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Effects of sawdust amendment on forest soil fungal community and infestation by cockchafers

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Abstract: Effects of Scots pine wood amendment on the fungal community in forest soil infested with cockchafers and foraged or non-foraged by wild boars and hares were investigated. We hypothesized that sawdust amendment would increase the abundance of entomopathogenic and insect-associated species effective in cockchafer predation. The soil dilution method and morphotyping were used for fungal isolation and identification in order to quantify and qualify the viable components of the microbiota that are important for evaluating soil functions. There was usually increased abundance and decreased diversity of soil fungi one year after sawdust amendment. Application of pine sawdust more often increased than decreased the abundance of some insect-associated fungi or dermatophytes and keratinophilic species and decreased the number of cockchafer larvae. Abundance of *Geomyces pannorum*, *Mortierella* spp. + *M. luteus, Pseudogymnoascus roseus, Tolypocladium inflatum* and *Trichoderma koningii* increased, at least locally, whilst *Chrysosporium merdarium, Penicillium* spp. (including the most common *P. citrinum, P. daleae* and *P. janczewskii*), *Sporothrix schenckii* and *Trichoderma* spp. decreased. Application of pine sawdust under roots of 1-year-old Scots pine seedlings significantly increased the abundance of *Phoma* + *Pyrenochaeta* spp. in neighbouring soil, thus increasing the risk from *Phoma* blight. *Trichoderma strigosum* was among the dominants (frequency >5%). Another six and two *Trichoderma* species were among the subdominants (frequency 1–5%) and subrecedents (frequency <1%), respectively. Dermatophytes, coprophilous and keratinophilic species, e.g. *Cephalotrichum, Chrysosporium merdarium* or *S. schenckii*, occurred only or mostly in foraged plots. The altered communities resulting from pine sawdust amendment may contribute to biological control of the cockchafer larvae.

Keywords: cockchafers, forest soil, fungal communities, sawdust amendment

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Introduction

Cockchafers are European beetles of the genus *Melolontha*. In the last 20 years the common cockchafer (*Melolontha melolontha* L.) and the forest cockchafer (*M. hippocastani* Fabr.) were found to occur in central Europe on more than 200 000 hectares, causing considerable losses in plant and wood pro-

duction (Keller & Brennen, 2005; Keller & Zimmermann, 2005; Malinowski, 2007; Oltean et al., 2010; Svestka, 2010).

In Poland, in 1995–2012, progressive increases in *M. melolontha* and *M. hippocastani* were reported in 15–120 000 ha of forests; particularly in central-eastern Poland. Severe damage in nurseries and plantations was observed in 175 forest districts on 31 896 ha (Woreta, 2013). Restocking the infested areas by fill-planting or under-planting does not improve the condition of the forests.

Adult cockchafers injure the leaves and flowers of many deciduous trees, shrubs and herbaceous plants, but rarely cause any serious damage. However, their fat, white larvae, 40–45 mm long, feed on plant roots and this results in stunted growth, wilting, necrosis and premature shedding of leaves. A density of 5–40 beetles per m^2 may cause up to 25% plant damage. Entire forest stretches may be defoliated. Cockchafers often occur on pedunculate oak (*Quercus robur* L.) and sessile oak (*Q. petraea* (Mattuschka) Liebl.), less often on leaves of *Aesculus*, *Acer*, *Betula, Carpinus*, *Fagus, Populus, Salix* and *Sorbus* and on needles of *Larix,* and occasionally on flowers of *Abies, Picea* and *Pinus* (Luterek & Szmidt, 1997).

The cockchafer usually has a 3-year life cycle. After mating, the female lays 10–20 eggs in soil at 20 cm depth. The eggs hatch after 21 days and the larvae remain in the soil for two years before changing into adult beetles. The young larvae consume humus and small tender roots, mainly of grasses. The older larvae injure the roots of seedlings and young trees (Blum, 1985).

