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Ectomycorrhizal root development in wet Alder carr forests in response to desiccation and eutrophication

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Abstract Effects of desiccation and eutrophication on ectomycorrhizal (ECM) root development in wet Alder carr forests in The Netherlands were studied. In northwestern Europe, wet Alder carr forests are found mostly in peatlands and along streams, forming an important component of wetland ecosystems. The dominant tree species in wet Alder carr forests is *Alnus glutinosa* (L.) Gaertn. (Black alder), which associates with ectomycorrhizal fungi. During recent decades, wet Alder carr forests in Europe have declined because of desiccation and eutrophication, particularly in The Netherlands. In the present study, the number of root tips of *A. glutinosa* trees was highest in an undisturbed wet Alder carr forest in a peatland area. Eutrophication in the peatland area significantly inhibited ectomycorrhizal (ECM) root development of *A. glutinosa*. In the eutrophied forest, ECM root tips were observed only close to *A. glutinosa* trees growing on hummocks. The concentrations of nitrate and potassium in soil water of the eutrophied forest were significantly higher than in the undisturbed forest, while magnesium and iron concentrations and the pH were significantly lower. The number of ECM root tips of *A. glutinosa* in a desiccated forest along a stream was generally lower than in an undisturbed wet Alder carr forest on waterlogged soil in the same area. The sulphate concentration in soil water in the desiccated forest was significantly higher than in the forest on waterlogged soil. ECM root development of *A. glutinosa* may have been negatively affected by the chemical composition of the soil water.

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Introduction

Alnus species occur worldwide and over 30 species have been described (Heywood 1993). Unusually, this genus associates with ectomycorrhizal (ECM) fungi, arbuscular mycorrhizal (AM) fungi and nitrogen-fixing actinomycetes (Molina 1979, 1981; Chatarpaul et al. 1989). A few *Alnus* species are able to grow under wet conditions and *A. glutinosa*, in particular, is well adapted to inundation and waterlogged soils (Weeda et al. 1985; Huss-Danell 1997; Stortelder et al. 1998). This species is indigenous to a large part of Europe and associates abundantly with ECM fungi (Pritsch et al. 1997a, b; Baar et al. 2000; Dilly et al. 2000).

A. glutinosa is the dominant tree species of forests commonly found in peatlands and along streams, the wet Alder carr forests (Weeda et al. 1985; Stortelder et al. 1998). The soils in these forests are permanently waterlogged and the *A. glutinosa* trees generally grow on hummocks (Weeda et al. 1985; Stortelder et al. 1998). The conservation value of the wet Alder carr forests is high. Sporocarp surveys in such forests in peatlands and along streams in The Netherlands recorded at least 22 characteristic symbionts of *A. glutinosa*, including *Alnicola escharoides* (Fr.: Fr.) Romagn., *Cortinarius alnetorum* (Velen.) Mos. and *Lactarius obscuratus* (Lasch: Fr.) Fr., In recent decades, however, wet Alder carr forests in Europe have become threatened by desiccation and eutrophication (Stortelder et al. 1998). Desiccation of wet forest ecosystems is the result of the lowering of groundwater and surface water levels by a few decimetres or up to several meters. Eutrophication is caused by the ingress of water from outside sources to compensate for water shortage in desiccated Alder carr forests. Drainage of agricultural areas also can result in an increased water level in wet Alder carr forests, enhancing nutrient concentrations. Furthermore, wet Alder carr forests are eutrophied by nutrient-enriched groundwater originating from intensive agricultural practices (Stortelder et al. 1998).

Field observations in The Netherlands have revealed that sporocarp formation of ECM symbionts of *A. glutinosa* has decreased in desiccated and eutrophied areas (Arnolds et al. 1995). Stortelder et al. (1998) reported a decline in sporocarps of ECM fungi in desiccated Alder carr forests along streams. Desiccation and eutrophication of wet Alder carr forests may also reduce belowground development of ECM symbionts of *A. glutinosa*, with consequences for mycorrhizal function. However, the effects of desiccation and eutrophication on ECM fungi below ground in wet Alder carr forests have not been studied and are still unknown.

Therefore, in the present study we examined the effects of desiccation and eutrophication on the development of ECM fungi below ground in various wet Alder carr forests in The Netherlands. We hypothesised that desiccation and eutrophication reduce the formation of *A. glutinosa* ECM root tips in wet Alder carr forests along streams and in peatlands, respectively.

