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Fungal communities in barren forest soil after amendment with different wood substrates and their possible effects on trees', pathogens, insects and nematodes

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Abstract: Scots pine sawdust, composted bark or coarse, post-harvest woody debris from conifers had been spread over the surface of barren forest soil before planting with Scots pine. The effects of the Scots pine sawdust, composted bark or coarse, post-harvest woody debris from conifers on the abundance and diversity of culturable fungi were investigated. The amendments were aimed at increasing the soil suppressiveness to *Armillaria* and *Heterobasidion.* The classical soil-dilution method was chosen for qualitative and quantitative analyses of fungal communities in soils because of its proven reliability and consistency. The soil was inhabited by saprotrophic fungi from Ascomycota and Zygomycota, including species known to be potential antagonists of *Armillaria* or *H. annosum* (i.e. *Clonostachys* + *Trichoderma* spp., *Penicillium commune, P. daleae, P. janczewskii*) or stimulants of *Armillaria* (i.e. *Pseudogymnoascus roseus*, *Trichocladium opacum*). Eleven years after treatment, the abundance and diversity of fungi, the abundance of *P. commune*, and locally the abundance of *P. janczewskii* increased, while *Clonostachys* + *Trichoderma* spp., and locally, *P. daleae* and *T. opacum* decreased. Amending the barren soil with organic matter does not guarantee effective, long-term suppressiveness of the sandy loam soil to *Armillaria* and *Heterobasidion.* Increased abundance of entomopathogenic and nematophagous species, 11 years after treatment, does suggest the long-term possibility of insect or nematode control in soil.

Key words: *Armillaria, Heterobasidion,* insects, nematodes, organic matter, soil fungi

Introduction

Poland has a 28.8% forest cover, which is less than the European average of 32%. At the same time, 25% of land (4.6 million ha) that is potentially available for arable use is unsuitable for agricultural production. Considering Poland's present stage of development, land-use structure, and environmental, geographic and climatic conditions, forest cover should be 33–34%. In 2002, the National Program for the Augmentation of Forest Cover (KPZL) was revised. The Program anticipates afforestation of 1.5 million ha of post-agricultural land and an increase in forest cover to 30% by 2020 and 33% by 2050 (Zając and Kwiecień 2001). Land designated for afforestation includes 2.3 million ha of marginal land. Such lands include soils which are non-fertile, contaminated with chemicals, destroyed or mechanically transformed, and fallow land or land with unfavourable natural and topographic conditions.

First and second generation Scots pine (*Pinus sylvestris* L.) grown on soils formerly under agricultural use become seriously damaged by *Heterobasidion annosum* (Fr.) Bref. Young Scots pine plantations also become infested with *Armillaria* spp. (Fr. Fr.) Staude (Sierota 1995, 2013).

Armillaria ostoyae (Romagn.) Herink and *H. annosum* are among the most dangerous pathogens causing butt and root rot in conifers of temperate and boreal regions of the world, in Europe, North America, and Asia (Gregory *et al*. 1991; Korhonen and Stenlid 1998; Korhonen *et al*. 1998; Dettman and van der Kamp 2001; Kile *et al*. 2001; Dai *et al*. 2003, 2006; Żółciak 2007; Otrosina and Garbelotto 2010; Worrall *et al*. 2010). In Europe alone, *H. annosum* is responsible for the loss of 800 million euros annually. In Poland, in 2013, serious occurrence of butt and root rot caused by *Armillaria* and *H. annosum* were recorded on 99,215 and 146,379 ha, respectively (Tarwacki 2013).

Favourable conditions for pathogens include alkaline soils, soils with a disease history, and soils with a deficiency of competitive and antagonistic microorganisms. In these conditions, saplings of conifers can become infected very soon after being planted.

Damage by *H. annosum* is greater on former agricultural and fallow lands partly because of their higher nitrogen content, lower C : N ratio, deficiency in potassium and phosphorus, higher pH, lower capillary ascent of water and lower aggregate stability of soil under the "plough layer". Shortage of lignified tissues, which are the specific carbon and energy source for the forest soil microbiota,

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creates an unfavourable habitat for microorganisms that could contribute to forest site-forming processes (Trojanowski and Heider 1975).

The aim of the research was to determine the abundance, diversity, and temporal distribution of culturable fungi in barren forest soils amended with different forms of organic matter, i.e. Scots pine sawdust, composted bark and coarse, post-harvest coniferous woody debris. The hypothesis was that organic matter amendment would change the physical and chemical properties of soil, which in turn would initiate and stimulate microbiological shifts that would increase the long-term suppressiveness of soil to *Armillaria* and *Heterobasidion*. The classical soil-dilution method was chosen for qualitative and quantitative analyses of fungal communities in soils because of the method's proven reliability and consistency. The affiliation, activity and microbiological relationships of many soil fungi detected with such culture-based methods are already known. The microbiological status of soils so determined, can be used for comparing habitat qualities/properties. Alternative methods of profiling based on DNA sequences were not used because they tend to detect different communities. The communities most often detected are of non-culturable or slow-growing, non-sporulating organisms with unknown affiliation or activity and less obvious relevance in microbiological interactions (Kwaśna *et al*. 2008; van Elsas and Boersma 2011).

Materials and Methods

Site description and treatments

The experiment was established on barren, uncultivated soil in the Bielsk Forest District (north-east Poland, 23°11′11″E, 52°45′54″N). In October 2001, an area of 0.4 ha was divided into 20 plots of 200 m^2 (10 × 20 m). Scots pine sawdust, composted bark or coarse, post-harvest coniferous woody debris was spread on the surface of the plots, along the proposed planting rows. The amount of amendment used was 7.5 dm^3 per 1 m × 0.4 m area. One-year-old Scots pine seedlings were planted 0.6 m apart, in rows 1.2 apart. The seedlings were planted in spring 2002 which was eight months after amendments were applied. In an additional treatment, 0.4 dm^3 of composted bark was placed under the roots of each of the one-year-old Scots pine seedlings while they were being planted in spring 2002. In control plots, Scots pine seedlings were planted into non-amended soil. Treatment and control plots were replicated four times in a randomised block design.

