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Microfungi in the soil of Scots pine forest in Poland and Germany

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The still interoding in two 17-year-old Scots pine forest soils were surveyed. One forest was located in Polsad, and the other in Germany, 900 km apart. The total number of lungal man detected was So and included 11 groupredes, 11 accompanies and 4 mitopaper (angl. From the Polsh and German soils, 145 and 122 foldates representing 44 and 32 fungs justices, 12 minutes, 12 minu

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INTRODUCTION

Forest soils are a natural ecological habitat for fungi. They play a fundamental role in the decomposition processes of wood, leaf and needle litter (Swift et al. 1979; Rayner and Boddy 1988). The specific composition of microfungal communities influences the biological activity of the forest soil (Kwašna 1995; 2001; Kwašna et al. 2000). The composition of microfungal communities and their spatial heterogeneity show ecological and geoclimatic specificity in response to environmental variables, e.g. vegetation, type and quality of soil, its physical properties, its temperature and moisture content and mutual bacterial and fungal interactions fleadurs and Badurous and Baduro

sity of forest soil fungi has been infrequent. Prominent in such studies were workers in Wisconsin (Tresner et al. 1954; Orpurt and Curtis 1957; Christensen 1960; Christensen and Novak 1962; Christensen et al. 1962; Christensen and Whittingham 1965; Gochenaur and Backus 1967; Gochenaur and Whitting ham 1967) who concluded that forest soil microfungi in the USA do not form discrete communities, but rather continua, similar to those of the higher plants. Later, Gochenaur (1978, 1984) studied the forest soil microfungi on Long Island, and Widden (1984, 1986 a, b, c) in southern Canada. In Europe, studies on the biodiversity of forest soil microfungi were carried out by Manka and Chwalinski (1960), Badura and Badurowa (1964), Gierczak (1967), Mańka and Kowalski (1968), Mańka (1974), Söderström (1975), Söderström and Bääth (1978), Widden (1987) and Kwasna (1995). None of them, however presents a comparison between communities from similar habitats.

The main objective of the present study was the evaluation of differences in diversity of microfungal communities and density of the individual taxa in soil of similar type, covered by Scots pine forests, in two countries, Poland and Germany. These two forests of similar age are located 300 km apart and have similar environmental conditions within the temperature zone.

MATERIALS AND METHODS

In April 1992, two samples of soil were collected from 17-year-old Scots pine (Pinus sylvestris L.) stands, one in central-west Poland, near Poznań, Zielonka Forest District (17º10'E, 52º50'N), and the second 300 km to the west, in central-east

Germany, near Berlin, Müggelheim Forest District (13º40'E, 52º50'N). The soils were similar regarding type and vegetation. They were created in the same glacial period and were of podzol type (cryptopodzol) with lightly clayed sand of various granulations with a small skeletal admixture in the horizons A and B. Descriptions of the soil profiles are presented in Table 1.

Table 1

Horizon	Depth (cm)	Zielonka forest	Müggelheim forest
A _{cc}	2	Leaf and litter layer	Leaf and litter layer
A,	2-6	Dark humified organic matter mixed with bleached sand grains	Humified litter and root debris mixed with sand grains
A	10-12	Grey-brown, semi-compact, silty sand	Brownish, semi-compact, silty sand
В	20-24	Orange-brown compact sand, coloration decreasing with depth, transitional to C	Orange-brown compact sand, coloration decreasing with depth, transitional to C
C	Below 30	Light yellow compact sand	Light yellow compact sand

The sand was mainly quartz and contained only small quantities of other minerals such as orthoclase and mica. The soils had little structure and were coarsely grained. Humus stains reached to a depth of 40 cm and decalcification was observed below 200 cm. The low fertility of both soils was intensified by rapid percolation of rain water leaching plant nutrients (Tab. 2). The main tree species was 17-year-old Scots pine mixed with a few single oaks (Quercus robur L.). The soil in Poland was

covered mainly with Deckampsia flexuosa (L) Trin. and single patches of Brachpool dum and Calampsoxis epgies (a). Host h. The soil in Germany was only partly covered with patches of D. flexuosa. Evidence suggested that the podzol had developed as a result of the ackwood cover and its replacement by Callinav sulgaris (L.) Hull. The accumulation of an acid litter intensified the leaching of sesquioxides from the previously forested soil, leading to the development of a podzel profile.