Materials and methods

Site description

We selected two relatively large Alder carr forests containing undisturbed wet areas (Stortelder et al. 1998). In the peatland area “Westbroekse Zodden” in the western part of the Netherlands (52° 9′N; 5° 7′E), two natural 60-year-old wet Alder carr forests (500 m^2) were selected. One forest was undisturbed and fed with groundwater, whilst the other forest was eutrophied. In the southeastern part of The Netherlands, two natural wet Alder carr forests (500 m^2) were selected in the wet forest ecosystem “Koelbroek” along a stream (51° 22′N; 6° 7′E). These groundwater-fed forests have been present since the middle-ages. One forest was undisturbed with a relatively high conservation value, while the other had suffered desiccation for several decades. In all forests, *A. glutinosa* was the only tree species, with trees growing on hummocks where the soils were waterlogged.

Root sampling

In each forest, five *A. glutinosa* trees of the same size and growing at least 5 m apart were selected randomly within an homogenous area (12×35 m^2). From August to November 2000, soil cores (each 95.52 cm^3) were taken at 0, 0.4, 0.8 and 1.2 m in a straight line from the selected trees. Thus, for the five selected trees per forest, five lines of sample points were laid out. The direction of the sample points was chosen such that each sample point was situated at least 5 m from the nearest tree. For *A. glutinosa* trees growing on hummocks, the cores at 0 and 0.4 m were taken from soil in the hummocks. The remaining sample points were located outside the hummocks. The soil cores were taken to a soil depth of 10 cm for all sample points and one soil core per sample point was collected. The cores contained only *A. glutinosa* roots. ECM root tips were defined as roots with a mantle, external mycelium and no root hairs, based on the characteristics described by Pritsch et al. (1997b). No attempt was made to identify the ECM root tips to fungal species.

After sampling, the soil in each core was sieved through a 2-mm sieve. Dead roots, defined as desiccated, shrunken and highly fragile, were removed. ECM and non-ECM root tips were separated from the root systems, counted and freeze-dried for 48 h. The remaining root systems were also freeze-dried for 48 h. After freeze-drying, the biomass of the root tips and the remaining roots was determined. The total number of ECM and non-ECM root tips was obtained from the numbers of root tips at four distances per tree recalculated to a soil volume of 100 cm^3. The remaining data, including total root biomass, were recalculated also to a soil volume of 100 cm^3.

Analysis of soil water

Five ceramic cups were placed in the upper 10 cm of the soil in each forest. Pore water was collected by connecting the ceramic cups to vacuum serum bottles. In the laboratory, the pH of the sampled soil water was determined with a standard KCl pH electrode. The concentrations of ammonium, nitrate, ortho-phosphate, sulphur, potassium, calcium, magnesium and iron in the soil water samples were measured according to Lamers et al. (1998).

Statistics

Normally distributed data were analysed by *t*-test (Sokal and Rohlf 1995). The Mann-Whitney-U test was applied to data not normally distributed (Siegel and Castellan 1988). Associations between root parameters and soil water data were determined using the Spearman rank-order correlation coefficient (Siegel and Castellan 1988).

Results

Root growth and ECM development

In the peatland area, the number of ECM root tips was significantly higher in the undisturbed wet Alder carr forest than in the eutrophied forest (Fig. 1a). In the eu-

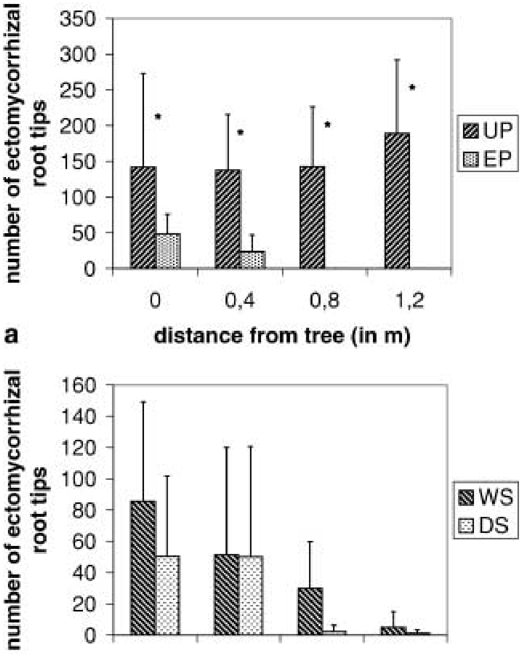

Fig. 1a, b Average numbers of ectomycorrhizal (*ECM*) root tips per 100 cm^3 soil. *DS* desiccated forest along the stream, *EP* eutrophied forest in the peatland area, *UP* undisturbed forest in the peatland area, *WS* forest on waterlogged soil along the stream, significant differences are indicated by * $P<0.05$ (Mann-Whitney U), *ns* = not significant, *SED* = standard error of difference