Composted bark was prepared in windrows (4–5 m wide, 2.5–3 m high) and consisted exclusively of Scots pine bark. Composting occurred in three phases: 1) an initial phase of 1–2 days during which easily degradable compounds were decomposed; 2) a thermophilic phase, lasting several months, with temperatures 40–80°C, in which cellulose was primarily degraded; 3) a stabilisation phase, with a decreased rate of decomposition, lower temperature and colonisation by mesophilic microorganisms. Composted bark contained 5% readily degradable carbon (cellulose).

The soil in the plots was sandy-loam (10.8% clay, 13.6% silt, and 75.6% sand), with 0.353% organic carbon content, and a pH of 4.41 in water (Table 1). Increased amounts of carbon, nitrogen, phosphorus (3–4×), and potassium were present 11 years after treatment. The first year of the experiment (2002) was slightly warmer (mean annual temperature 8.25°C) and dryer (total annual rainfall 489.4 mm) than the last year (2012) when the mean temperature was 7.0°C and total annual rainfall was 611.0 mm.

Table 1. Physicochemical properties of the barren forest topsoil (3–20 cm layer) before organic substrate amendments (2001) and 11 years after amendments (2012)

Soil characteristics	Barren forest soil (2001)	11-year-old *Penicillium sylvestris* plantation (2012)				
		Scots pine sawdust-spread	composted bark		coarse coniferous woody debris-spread	the control
			under roots	spread		
pH in KCl[1]	3.970	3.945	4.030	3.940	3.955	4.000
pH in H_2O	4.410	4.710	4.720	4.695	4.510	4.585
Organic carbon (%)[2]	0.353	0.470^{abcd}	0.357^{aefg}	0.553^{behi}	0.419^{cfhj}	0.453^{dgij}
Total nitrogen (%)[3]	0.029	0.049	0.040	0.056	0.046	0.048
C : N	12.10	9.40	9.15	9.80	9.15	9.15
Extractable phosphorus P_2O_5 [mg · kg^{-1}][4]	48.0	193.0	172.0	207.0	195.0	200.0
Extractable potassium [mg · kg^{-1}][5]	7.00	11.70	10.95	12.10	10.00	11.25
Extractable magnesium [mg · kg^{-1}][5]	1.00	1.10	1.05	1.35	1.00	1.20
Extractable calcium [mg · kg^{-1}][5]	7.00	8.00^{abc}	4.30^{adef}	13.10^{bdgh}	5.10^{cegi}	8.25^{fhi}
Extractable sodium [mg · kg^{-1}][5]	4.00	1.65^{abc}	0.75^{a}	1.00^{b}	0.90^{c}	0.90^{d}

[1]analysed with potentiometer according to norm PN-ISO 103390:1997

[2]analysed chemically according to norm PN-ISO 10694:2002

[3]analysed chemically according to norm PN-13878:2002

[4]analysed chemically with method of Egner-Riehm

[5]analysed chemically according to procedure PB-05 ed. 2

The same letter indicates a statistically significant difference according to one-way ANOVA at $p \leq 0.001$ or at $p \leq 0.05$

Collection of samples

In September 2002 (10 months after treatment) and 2012 (11 years after treatment), six 7.5-cm-diam. cores of non-rhizosphere soil were collected from the A horizon (0–20 cm deep) along a row of the Scots pine trees in each of the four randomised plots in each treatment. The sample from a single plot were bulked together and mixed by rotating for 10 h.

Isolation and identification of fungi

Soil fungi were isolated using the soil-dilution method (Warcup 1950, 1955). From the four bulked sub-samples of each plot, 1 g of soil was diluted in 149 g of sterile quartz sand and 0.02 g of the mixture was put into a Petri dish, then covered with liquid (50°C) Johnson-Martin's agar (JMA; glucose 10 g · l^{-1}, peptone 5 g · l^{-1}, KH_2PO_4 1 g · · l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g · l^{-1}, rose Bengal 0.03 g · l^{-1}, aureomycin 0.0025 g · l^{-1}, agar 20 g · l^{-1}). Eight replicates (Petri dishes) were made for each plot (= 32 Petri dishes for a treatment). All plates were incubated for 20 days at 25°C.

All colonies on each plate were examined macro- and microscopically and distinguished on the basis of colour, growth rate, hyphal characteristics, and sporulation. Colonies of each species were counted and representatives of fungi were identified by morphotyping on Potato Dextrose Agar (PDA; Difco PDA 39 g · l^{-1}, pH 5.5) and Synthetic Nutrient Agar (SNA; KH_2PO_4 1 g · l^{-1}, KNO_3 1 g · l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g · l^{-1}, KCl 0.5 g · l^{-1}, glucose 0.2 g · l^{-1}, sucrose 0.2 g · l^{-1}, agar 20 g · l^{-1}). The *Aspergillus* and *Penicillium* species were identified on Czapek Yeast Autolysate agar (CYA; sucrose 30 g · l^{-1}, powdered yeast extract 5 g · l^{-1}, KH_2PO_4 1 g · l^{-1}, Czapek concentrate 10 ml · l^{-1}, agar 15 g · l^{-1}) and 2% Malt Extract Agar (MEA; powdered malt extract 20 g · l^{-1}, glucose 20 g · l^{-1}, peptone 1 g · l^{-1}, agar 20 g · l^{-1}). Identification was made using mycological keys (Raper and Thom 1949; Barron 1968; Domsch *et al.* 1980; Pitt 1979; Klich and Pitt 1992). Fungi abundance was defined as the number of colony forming units (*cfu*) in a treatment. Frequency of an individual species or group of species was defined as percentage of isolates in the total number of isolates. Diversity was defined as the number of species in a treatment. A species, or group of related species of fungi, was considered as: (i) eudominant, with a frequency of 10–100%; (ii) dominant, with a frequency of 5–10%; (iii) subdominant, with a frequency of 2–5%; (iv) recedent, with a frequency of 1–2%; (v) subrecedent, with a frequency of 0–1%, in at least one treatment (Tischler 1949).