T a b l c 2
Textural and chemical analyses of the soil from the 17-year-old Scots pine forests

Parameter	Zielonka soil	Müggelheim soi
Sand	93.1	93.6
Silt	5.6	5.1
pH	3.9	3.9
Soil organic matter (mg kg 1)	3.4	2.8
N (mg kg 1)	3.8	3.1
Mg (mg kg 1)	0.7	0.5
K,0 (mg kg · 1)	2.0	1.4
P ₁ 0 ₄ (mg kg ⁻¹)	12.6	5.1
Water soluble fraction (mg KCl kg 1)	18.9	18.6
Chloride (mg kg 1)	trace	trace
Nitrate (mg N kg 1)	0	0
Sulphate (mg kg 1)	0	0
Weight/volume (g/L ⁻¹)	137.4	148.4

Each soil was sampled to a depth of 10-15 cm, from six different sites situated 5 to 6 m apart. The six subsamples were mixed together and than air-dried on a clean workbench until the soil could be pressed through a 0.6-mm-mesh sieve. Subsequently the soil samples were rotated for 6 h to ensure thorough mixing. Twenty particles of each soil were placed centrally on synthetic low nutrition agar (SNA) (Nirenberg 1976; Nirenberg and Metzler 1990) in individual 9-cm plastic Petri dishes. The growth of bacteria was suppressed by adding antibiotics (chlorotetracycline 10 mgl⁻¹, dihydrostreptomycin-sulphate 50 mgl⁻¹, penicillin G 100 mgl⁻¹). For the first 5 days the dishes were incubated at 17°C in darkness. Afterwards, they were placed under continuous black light (Philips 40/80W) at 20°C for 7 days and thereafter in the laboratory under natural day-night cycle at 20-25°C for the following 30 days. After 5, 12 and 42 days the plates were viewed under a microscope (magnification 100x and 250x). If necessary for identification, the fungi growing out of the soil particles were transferred onto special media, e.g. 2% potato-dextrose agar (PDA), Czapek solution agar (CzA), Czapek veast autolysate agar (CYA), 2% malt extract agar (MEA), 1% carrot decoct agar (CDA) or SNA.

The density of fungi was defined as the absolute number of isolates, and the diversity as the absolute number of species in the standardized sample consisting of 20 soil particles. Taxa with percentage > 3% in the fungal community were classified as dominant fungi.

Soil parameters. The soils contained similar amounts of sand and silt (Tab. 2). Both soils were acidic with the same pH=3.9, and had similar water-soluble fractions. The humus as well as the N, Mg, K.O, P.O, contents were higher (P.O, more

than twice) in the Polish soil. There were only traces of chloride, and no nitrate and sulphate in either soil.

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Statistical analysis used. χ^2 -test was performed to compare the numbers of isolates and species as well as the percentages of single taxa in the two communities.

RESULTS

Density and diversity of fungal species per sample. 145 and 122 fungal isolates belonging to 43 and 32 species representing 11 zygomycetes, 1 ascomycet and 43 mitosporic fungi, were recorded from the Polish and the German ITyear-old Scots pine forest soils, respectively. The list of fungal species and the percentages of the most common genera in the fungal communities are given in Tables 3 and 4,7 test values showed no significant difference between the Polish and German soils in terms of density, expressed as the total number of isolates, and diversity, expressed by the number of species (Tab. 3). The numbers of genera were also similar in the Polish and the German soils. The most common genera were Periodilium (25% and 15) and 15 miles and 15 miles periodic periodi

