody density and will probably give better correlations. Although significant, the correlation between fruitbody density and mycorrhiza density is not very high. This makes fruitbody density an unreliable estimator of mycorrhiza density.

Succession in mycorrhizal fungi has been reported before. Mason *et al.* (1982) have found an increase in number of species and fruitbodies with age of *Betula* saplings in the second to sixth year after planting. Succession of mycorrhizal types has also been reported by Fleming *et al.* (1984). Dighton *et al.* (1986) have reported a decline of number of fruitbodies and species of mycorrhizal fungi with increasing stand development, i.e. with age, in stands of *Pinus contorta* and *Picea sitchensis*. However, their data comprise only stands of 11–27 years old. Bendiksen (1981) has reported, from Norwegian *Picea abies* woods, a decrease in fruitbody density by half, when a 20-year-old stand and an 'old' stand were compared, but the diversity in number of species remains the same. Vogt *et al.* (1983) have reported from Douglas fir stands the highest mycorrhiza frequency (92–95%) at canopy closure, that is at ages of 45–65 years. When older, the mycorrhiza frequency dropped to 61–88% in stands of 160 years old.

It is obvious that the number of mycorrhizas, mycorrhizal species and fruitbodies increases in the first years of the life of a tree or a stand, and that the highest numbers are reached at the age of canopy closure. After canopy closure, mycorrhiza frequency and fruitbody production drop slowly. In Douglas fir stands in The Netherlands, canopy closure and also highest mycorrhiza frequency is reached at a stand age of less than 20 years. The decrease after canopy closure, however, is not a slight but a strong decrease, and in stands older than 40 years very few mycorrhizas and fruitbodies were observed. This strong decline of density and diversity of fruitbodies and mycorrhizas was not expected. This phenomenon cannot be explained yet and deserves more attention. Whether there is a relation with tree vitality and 'air pollution' is being studied.

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REFERENCES

Agerer, R. (1985): *Zur Oekologie der Mykorrhizapilze*. Cramer, Vaduz.

Arnolds, E. (1984): Standaardlijst van Nederlandse macrofungi. *Coolia* **26** (Suppl.).

— (ed.) (1985): *Veranderingen in de paddestoelenflora (mycoflora)*. Wet. Meded. 167, KNNV, Hoogwoud.

Barkman, J.J. (1987): Methods and results of mycocoenological research in The Netherlands. In: Pacioni, G. (ed.). *Studies on Fungal Communities:* 7–38. L'Aquila.

Bendiksen, E. (1981): Mykorrhizasopp i forskjellige suksesjonsstadier av granskogssamfunn i Lunner, Oppland. *K. Norske Vidensk. Selsk. Mus. Rapp. Bot. Ser.* **1981–5:** 246–258.

Dighton, J., Poskitt, J.M. & Howard, D.M. (1986): Changes in occurrence of basidiomycete fruitbodies during forest stand development: with specific reference to mycorrhizal species. *Trans. Br. Mycol. Soc.* **87:** 163–171.

Fleming, L.V., Deacon, J.W., Last, F.T. & Donaldson, S.J. (1984): Influence of propagating soil on the

mycorrhizal succession on birch seedlings transplanted to a field site. *Trans. Br. Mycol. Soc.* **82:** 707–711.

Fogel, R. (1980): Mycorrhizae and nutrient cycling in natural forest ecosystems. *New Phytol.* **86:** 199–212.

— & Hunt, G. (1979): Fungal and arboreal biomass in a western Oregon Douglas-fir ecosystem: distribution patterns and turnover. *Can. J. For. Res.* **9:** 245–256.

— & Hunt, G. (1983): Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. *Can. J. For. Res.* **13:** 219–232.

Jansen, A.E. & de Vries, F.W. (1987): *Project 'Qualitative and quantitative research on the relation between ectomycorrhiza of* Pseudotsuga menziesii, *vitality of the host and acid rain' (1985–1986).* Report 25-01. Dutch Priority Programme on Acidification (RIVM, Bilthoven), Agricultural University, Wageningen.

Mason, P.A., Last, F.T., Pelham, J. & Ingleby, K. (1982): Ecology of some fungi associated with an ageing stand of Birches (*Betula pendula* and *B. pubescens*). *Forest Ecol. Management* **4:** 19–39.

—, Wilson, J. & Last, F.T. (1984): Mycorrhizal fungi of *Betula* spp.: factors affecting their occurrence. *Proc. R. Soc. Edinburgh* **85B:** 141–151.

Richardson, M.J. (1970): Studies on *Russula emetica* and other agarics in a Scots pine plantation. *Trans. Br. Mycol. Soc.* **55:** 217–229.

Vogt, K.A., Moore, E.E., Vogt, D.J., Redlin, M.J. & Edmonds, R.L. (1983): Conifer fine root and mycorrhizal biomass within the forest floors of Douglas-fir stands of different ages and site productivities. *Can. J. For. Res.* **13:** 429–437.

Winterhoff, W. (1984): Analyse der Pilze in Pflanzengesellschaften, insbesondere der Makromyzeten. In: Knapp, R. (ed.). *Sampling Methods and Taxon Analysis in Vegetation Science:* 227–248. Junk, The Hague.