Statistics

Differences in soil chemical properties and numbers of *cfu* from two different samples, were analysed with one-way analysis of variance (ANOVA) using Statgraphics™ Centurion (Statpoint Technologies, Inc. Warrenton, VA, USA). Diversity within and between microbial communities was compared using diversity indices calculated for each community (Magurran 1988). Species richness was indicated by the total number of species in the community, Margalef's index (DMg), Shannon's diversity index (H'), and Simpson's diversity index (D). Evenness and dominance were indicated by Shannon's evenness index (E), and Berger-Parker's index (d). Similarity between fungal communities in two systems was determined by calculating the qualitative Sorensen's similarity index (CS). Relationships between the soil chemical properties and the abundances of fungi were estimated using Pearson's correlation coefficient.

Results

A total of 93 species of saprotrophic soil fungi from Ascomycota and Zygomycota were recorded in the barren, non-rhizosphere forest soil 10 months and 11 years after amending the soil with Scots pine sawdust, composted bark or coarse coniferous woody debris and after having been planted with Scots pines (Table 2). There were increases between 2002 and 2012 in total abundance of fungi, diversity of fungi, abundance of *Acremonium* + *Sagenomella* spp., *Aspergillus* spp., *Oidiodendron* spp. and *Penicillium* + *Talaromyces* spp., and often of the entomopathogenic and nematophagous species. There were decreases in abundance of *Mortierella* spp. and *Clonostachys* + *Trichoderma* spp. (Fig. 1, Table 3). There were 16 eudominant, 28 dominant, eight subdominant, five recedent, and 36 subrecedent species. Twenty-one species, including *A. kiliense* Grütz, *A. pteridii* W. Gams, *A. strictum* W. Gams, *A. ochraceus* Wilhelm, *C. albicans* Berkhout, *C. merdarium* (Link ex Grev), *C. candelabrum* (Bonord.) Schroers, *Mortierella* spp., *P. lilacinus* (Thom) Samson, *P. adametzii* Zaleski, *P. brevicompactum* Dierckx, *P. citreo-viride* Biourge, *P. cyclopium* Westling, *P. lilacinum* Thom, *T. opacum* (Corda) Hughes, *T. polysporum* (Link ex Pers.) Rifai, *T. mentagrophytes* (Robin) Blanchard, and *U. nana* (Linnem.) Arx already occurred 10 months after the treatment. Fifty-six species, including *A. fusidioides* (Nicot) W. Gams, *A. niger* Tiegh., *C. cladosporioides* (Fresen.) G.A. de Vries, *O. griseum* Robak, *P. commune* Thom, *P. steckii* Zaleski and *P. vinaceum* J.C. Gilman & E.V. Abbott (eudominants and dominants), and all subdominants, recedents and subrecedents, only began to occur 11 years after treatment. The most frequent *Penicillium* species were *P. commune*, *P. daleae* Zaleski, *P. janczewskii* Zaleski, *P. lilacinum* Thom, *P. steckii*, and *P. vinaceum*. From the *Trichoderma*, only *T. polysporum* was among the eudominants. Another three *Trichoderma* species, i.e. *T. hamatum* (Bonord.) Bain, *T. koningii* Oudem., and *T. viride* Pers. were among the dominants.

The relatively small number of fungal taxa and the infrequent occurrence of many taxa soon after treatment, resulted in relatively small diversity indices based on species richness (DMg) and proportional abundance of species (H') (Table 4). There was significantly more diversity after 11 years than 10 months after treatment. Communities were more evenly distributed after 11 years. Dominance, to a greater or lesser extent, of single taxa after 11 years resulted in smaller values for Shannon's evenness index (E) and often higher values for the dominance index (d). Sorensen's qualitative similarity index (CS), when used for comparing fungal communities between the two samples, showed there was the most similarity after the

Table 2. Frequency (%) of fungi in barren forest soil recently amended with organic substrates and planted with Scots pines (2002), and the frequency 11 years after amending (2012)