T a b 1 e 3
Fungi in the 17-year-old Scots pine forest soils

Species of fungi	Number of isolates out of 20 soil particles		
	Zielonka soil	Müggelheim soi	
Absidia cylindrospora Hagem	.5	1	
Absidia glauca Hagem	0	2	
Acremonium apii (M. A. Smith et Ramsey) W. Gams	1	0	
Acremonium bacillisporum (Onions et Barron) W. Gams	1	4	
Acremonium butyri (van Beyma) W. Gams	1	0	
Alternaria alternata (Fr.) Keissler	2	0	
Candida humicola (Daszewska) Diddens et Lodder	2	2	
Chaetomium globosum Kunze: Fries	1	0	
Chromelosporium fulvum (Link) Mc Ginty, Hennebert et Korf	1	0	
Clonostachys candelabrum (Bonorden) Schroers	1	0	
Clonostachys rosea (Link: Fr.) Schr., Samuels, Seif. et W. Gams	1	0	
Circinella pinicola Kwaśna et Nirenberg	9	0	
Coniothyrium fuckelii Sacc.	0	2	
Cylindrocarpon destructans (Zinssm.) Scholten	3	0	
Exophiala dermatitidis (Kano) de Hoog	0	2	
Exophiala jeanselmei (Lang.) McGinnis et Padhybe var. lecanii- comi (Benedek et Specht) de Hoog	0	1	
Exophiala moniliae de Hoog	0	1	
Exophiala pisciphila McGinnis et Ajello	1	0	
Geomyces auratus Traaen	1	4	
Geotrichum candidum Link ex Leman	5	0	
Gliocladium catenulatum Gilman et Abbott	1	0	
Heteroconium sp.	0	1	
Mortierella alpina Peyronel	2	0	
Mortierella gamsii Milko	2	0	

Mortierella humilis Linnemann ex W. Gams	6	1
Mortierella parvispora Linnemann	2	3
Mucor hiemalis Wehmer	2	1
Oidiodendron griseum Robak	15	11
Paecilomyces inflatus Matsushima	2	0
Penicillium adametzii Zaleski	3	4
Penicillium arenicola Chalabuda	1	0
Penicillium camembertii Thom	0	4
Penicillium chrzaszczii Zaleski	1	17
Penicillium daleae Zaleski	15	1
Penicillium janczewskii Zaleski	5	14
Penicillium janthinellum Biourge	1	0
Penicillium piscarium Westling	3	0
Penicillium sacculum Dale	0	1
Penicillium simplicissimum (Oudem.) Thom	4	0
Penicillium spinulosum Thom	1	10
Penicillium stoloniferum Thom	1	0
Penicillium vinaceum Gilman et Abbott	2	0
Penicillium spp.	. 0	3
Pochonia bulbillosa (W. Gams et Malla) Zare et W. Gams	4	6
Pochonia suchlasporia var. catenata (W. Gams et Dack.) Zare et W. Gams	0	1
Sporothrix schenckii Hectoen et Perkins	0	2
Tolypocladium inflatum W. Gams	4	3
Trichoderma crassum Bissett	0	2
Trichoderma koningii Oudemans	3	0
Trichoderma pseudokoningii Rifai	2	1
Trichoderma viride Pers. ex Gray	3	0
Umbelopsis isabellina (Oudemans) W. Gams	2	7
Umbelopsis vinacea (Dixon-Stewart) von Arx	20	10
Zygorhynchus moelleri Vuillemin	2	.0
Unidentified isolates	1	0
Identified isolates	144	122
Number of isolates	145	122 (1.98)
Number of species	43	32 (1.61)
Number of genera	22	15 (1.32)
Number of isolates per soil particle	7.3	6.1 (0.1)

44%) with 11 and 8 species, *Umbelopsis* (15% and 14%) with 2 species, *Oidiodendron griseum* (10% and 9%), *Mortierella* (8% and 3%) with 4 and 2 species, and *Trichoderma* (6% and 2%) with 3 and 2 species, in the Polish and German soils, respectively (Tab. 4).