Table 1 Averages of total root system biomass (g dry wt. per 100 cm^3 soil), number of non-ectomycorrhizal (*N-ECM*) and ectomycorrhizal (*ECM*) root tips per 100 cm^3 soil, biomass of ECM root tips (g dry wt. per 100 cm^3 soil) and number of ECM root tips per g dry wt. root biomass (*DS* desiccated forest along the stream, *EP* eutrophied forest in the peatland area, *UP* undisturbed forest in the peatland area, *WS* forest on waterlogged soil along the stream)

Site	Forest type	Root biomass	N-ECM tips	ECM tips	Biomass of ECM tips	ECM tips per g dry wt.
Peatland	UP	1.15	0.8	610.9	0.16	1442.6
	EP	2.03	12.3	117.9	0.02	66.8
		n.s.	*	*	n.s.	*
	SED	1.40	7.7	319.0	0.16	964.8
Stream	WS	0.94	9.4	172.0	0.01	245.0
	DS	0.12	50.3	103.7	0.01	792.3
		*	*	n.s.	n.s.	n.s.
	SED	0.68	42.6	103.1	0.01	539.4

* Significant difference $P<0.05$ (t-test), *ns* not significant, *SED* standard error of difference

Table 2 Soil water nutrient concentrations (μmol l^{-1}) and pH in wet Alder carr forests. Abbreviations as in Table 1

		NH_4^+	NO_3^-	*o*-PO_4	S	K^+	Ca^{2+}	Mg^{2+}	Fe^{3+}	pH
Peatland	UP	19.2	1.5	0.9	164.5	29.5	838.8	402.2	64.0	6.5
	EP	41.1	4.0	1.4	210.4	54.5	503.0	108.6	31.5	6.1
		n.s.	*	n.s.	n.s.	**	n.s.	**	**	*
	SED	19.7	1.5	0.7	36.2	14.0	291.0	162.7	18.4	0.3
Stream	WS	352.2	2.3	5.6	980.5	107.8	3010.0	347.6	375.7	6.5
	DS	124.4	5.4	0.8	3889.8	208.3	3783.0	734.0	124.2	6.5
		n.s.	*	n.s.	***	n.s.	n.s.	**	n.s.	n.s.
	SED	197.8	2.1	4.7	1617.9	94.1	722.5	221.2	237.7	0.2

*$P<0.05$, **$P<0.01$, ***$P<0.001$, *ns* not significant, *SED* standard error of difference

Table 3 Associations between measured root parameters and soil variables. + positive association, – negative association

Site	Parameter	NH_4^+	NO_3^-	*o*-PO_4	S	K^+	Ca^{2+}	Mg^{2+}	Fe^{3+}	pH
Peatland	Total root biomass		n.s.			n.s.	–**	n.s.	n.s.	n.s.
	Total ECM tips		n.s.			–*	n.s.	+**	+**	n.s.
	ECM tips at 0.8 m		–*			n.s.	n.s.	n.s.	n.s.	+*
	ECM tips at 1.2 m		n.s.			–**	n.s.	+**	+**	+*
Stream	Total root biomass	+*		+*	–*					
	Total ECM tips	+*		n.s.	–*					

*$P<0.05$, **$P<0.01$, *ns* = not significant

trophied forest, ECM root tips were found only close to the trees (0 and 0.4 m) in the hummocks where the *A. glutinosa* trees were growing. No roots were observed at further distances (0.8 and 1.2 m) from the trees. The total number of ECM root tips and the total number of ECM root tips per gram dry weight of root biomass were significantly lower in the eutrophied forest than in the undisturbed forest (Table 1). Reduced ECM development in the eutrophied forest was related to changes in the chemical composition of the soil water (Tables 2, 3).

Along the stream, the number of ECM root tips was generally higher in the wet Alder carr forest on waterlogged soil than in the desiccated forest, especially close to the trees (0 m) and at 0.8 m (Fig. 1b). Furthermore, the total root biomass and the total number of ECM root tips were generally lower in the desiccated forest than in the forest on waterlogged soil, while the total number of non-ECM root tips was significantly higher (Table 1). Root biomass and ECM development were associated negatively with sulphate concentration in the soil water, but positively with the ammonium and ortho-phosphate concentrations (Table 3).