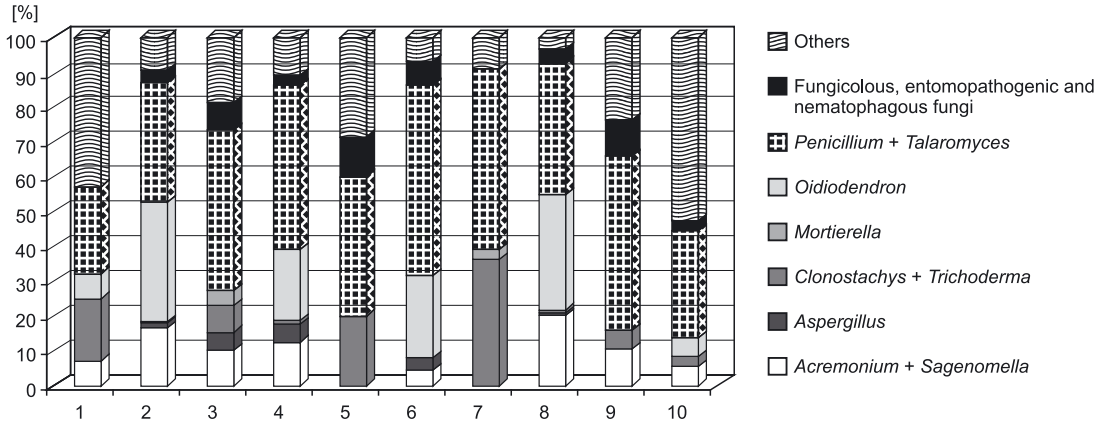
No.	Taxon	Organic matter amendment									
		Scots pine sawdust-spread		composted bark				coarse coniferous woody debris-spread		the control	
				under roots		spread					
		2002	2012	2002	2012	2002	2012	2002	2012	2002	2012
		Eudominants (frequency 10–100% in at least one treatment)									
1	*Acremonium fusidioides* (Nicot) W. Gams	–	15.1^{ab}	–	9.1^{c}	–	3.7^{a}	–	18.6^{c}	–	5.6^{b}
2	*Cladosporium* spp.	–	–	–	–	–	0.5^{a}	3.5	0.7^{b}	–	13.8^{ab}
3	*Clonostachys candelabrum* (Bonord.) Schroers	–	–	–	–	–	–	25.9	–	–	–
4	*Oidiodendron tenuissimum* (Peck) S. Hughes	7.1^{a}	32.8^{abe}	–	11.4^{bcdf}	–	21.6^{cg}	–	26.6^{dh}	–	5.6^{efgh}
5	*Penicillium citrinum* Thom	–	–	–	–	–	–	–	–	10.4	–
6	*P. commune* Thom	–	10.6^{ab}	–	22.8^{ad}	–	31.6^{bce}	–	18.2^{ce}	–	8.2^{de}
7	*P. cyclopium* Westling	–	–	–	–	5.7^{a}	–	–	–	23.6^{a}	–
8	*P. daleae* Zaleski	–	–	7.7	–	5.7	–	29.2	1.0	–	–
9	*P. janczewskii* Zaleski	7.1^{a}	6.6	10.1^{b}	0.5^{bef}	11.5^{c}	2.9^{c}	17.2^{ad}	8.8^{df}	–	5.6^{e}
10	*P. lilacinum* Thom	–	–	28.3	–	17.1	–	–	–	–	–
11	*P. purpurescens* Sopp	25.1^{a}	–	–	–	–	–	–	–	10.4^{a}	–
12	*P. steckii* Zaleski	–	1.4^{a}	–	10.3^{abc}	–	1.1^{b}	–	2.6^{c}	–	5.6
13	*P. vinaceum* J.C. Gilman & E.V. Abbott	–	12.6^{a}	–	9.7^{b}	–	16.9^{bcd}	–	4.3^{ace}	–	8.2^{de}
14	*Pseudogymnoascus roseus* Raillo	7.1	–	–	–	–	0.8^{a}	–	–	13.2^{a}	19.4
15	*Trichocladium opacum* (Corda) Hughes	14.4^{a}	–	–	–	–	–	–	–	5.3^{a}	–
16	*Trichoderma polysporum* (Link ex Pers.) Rifai	17.9^{ab}	–	–	–	14.3^{ac}	–	6.9^{bc}	–	–	–
		Dominants (frequency 5–10% in at least one treatment)									
17	*A. kiliense* Grütz	–	–	10.0		–	–	–	–	5.3	–
18	*A. pteridii* W. Gams	7.1	–	–	–	–	–	–	–	–	–
19	*A. roseogriseum* (S.B. Saksena) W. Gams	–	0.4	–	–	–	0.3	–	–	5.3	–
20	*A. strictum* W. Gams	–	–	–	–	8.6	–	–	–	–	–
21	*Alternaria alternata* (Fr.) Keissl.	7.1	–	–	–	–	–	–	–	–	2.8
22	*Aspergillus niger* Tiegh.	–	1.0	–	5.2	–	2.9	–	0.7	–	–
23	*A. ochraceus* Wilhelm	–	–	5.2	–	–	–	–	–	–	–
24	*Candida albicans* Berkhout	–	–	–	–	5.7	–	–	–	–	–
25	*Chrysosporium merdarium* (Link ex Grev)	–	–	–	–	5.7	–	–	–	–	–
26	*C. cladosporioides* (Fresen.) G.A. de Vries	–	0.7	–	–	–	–	–	0.7	–	5.6
27	*Lecanicillium lecanii* (Zimm.) Zare & W. Gams	–	0.4	7.7	2.9	5.7	6.4	–	3.2	–	–
28	*Leptographium lundbergii*	–	–	–	–	–	–	–	–	5.3	–
29	*Mortierella macrocystis* W. Gams	–	–	5.2	–	–	–	–	–	–	–
30	*M. spinosa* Linnem	–	–	7.7	–	–	–	–	–	–	–
31	*O. griseum* Robak	–	1.8	–	9.1	–	1.9	–	5.8	–	–
32	*Paecilomyces carneus* (Duché & R. Heim) A.H.S. Br. & G. Sm.	–	1.4	–	0.5	–	–	–	0.3	5.3	–
33	*P. lilacinus* (Thom) Samson	–	–	–	–	5.7	–	–	–	–	–
34	*P. adametzii* Zalewski	–	–	5.2	–	–	–	–	–	–	–
35	*P. brevicompactum* Dierckx	–	–	–	–	8.6	–	–	–	–	–
36	*P. canescens* Sopp	–	–	–	–	–	–	–	–	5.3	–
37	*P. citreo-viride* Biourge	–	–	–	–	–	–	5.2	–	–	–
38	*Simplicillium lamellicola* (F.E.V. Sm.) Zare & W. Gams	–	–	–	–	–	–	–	–	5.3	–
39	*Trichoderma hamatum* (Bonord.) Bain	–	–	–	–	–	–	–	–	5.3	–
40	*T. koningii* Oudem.	–	–	–	–	5.7	–	3.5	0.7	–	2.8
41	*T. viride* Pers.	–	–	7.7	–	–	–	–	0.3	–	–
42	*Trichophyton mentagrophytes* (Robin) Blanchard	7.1	–	–	–	–	–	–	–	–	–
43	*Ulocladium consortiale* (Thüm.) Simmons	–	–	5.2	–	–	–	–	–	–	–
44	*Umbelopsis nana* (Linnem.) Arx	–	–	–	–	–	–	8.6	–	–	–
		Subdominants (frequency 2–5% in at least one treatment)									
45	*Acrodontium salmoneum* de Hoog	–	–	–	–	–	–	–	–	–	2.8
46	*Cladosporium herbarum* (Pers.) Link	–	0.4	–	–	–	0.5	–	0.7	–	2.8
47	cf. *Cylindrocladiella hahajimaensis* (Ts. Watanabe) Inderb., R.M. Bostock & K.V. Subbarao	–	–	–	–	–	0.5	–	–	–	–
48	*Metacordyceps chlamydosporia* (H.C. Evans) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora	–	–	–	–	–	–	–	–	–	2.8
49	*Metarhizium anisopliae* (Metschn.) Sorokīn	–	2.1	–	0.5	–	0.5	–	–	–	–
50	*Paraconiothyrium fuckelii* (Sacc.) Verkley & Gruyter	–	2.1	–	1.2	–	–	–	0.3	–	–
51	*Sagenomella diversispora* (J.F.H. Beyma) W. Gams	–	–	–	3.4	–	0.5	–	0.7	–	–
52	*Talaromyces variabilis* (Sopp) Samson, Yilmaz, Frisvad & Seifert	–	–	–	2.8	–	–	–	0.3	–	–