Fungal communities. The total number of fungal taxa detected in both soils was 55. Only 18 taxa (32.7%) were recorded in both soils. Twenty five separate taxa (45.5%) were recorded only in the Polish, and 12 taxa (21.8%) only in the Ger-

Table 4

The numbers and the percentages of the most common taxa in fungal communities from the forest soils

Taxon	Number of species		Number of isolates		Percentage in fungal community	
Tuxon	Zielonka	Müggelheim	Zielonka	Müggelheim	Zielonka	Müggelheim
Mortierella spp.	4	2 (0.7)"	12	4 (3.1)	8	3 (2.2)
Mucor spp.	2	1 (0.3)	115	1 (8.3)	85	1 (5.4)
Oidiodendron griseum	1	1(0)	15	11 (0.6)	10	9 (0.05)
Penicillium spp.	11	8 (0.4)	37	54 (3.2)	25 ^b	44 (5.2)
Umbelopsis spp.	2	2(0)	22	17 (0.6)	15	14 (0.1)
Trichodenna spp.	3	2 (0.2)	8	3 (2.3)	6	2 (2.0)

Explanations: "z' values are shown in parentheses; 'Significant difference at Ps0.005
man soil. Only the density and percentage of Mucor were significantly greater in the

man soil. Only the density and percentage of Mucor were significantly greater in the Polish soil (P<0.001), and the percentage of Penicillia was significantly greater in the German soil (P<0.05) (Tab. 4).

Dominant lungs. Only a few species were dominant, with percentage >3% in the fungal community. Three dominant species occurring in both the Polish and German soils were U vancee (13.8% and 8.2%). O griscum (10.3% and 9%) and P plancewski (3.4% and 11.5%). Five dominant species occurring only in the Polish soil were P dalace (10.3%). C pinicola (6.2%), M, limitlis (4.1%). A, cylindrospon (3.4%) and G. candidam (3.4%) begind to candidam (3.4%) begind to candidam (3.4%) c pinicola (6.2%), M, buttlis (9.5%). P better begind to the property of the property

DISCUSSION

The increased interest in biodiversity of fungi, not only in global but also in national and local assessments, prompted the authors to compare two microfungal communities from Scots pine forest soils located in the same temperate region but 300 km apart and created in the same glacial period. Apart from contributing to knowledge of the active fungi inhabiting forest soils, the main value of this investigation lies in the parallel study of the fungal populations of two similar soils, though located apart. Studies on soil microfungal communities have only been rarely carried out in Poland and Germany. Considering the changes in the taxonomic classification of soil fungi and isolation methods it is necessary to carry out these studies continually.

continually.

The fungi were isolated after plating single soil particles from each sample onto SNA, according to the soil particle plating method of Nirenberg and Metzler (1990). Though conventional, the method proved to be very efficient. The number of fungal species found in Scots pine forest soils was up to 70% higher compared to results with other methods for solation of fungi (Kwasina and Nirenberg 1994).

Molecular techniques such as polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGE) and temperature gradient gel electrophoresis (TGGE) are more and more often used for detection and characterisation of soil microfungi (Muyzer and Smalla 1998); van Tuinen et al. 1998). They will, however, caused provide a precise indication of the density and diversity of fungal species in soil, and the application of conventional techniques is still necessary. Via u d et al. (2000) who studied microfungi in a single agricultural soil, were able to find 51 fungal taxa using PCR-RFLP of the ITS region and 67 taxa culturable on agar media using the soil dilution method. Only two species were identified by both techniques. Aurther 50 and 66 taxa were recorded only with PCR-RFLP and the soil dilution method, respectively.

The soil particle plating method enabled the fast isolation of 267 isolates within 55 different fungal taxa from both soils. All fungi isolated, except one, were able to grow in pure culture. The average number of species found in both soils (375) is higher than that recorded by Christen sen (1981) who, using four non-nolecular techniques, found on average 33 microfungal species among 180-200 isolates from confire-hardwood forest soils of the USA.

The small number of taxa common for both soils (18 fungal species out of 55 = 3.2%), the small number of dominant species common for both soils (3 species out of 55 = 5.5%), and the high number of species restricted to only one soil (25 = 5.5%) and polish and German soils) repetitively suggest that there is low diversity among the dominant and high diversity among the infrequent fungi in Scots pine foorest soils of temperate zone. Considerable spatial heterogeneity of the fungal communities in forest soils was noticed earlier by Kwsfan (1995, 1997). The main reasons are usually the existence of microsites and ecological niches created by differences in vegetation, the distribution of plant roots, the movement of water and soil funan, and the specific tive of funeal soortalation.