Composition of soil water

The magnesium and iron concentrations and the pH of the soil water in the eutrophied wet Alder carr forest of the peatland area were significantly lower than in the undisturbed forest (Table 2). Nitrate and potassium concentrations of the soil water in the eutrophied forest were significantly higher than in the undisturbed forest.

Along the stream, the sulphate concentration in the soil water of the desiccated forest was about four times higher ($P<0.001$) than that of the undisturbed forest (Table 2). Nitrate and magnesium concentrations in the soil water of the desiccated forest were significantly higher than in the undisturbed forest, while concentrations of ammonium, ortho-phosphate and iron were generally lower (Table 2).

Discussion

The decreased ECM root tip formation in the eutrophied wet Alder carr forests in the peatland area was associated with changes in the chemical composition of the soil water. The relatively low iron and calcium concentrations and the reduced pH in the eutrophied forest indicate a decline in the groundwater influence. The increased nitrogen concentration in the soil water of the eutrophied forest suggests that nutrient enrichment from agricultural activities in adjacent areas was a source of eutrophication (Roelofs et al. 1996). The influx of water from outside sources was another possible source of eutrophication of the wet Alder carr forest in the peatland area. Water influx from outside sources changes soil decomposition processes and enhances reduction processes in the anoxic peaty soils, which results in internal eutrophication (Smolders and Roelofs 1993; Bellemakers and Maessen 1998).

The reduced root biomass and number of ECM root tips in the desiccated Alder carr forest along the stream were associated with relatively high sulphate concentrations in soil water. The sulphate originates from pyrite (FeS_2) layers in the soil often formed in wet Alder carr forests on anoxic peaty soils fed with iron-rich groundwater (Stortelder et al. 1998).

The eutrophication effects on ECM development in the peatland area were more significant than the desiccation effects along the stream. A possible explanation is that the changes in soil water chemistry were inhibitory for the ECM fungi, in combination with limited oxygen availability in the waterlogged soils. A severe decrease in ECM growth below ground, as observed in the eutrophied wet Alder carr forest on waterlogged soils, could diminish linkage of the *A. glutinosa* roots with the peaty soils, which results in decreased nutrient uptake.

The number of non-ECM root tips in the disturbed forests was higher than in the undisturbed forests in the present study. A likely explanation is that desiccation and eutrophication inhibited ECM association with the *A. glutinosa* roots. However, it is possible that the soil chemical processes caused by desiccation and eutrophication favoured other fungi, including AM or parasitic fungi. Our observations in roots of *A. glutinosa* trees at the study sites did not reveal associations with AM fungi (unpublished data). Further studies are needed to determine whether infection of *A. glutinosa* trees with parasitic fungi is affected by desiccation and eutrophication.

In the present study, ECM development was observed outside the hummocks at great distance from the *A. glutinosa* trees in the anoxic waterlogged soils, particularly in the undisturbed wet Alder carr forest of the peatland area. Outside the hummocks, nutrient availability is unlimited and apparently ECM fungi are able to grow under oxygen-poor soil conditions. Baar et al. (2000) hypothesised about the mechanisms enabling ECM fungi to develop in oxygen-poor, waterlogged soils.

Mycorrhizal root tips occurred abundantly in the undisturbed wet Alder carr forests, while the number of non-mycorrhizal root tips was relatively low. This is in accordance with observations by Pritsch (1997a), who reported that over 96% of *A. glutinosa* roots were colonised by ECM fungi in a 60-year-old *A. glutinosa* forest in northern Germany. However, the numbers of ECM root tips in the wet Alder carr forests investigated in the present study were lower than in undisturbed coniferous forests in The Netherlands and Scandinavia (Ohtonen et al 1990; Baar 1997; Jonsson et al. 1999).

In conclusion, ECM root tip formation was reduced by desiccation and eutrophication in wet Alder carr forests in a peatland area and along a stream in The Netherlands. The inhibition of ECM development was more significant in the eutrophied forest than in the desiccated forest. Changes in soil water composition under oxygen-poor conditions may have caused the severe reduction of ECM development in the eutrophied forest. We expect that desiccation and eutrophication in wet Alder carr forests also affect the diversity of ECM symbionts of *A. glutinosa*. Molecular techniques could be applied to describe the ECM communities in the desiccated, eutrophied and undisturbed wet Alder carr forests.

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