Table 2. Frequency (%) of fungi in barren forest soil recently amended with organic substrates and planted with Scots pines (2002), and the frequency 11 years after amending (2012) – *Continuation*

No.	Taxon	Organic matter amendment									
		Scots pine sawdust-spread		composted bark				coarse coniferous woody debris-spread		the control	
				under roots		spread					
		2002	2012	2002	2012	2002	2012	2002	2012	2002	2012
		Recedents (frequency 1–2% in at least one treatment)									
53	*Haptocillium sphaerosporum* (Goodey) Zare & W. Gams	–	–	–	1.7	–	–	–	0.3	–	–
54	*Penicillium ochrochloron* Biourge	–	1.7	–	–	–	–	–	–	–	–
55	*P. spinulosum* Thom	–	–	–	–	–	–	–	2.0	–	–
56	*Talaromyces pinophilus* (Hedgc.) Samson, Yilmaz, Frisvad & Seifert	–	0.4	–	–	–	1.3	–	–	–	2.8
57	*T. deliquescens* (Sopp) Jaklitsch	–	0.4	–	1.1	–	–	–	–	–	–
		Subrecedents (frequency 0–1% in at least one treatment)									
Total abundance – number of colony forming units (*cfu*) in a sample		84^{afghi}	$28^{amn\circ}$	117^{bfj}	176^{bmprs}	105^{cgk}	379^{cnptu}	174^{dhjkl}	$307^{d\circ rtw}$	114^{eil}	36^{esuw}
Diversity – number of species		9^{a}	36^{ae}	11^{b}	27^{b}	12^{c}	31^{c}	8^{d}	29^{d}	12	16^{e}

Subrecedents (with frequency < 1%) included *Apiospora montagnei* Sacc., *A. fumigatus* Fresen., *A. versicolor* (Vuill.) Tirab. *C. pannicola* (Corda) Oorschot & Stalpers, *Chrysosporium* sp., *Fusarium oxysporum* Schltdl., *Geomyces pannorum* (Link) Sigler & J.W. Carmich., *Humicola fuscoatra* Traaen, *H. grisea* Traaen, *Nectria inventa* Pethybr., *O. echinulatum* G.L. Barron, *P. variotii* Bainier, *P. dierckxii* Biourge, *P. expansum* Link, *P. megasporum* Orpurt & Fennell, *P. raistrickii* G. Sm., *Phialophora bubakii* (Laxa) Schol-Schwarz, *S. striatispora* (Onions & G.L. Barron) W. Gams, *T. flavus* (Klöcker) Stolk & Samson, *T. islandicus* (Sopp) Samson, Yilmaz, Frisvad & Seifert, *T. rugulosus* (Thom) Samson, Yilmaz, Frisvad & Seifert, *Trichothecium roseum* (Pers.) Link, and *U. isabellina* (Oudem.) W. Gams, non-sporulating – 13 species

The same letter indicates a statistically significant difference according to one-way ANOVA at $p \leq 0.001$ or at $p \leq 0.05$



1, 2 – Scots pine sawdust-spread (2002, 2012)
3, 4 – composted bark under roots (2002, 2012)
5, 6 – composted bark-spread (2002, 2012)
7, 8 – coarse post-harvest coniferous woody debris-spread (2002, 2012)
9, 10 – the control (2002, 2012)

Fig. 1. Frequencies of the most common taxa in forest barren soil (2002) and in 11-year-old Scots pine plantation (2012) after organic substrate amendment

application of coarse woody debris (Table 5). Comparable similarity among all treatments, 11 years after their application was also shown, however, with the CS index.