The number of fungal species common for both locations (32.7%) is similar to the number of species common for the two most studied fungal communities e.g. in Esber Common in Surrey and in Slapton Ley National Nature Reserve in Devon, UK. Both sites located in southern England have very different soils and habitats. Less than 40% of microfungal species are recorded in both (Cannon et al. 2001). The similar percentage of shared fungal species 40%) among most of 10 stands of upland boreal forests in Canada was recorded by Morrall and Vanterpool (1968).

The two soil microfungal communities are similar in composition to those in the northern Wisconsia conifer-hardwood forests (Christensen 1981). 40% of the recorded species, including, 4. c/findrospora, A. glauca, Allemaria, Chaetomium, Coniodnyium, C. roseum, Cylindrocarpon, G. candidum, M. alpina, M. hiemalis, O. griseum, P. adametti, P. elrazaccii (e. P. steckii), P. fonzerosskii, P. phinhaellum, P. simplicisimum, P. spinulosum, P. stoloniferum, S. schenckii, U. isabellina, U. vinacea and Z. moellerii vivee found by Christensen (1981).

The group of three dominant fungal taxa present in both soils, i.e. *U. vinacea*, *O. grisum* and *Penicillia* (*P. chrauszczii*, *P. daleue*, *P. janczewskii*, *P. spinulosum*) readily distinguishes forest soils from others (Morrall and Vanterpool 1968; authors, unpublished). The small number of the dominant taxa recorded supports the theory of Thornton (1966), who came to the conclusion that, in relatively undisturbed

forest soils 'only a small number of soil fungi assume dominance as a result of particularly favourable conditons'.

The number of dominant fungal species shared by the two soils (= 3, e.g. U. vinacea + O. griseum + P. janczewskii) is smaller than the average number of dominant fungal species shared by two forest soils (= 7.6) in 13 locations studied by Christensen (1981). This is, however, due to the greater number of techniques (= 4) used by Christensen for the fungal isolation.

Many of the species recorded, e.g. A. cylindrospora, G. auratus, M. alpina, M. gamsii, M. humilis, O. griseum, P. adametzii, P. janczewskii, P. spinulosum, P. bulbillosa, T. inflatus, U. vinacea, are prevalent forest fungi but occur also in other habitats (Domsch et al. 1981). Christensen (1981) found that the average numbers of soil microfungi shared by forest soil and desert, grassland, heath and tundra soil were 2.0, 4.6, 5.9 and 5.2, respectively. Among the dominant fungi, U. vinacea and O. griseum are very widely distributed in acidic, not only forest, but also agricultural soils, Penicillium janczewskii is considered to be one of the most wide-spread and abundant species of Penicillium in all kinds of soil (Christensen 1969; Domsch et al. 1980; Pitt 1979).

Almost all fungal taxa recorded in both soils belong to saprobic species which have long been recognized as less host-restricted and host-specific (Lindblad 2000). The only pathogenic fungus recorded in the Polish soil was C. destructans. This species often occurs in acidic forest soils in Poland (Kwaśna, unpublished), although many reports on its occurrence indicate that it has a preference for moist organic soils with higher pH values (Flan agan and Scarborough 1974; Bissett and Parkinson 1979; Widden and Parkinson 1979).

Seventeen taxa occurred only once in one soil sample indicating that many soil microfungi appeared to be highly restricted in occurrence. In studies by Christensen (1969), about 90% of the species were also rare and isolated from fewer than 4 out of 36 stands. The low density of certain species implies that either a particular species grows sparsely on the nutritional source or that it has a limited and discontinuous distribution (Willoughby 1962; Lee 2000). In both cases there is a likelihood of overlooking it in a soil sample.

The composition of both communities was, to a certain extent, affected by temperature, which is one of the major environmental factors. Since both communities were isolated in the middle of spring, they consisted of many species which, according to Widden (1986), are characteristic of the colder months in the temperate zone, e.g. Exophiala, Geomyces, Paecilomyces, Penicillium, T. koningii. The authors' opinion is, however, that they are characteristic of the temperate zone.