The fungal communities contained a few species known to be antagonists of *Armillaria* or *H. annosum*, i.e. *C. candelabrum, G. pannorum* (Link) Sigler & J.W. Carmich., *P. adametzii*, *P. commune*, *P. daleae*, *P. janczewskii*, and *Trichoderma* spp., or stimulants of *Armillaria*, i.e. *M. macrocystis* W. Gams, *P. adametzii*, *P. citrinum* Thom, *P. spinulosum* Thom, *P. roseus* Raillo, and *T. opacum* (Table 6). Eleven years after treatment there was an increase in all treatments of only one antagonist of *H. annosum*, i.e. *P. commune*, whilst the abundance of *P. janczewskii* increased slightly after the sawdust treatment. Overall, there were small decreases in populations of four *Armillaria* stimulants, i.e. *M. macrocystis*, *P. adametzii*, *P. roseus*, and *T. opacum*. But the effect was significant only for *T. opacum* after the sawdust treatment.

Table 3. Abundances (*cfu* per sample) of the most common taxa in barren forest soil recently amended with organic substrates and planted with Scots pines (2002), and 11 years after amending (2012)

No.	Taxon	Organic matter amendment									
		Scots pine sawdust-spread		composted bark				coarse coniferous woody debris-spread		the control	
				under roots		spread					
		2002	2012	2002	2012	2002	2012	2002	2012	2002	2012
1	*Acremonium* + *Sagenomella* spp.	6^{a}	47^{aefgh}	12^{b}	22^{beij}	0^{c}	17^{cfk}	0^{c}	62^{dgikl}	12^{d}	2^{dhjl}
2	*Aspergillus* spp.	0	4^{b}	6	9^{c}	0^{a}	14^{abd}	0	2^{c}	0	0^{d}
3	*Clonostachys* + *Trichoderma* spp.	15^{aef}	1^{a}	9^{bgh}	2^{b}	21^{cgij}	0^{c}	63^{dehi}	3^{d}	6^{fj}	1
4	*Mortierella* spp.	0	0	5	0	0	0	5	0	0	0
5	*Oidiodendron* spp.	6^{a}	98^{aef}	0^{b}	36^{beghi}	0^{c}	89^{cgj}	0^{d}	101^{dh}	0	2^{fij}
6	*Penicillium* + *Talaromyces* spp.	21^{afghi}	97^{ajkl}	54^{bf}	$83^{bmn\circ}$	42^{cg}	20^{cjmp}	90^{dh}	116^{dknp}	57^{ei}	$11^{el\circ}$
Entomopathogenic and nematophagous *Haptocillum* + *Lecanicillium* + *Metacordyceps* + *M. anisopliae* + *Paecilomyces* + *Simplicillium*[1]		0^{aefg}	10^{akl}	9^{eh}	5^{mn}	12^{bfi}	$26^{bkm\circ p}$	0^{chij}	$13^{c\circ}$	12^{dgj}	1^{dlnp}

Explanations – see table 2

[1]according to Domsch *et al.* 1980; Watanabe 1980; Rombach *et al.* 1986; Gams *et al.* 2004; Inglis *et al.* 2006; Sun and Liu 2006; Quesada-Moraga *et al.* 2014

Table 4. Diversity indices for fungi in barren forest soil recently amended with organic substrates and planted with Scots pines (2002), and 11 years after amending (2012)

Index	Organic matter amendment									
	Scots pine sawdust-spread		composted bark				coarse coniferous woody debris-spread		the control	
			under roots		spread					
	2002	2012	2002	2012	2002	2012	2002	2012	2002	2012
	Species richness indices									
Margalef's index (DMg)	2.40	6.19	2.72	5.02	3.09	5.05	1.72	4.88	3.02	4.18
Shannon's diversity index (H')	2.06	2.32	2.22	2.45	2.39	2.15	1.79	2.27	2.32	2.57
Simpson's diversity index (D)	0.14	0.16	0.13	0.11	0.10	0.18	0.19	0.15	0.11	0.09
	Evenness or dominance indices									
Shannon's evenness index (E)	0.94	0.65	0.93	0.74	0.96	0.48	0.86	0.67	0.93	0.93
Berger-Parker's index (d)	0.25	0.33	0.28	0.23	0.17	0.31	0.29	0.27	0.24	0.19
Sorensen qualitative similarity index (CS)	0.08		0.10		0.09		0.21		0.07	

Table 5. Sorensen qualitative similarity index (CS) between treatments for fungal communities in barren forest soil recently amended with organic substrates and planted with Scots pines (2002), and 11 years after amending (2012)

Treatments compared		Sorensen qualitative similarity index (CS)	
1	2	2002	2012
Scots pine sawdust – spread	composted bark under roots	0.04	0.44
Scots pine sawdust – spread	composted bark – spread	0.19	0.44
Scots pine sawdust – spread	coarse coniferous woody debris – spread	0.23	0.40
Composted bark under roots	composted bark – spread	0.34	0.41
Composted bark under roots	coarse coniferous woody debris – spread	0.23	0.50
Composted bark –spread	coarse coniferous woody debris – spread	0.40	0.40

Table 6. Abundances (*cfu* per sample) of antagonists and stimulants of *Heterobasidium annosum* and of *Armillaria* in barren forest soil recently amended with organic substrates and planted with Scots pines (2002), and 11 years after amending (2012)

Taxon	Organic matter amendment									
	Scots pine sawdust-spread		composted bark				coarse coniferous woody debris-spread		the control	
			under roots		spread					
	2002	2012	2002	2012	2002	2012	2002	2012	2002	2012
					Armillaria antagonists					
Clonostachys candelabrum[1]	0	0	0	0	0	0	45^{ab}	0^{a}	0^{b}	0
Penicillium janczewskii[1]	6^{acd}	19^{ajkl}	12^{bef}	1^{bjmn}	12^{gh}	$11^{m\circ p}$	30^{cegi}	$27^{kn\circ r}$	0^{dfhi}	2^{lpr}
Total	6^{ad}	19^{aij}	12^{be}	1^{bik}	12^{fg}	11^{l}	75^{cdefh}	27^{cklm}	0^{gh}	2^{jm}
					Armillaria stimulants					
Mortierella macrocystis[1]	0	0	6	0	0	0	0	0	0	0
P. adametzii[1]	0	0	6	0	0	0	0	0	0	0
P. citrinum[1]	0^{a}	0	0	0	0	0	0	0	12^{a}	0
P. spinulosum[1]	0	0	0	0	0	0	0	6	0	0
Pseudogymnoascus roseus[1]	6^{a}	0^{b}	0	0	0	3	0	0	15^{a}	7^{b}
Trichocladium opacum[1]	12^{a}	0^{a}	0	0	0	0	0	0	6	0
Total	18^{ade}	0^{af}	12^{bd}	0^{b}	0^{e}	3	0^{f}	6	33^{ce}	7^{cf}
					Heterobasidium annosum antagonists					
Geomyces pannorum[1]	0	1	0	0	0	0	0	0	0	0
P. adametzii[1]	0	0	6	0	0	0	0	0	0	0
P. commune[2]	0^{a}	30^{aefg}	0^{b}	40^{bhij}	0^{c}	120^{cehkl}	0^{d}	56^{dfikm}	0	3^{gjlm}
P. daleae[1]	0^{cd}	0	9^{ace}	0^{a}	6^{f}	0	51^{bdefg}	3^{b}	0^{g}	0
P. janczewskii[2]	6^{ac}	19^{aij}	12^{bde}	1^{biklm}	12^{fg}	$11^{kn\circ}$	30^{cdfh}	27^{lmnp}	0^{egh}	$2^{j\circ p}$
Trichoderma spp.[1,2,3]	15^{ab}	1^{a}	9^{cd}	2	21^{bcef}	0^{b}	63^{cbdeg}	3^{c}	6^{fg}	1
Total	21^{adefg}	$50^{an\circ pr}$	36^{dhij}	43^{stu}	39^{bekln}	$131^{bh\circ svw}$	144^{cfkm}	89^{ciptvz}	6^{glm}	6^{jruwz}

Explanations – see table 2

[1]according to Kwaśna 1995, 1997a, b, c, 2001, 2002; Kwaśna *et al*. 2001; Szwajkowska-Michałek *et al.* 2012

[2]according to Nona *et al.* 1968; Werner and Zadworny 2002

[3]according to Nikolajeva *et al.* 2012

Discussion

The application of organic substrates to soil usually results in short- or long-term shifts in microbial populations. If such population changes are directed and stimulated, the outcome may be an enhanced biological control of soil-borne pathogens.

Natural, organic substrates applied in forestry usually include sawdust, composted bark or chipped post-harvest woody debris. These have been previously applied with good biological control effects. In forest soils, coniferous sawdust effectively increased the density of *Trichoderma harzianum* Rifai, a potential antagonist of *Armillaria* spp. and *H. annosum* (Sierota 1995, 2013; Sierota and Kwaśna 1999; Kwaśna *et al.* 2000; Nikolajeva *et al*. 2012). Incorporation of sawdust into the soil or sawdust with *Trichoderma* into the soil, increased the survival of Scots pine seedlings affected by damping-off fungi; the effect was comparable to protection with chemical fungicides (Duda and Sierota 1987). Composted tree bark suppresses many soil-borne pathogens, eliminating the need to sterilise the media, and reducing fungicide applications. Composted tree bark has been most widely adopted for commercial, containerised production of nursery stock, particularly of ornamental plants and trees (Hoitink *et al.* 1975, 1976). Chipped post-harvest coniferous woody debris left on the soil surface or mixed with the topsoil had a long-term suppressive effect towards *Armillaria* and *Heterobasidion* on a Scots pine planted acreage (Kwaśna *et al.* 2014).

The abundance and diversity of fungi increased 11 years after organic matter amendment was used. These results are consistent with Werner *et al.* (2001) who observed that fungi were more abundant in arable soils richer in nutrients. However, the extent of the response of the resident fungi to cellulose inputs is currently unknown (Eichorst and Kusk 2012). The induction and production of cellulase by fungi, particularly in natural conditions, are not completely understood (Dashtban *et al*. 2011; Nayebyazdi *et al*. 2012). The abundance and diversity of cellulose-responsive fungi is generally unique to a certain soil type, suggesting a strong potential influence of multiple edaphic factors.

In the present study, total abundances of fungi were only weakly correlated (mainly because of low abundance in the control in 2012) with organic carbon and total nitrogen contents ($r = 0.57$ and 0.56, $p < 0.0001$), and negatively, with extractable potassium and sodium

(r = –0.49 and –0.28, p < 0.0001). The increased abundances of the common taxa were mostly not correlated with soil nutrient contents (i.e. *Acremonium* + *Sagenomella, Oidiodendron, Penicillium* + *Talaromyces*) or moderately or strongly negatively correlated with organic carbon, total nitrogen, extractable phosphorus, potassium, magnesium, and calcium contents (i.e. *Clonostachys* + *Trichoderma* spp., r = –0.53 – 0.97, p < 0.0001). The response of *Trichoderma* can be attributed to the negative effect of carbon and nitrogen on sporulation of the fungus (Rajput *et al.* 2014). Considering the above-mentioned relationships, the resulting positive correlation of the total abundances of fungi to soil nutrient content seemed to be due to the positive correlation of a large number of infrequent species recorded.