Less diversity of microfungi in the German soil could be related to the lower humus, N (nitrogen), P (phosphorus) and K (potassium) contents, and smaller diversity of herbaceous plants in a forest floor. Deficiency of N diminishes the incidence of microorganisms due to the inhibition of microbial activity and 0, consumption (Macura 1958; Trolldenier 1979). Nitrogen in soil increases the absorption and allocation of N in the growing parts of the mycelium and reduces the carbon requirements of germinating spores, therefore increasing fungal frequency (Griffin 1964, 1973; Pass and Griffin 1972; Dowding 1981). Phosphorus is required for the development of nucleic acids, membranes, protoplasm and proteins. Potassium is the major ionic component of protoplast and vacuole in the fungal mycelium. Lower N. P and K content usually decreases root exudation which additionally limits the growth of fungi (Tolldenier 1979). Therefore, the higher frequency of C. destructurs, M. hiemalis, P. influtus, P. janthinellum and T. wirde, as well as the lower frequency of Geomyces spp. in the Polish soil, may be due to its higher amounts of N and K. All these data are in accordance with Widden (1986c), who studied the importance of the main nutritional compounds on the structure of soil fungal communities.

The higher content of humus and of mineral nutrients in the Polish soil may be due to more intense decomposition, indicated by the higher frequency of Zegomyetes. They do not usually degrade cellulose or hemicellulose at the beginning of the decomposition process, but rather proteins at its end (Hudson 1968; Bääth and Söderström 1980, Osono and Take da 2002). The increase in frequency of Zegomyetes in a soil amended with easily utilizable nitrogens sources was noticed earlier by Bollen (1979). Umblopsis vinaceae may degrade cellulose in the presence of a higher level of nitrogen (Park 1976; Bäät hand Söderström 1980), and the higher frequency of the fungus in the Polish soil may result from a higher N content due to the more advanced decomposition.

The lower diversity of the fungal community in the German soil may also be due to dominance by species with allelopathic activities, e.g. Penicillia. Christensen (1969) provides some evidence that antibiosis may play a role in the distribution of U. isabellina, P. janczewski and Tichoderma. The first two are reported to be sensitive to Trichoderma antibiotics. Penicillian janczewski and U. isabellina have consistently lower densities in the Polish soil which is richer in Trichoderma. Penicillian janczewski, which is a producer of priscodulvin, inhibits the growth of a few fungal species in vitro (Kwaśna 1995, 1997). We also cannot exclude synergistic effects between funoi.

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Mikrogrzyby w glebie spod sosny zwyczajnej w Polsce i Niemczech

Streszczenie

Badano skład zbiorowski mikrogrzybów spol 17-letniej souny zwyczajnej w drzewostanych oddalonych o 300 km y Polsoc (Zichosha) 1 Niemzczek (Müggelheim). Ogolem wysiokowano 55 gatunkow zwyczejnej w drzewostanych oddalonych 200 km y Polsoc (Zichosha) 1 Niemzczek 154 jiż (ziolay przybów należące od 34 i 32 gatunków. W glebie z Zielonki i Müggelheim pajczęściej stwierdzano grzyby z rodzaju Perucililiam (25% 14%); reprezentowany przez 11 i 82 gatunków, Umkojeniej (15% i 14%); 2 gatunków, w glebie z Zielonki i Müggelheim najczęściej stwierdzano grzyby z rodzaju Perucililiam (25% 12%); 2 jaż tytkowany przez 11 i 82 gatunków, Umkojeniej (15% i 14%); 2 gatunkami, Oddodondon giacum (10% i 9%), Moriectela (5% i 3%) z 41 z gatunkami oraz Trichodema (6% i 25%) systapił y jlko w glebie z Zielonki i 12 usonów (21.8%) tyklo w glebie z Müggelheim. Trzy domininajce grzybo, o licebności 3 5% w oby glebac, należa-ży do Umkolopasi winoceu (13.5% i 8.2%), Oddodondom griesum (10.3% i 9%) i Piocililiam jazoczoskii (3.4% i 11.5%), Malsi Geta gatunków współych wysteplacych w bos glebach, swiadczy o dużym zróżnicowaniu gatunkowym mikrogrzybów w pojedynczym europejskim, cierum ekowstemie sown zwyczajenie sown zwyczajenie oson zwyczajenie owyczym europejskim, cierum ekowstemie sown zwyczajenie sown zwyczajenie owycznego control z dominia z domi