The seasonal abundances and diversity of fungi seemed to result from the degradation rate of the incorporated substrate and the succession due to specific degrading abilities. Many recorded fungi were soft-rot fungi, with cellulolytic activity and cellulase production (Stursová *et al.* 2012). Others, however, i.e. *Acremonium* + *Sagenomella, Oidiodendron* and *Penicillium* + *Talaromyces* spp. mostly utilise starch and pectin, which are released after earlier decomposition of cellulose (Bååth and Söderström 1980; Deacon *et al.* 2006), and they appear in the later phase of degradation. They appear after an absence of cellulose causes the elimination of *Clonostachys* + *Trichoderma* spp. which are common decomposers of cellulose and strong antagonists of other fungi; Deacon *et al*. 2006). The very frequent appearance of *Oidiodendron* in the soil, 11 years after the treatments, could have resulted, not only from its preferences for woody substrates (Nilsson 1973; Domsch *et al.* 1980; Dalpé 1991) but also from its ability to form ericoid mycorrhizas with *Calluna* and *Vaccinium* species (Couture *et al.* 1983), which were present on the site.

Classification of fungi into eudominants, dominants, subdominants, recedents, and subrecedents helps to show the scale of the functional potential of individual species. The relatively small numbers of frequent species and larger numbers of infrequent species in fungal communities agree with Rubino and McCarthy (2003), and Richard *et al.* (2004). The less frequent species were particularly common 11 years after treatments. They are particularly relevant for decomposition processes and ecosystem functioning. The less frequent species are often more active and efficient decomposers than those occurring more frequently (Deacon *et al.* 2006).

The most frequent species of *Penicillium (*i.e., *P. commune*, *P. daleae*, *P. janczewskii, P. lilacinum, P. steckii,* and *P. vinaceum*) are common in Polish forest soils and may not occur elsewhere in the world (Kwaśna and Nirenberg 1994; Grantina-Ievina *et al.* 2013).

The behaviour of the fungal communities (abundance of single species and diversity) was similar among all four treatments. These findings may indicate that the degradation of sawdust, composted bark, and even coarse coniferous woody debris proceeds at the same rate and that similar physico-chemical and biological effects can be expected after their application.

The fungal communities contained a few species known to be antagonists of *Armillaria* or *H. annosum*, i.e. *C. candelabrum, G. pannorum*, *P. adametzii*, *P. commune*, *P. daleae*, *P. janczewskii*, and *Trichoderma* spp., or stimulants of *Armillaria*, i.e. *M. macrocystis*, *P. adametzii*, *P. citrinum*, *P. spinulosum*, *P. roseus*, and *T. opacum* (Table 6). Mycelium and/or metabolites of the antagonists are known to: (i) inhibit growth of *Armillaria* and *Heterobasidion* in paired cultures *in vitro*; (ii) inhibit growth and ramification of *Armillaria* rhizomorphs *in vivo*; (iii) decrease the extent of root rot caused by *Armillaria* and decrease the extent of necrosis caused by *H. annosum* on *P. sylvestris* seedlings and other plants; (iv) stimulate growth of Scots pine seedlings inoculated with *Armillaria*; (v) effectively colonise the thin (< 5 mm diam) and thick (> 5 mm diam.) living roots and rhizosphere of younger and older coniferous and broadleaved trees and their stumps, which helped in competition with pathogens (Kwaśna 1995, 1997a, b, c, 2001, 2002; Kwaśna *et al.* 2001; Werner and Zadworny 2002; Nikolajeva *et al*. 2012; Szwajkowska-Michałek *et al*. 2012).

Abundance of species that are entomopathogenic [*M. anisopliae* (Metschn.) Sorokīn, *P. carneus* (Duché & R. Heim) A.H.S. Br. & G. Sm.] or nematophagous [*H. sphaerosporum* (Goodey) Zare & W. Gams] often increased after treatment and was weakly positively correlated with organic carbon and extractable calcium contents (r = 0.70 and 0.68, p < 0.0001), and negatively correlated with extractable potassium content (r = –0.82, p < 0.0001) (Table 3). These fungi may help control insects or nematodes in forest soil (Watanabe 1980; Inglis and Tigano 2006).

Since *Armillaria* and *Heterobasidion* continuously infect trees over a long period, useful antagonists are required to have long persistence in the ecosystem without the addition of nutrients or other amendments. Only one *H. annosum* antagonist: *P. commune*, was found to have an increased population 11 years after all soil amendment treatments. This fungus was previously found to occur at a low frequency in barren and abandoned farmland soils 1–2 years after amendment with Scots pine sawdust (Sierota and Kwaśna 1988, 1998). In the present study, *P. commune* became one of the eudominants 11 years after treatment, which theoretically suggests a long-lived functional potential. It should be mentioned, though, that only one of two tested isolates of *P. commune* was found to be antagonistic to *H. annosum in vitro* (Werner and Zadworny 2002), indicating functional diversity in its activity. Functional diversity of fungi had been observed earlier (van der Heijden *et al.* 1998; Szwajkowska-Michałek *et al*. 2012). Intra-specific variation in function in different conditions, can be high. Some organisms, e.g. *Scytalidium lignicola* Pesante, have remarkable antagonistic activity towards *Heterobasidion* only *in vitro*, while others, e.g. *Resinicium bicolor* (Alb. & Schwein.) Parmasto, have activity only on/in wood (Highley 1990, 1994). So far, similar antagonistic activity towards *H. annosum in vitro* and *in vivo* has been observed in *P. adametzii* (Szwajkowska-Michałek *et al.* 2012).

Conclusion

Spreading Scots pine sawdust, composted bark or coarse, post-harvest coniferous woody debris on the soil surface of barren forest land increased soil nutrients and

the abundance and diversity of culturable fungi in the long term (after 11 years). The increased abundance of only two known antagonists of *Armillaria* and *Heterobasidion* (particularly where sawdust or composted bark was spread on the soil surface) does not guarantee the effective long-term suppressiveness of soil. The increased abundance of entomopathogenic and nematophagous species does suggest the possibility of long-term control of insects and nematodes.

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