The current expansion of cockchafers may be a result of increased temperature (with global warming) and intensive forest management, i.e. deforestation over larger continuous areas (Niemczyk & Neyko, 2009).

Until recently, cockchafer larvae were controlled directly by the use of soil-applied insecticides containing carbofuran, carbosulfan, chloropyrifos or diazinon. After recent EC regulations prohibited the use of chemicals in forest nurseries (EC No 1107/2009 of the European Parliament) the recommendations for control of cockchafers include: (i) stimulation of growth of healthy and resistant plants by appropriate field tillage (summer ploughing of the soil, causing mechanical damage of larvae and their exposure to predation by birds); (ii) the use of high quality seeding and planting material for reforestation; (iii) correct choice of tree and bush grades; (iv) adequate watering and fertilizing; (v) annual or bi-annual fallowing; (vi) weed control; (vii) catching beetles with light traps (Woreta, 2015). Mechanical control of cockchafers is difficult and fallowing impossible in forests, where the alternative option is a more direct form of biological control.

Some entomopathogenic fungi, e.g. *Beauveria bassiana* (Bals.-Criv.) Vuill., *B. brongniartii* (Sacc.) Petch, *Metarhizium anisopliae* (Metschn.) Sorokīn and *Tolypocladium* species, entomopathogenic bacteria, e.g. *Bacillus thuringiensis* and *Coccobacillus* sp., microsporidia, e.g. *Pleistophora melolonthae* H. and *Telohania* sp., and nematodes may occur on all developmental stages of cockchafers, but especially on larvae and pupae (Shapiro-Ilan et al., 2002; Enkerli et al., 2004; Laengle et al., 2005; Lakatos & Tóth, 2006; Łabanowska & Bednarek, 2011).

The list of other insect-pathogenic or insect-associated fungi includes species of *Absidia, Acremonium, Aspergillus Chaetomium, Cladosporium, Clonostachys, Fusarium, Geomyces, Lecanicillium, Mortierella, Mucor, Nomuraea, Paecilomyces, Penicillium, Phialophora, Pseudogymnoascus, Rhizopus, Talaromyces*, *Trichoderma* and *Williopsis* (Visser et al., 1987; Gunde-Cimerman et al., 1998; Ali-Shtayeh et al., 2002; Deshmukh, 2002; Toledo et al., 2006; Domsch et al., 2007; Sun & Liu, 2008; Sun et al., 2008). Sun and Liu (2008) recorded 46 species from 27 genera. Insect-associated fungi may be opportunistic pathogens, which occur on predisposed insects causing epizootics, or secondary colonizers which come next in fungal successions (Thomas et al. 2003; Sun & Liu, 2008).

Fungal epizootics in soil insect populations are well documented and illustrated (Samson et al., 1988; Keller & Zimmerman, 1989; Klingen & Haukeland, 2006). Mechanisms of successful fungal pathogenesis include contact of conidia with the cuticle of the host insect, germination of conidia, growth of hyphae and production and transfer of enzymes and mycotoxins that suppress the host's immune system (e.g. beauverin, destruxins and efrapeptins produced, respectively, by *B. bassiana, M. anisopliae* and *Tolypocladium*) (Huxham et al., 1989; Hajek & St. Leger, 1994; Mazet et al., 1994; Clarkson & Charnley, 1996; Vilcinskas et al., 1997; Bandani et al., 2000; Hummel et al., 2002; Zimmermann, 2007).

Effective products based on *B. brongniartii* have been approved for use and are marketed in several European countries (including Austria, Italy and Switzerland). However, none of the strains currently used commercially is effective against larvae of *M. melolontha* and *M. hippocastani*. None of the commercial products has yet been approved for use in Poland.

The aim of the research was to study abundance, diversity, spatial and temporal distribution and effects of fungi, including insect-associated and dung-associated species, in forest soils infested with cockchafers, foraged by wild boars and hares and amended with Scots pine sawdust.

The sawdust application was intended, by causing changes in the physical and nutritional or other chemical properties of soil, to initiate and stimulate microbiological changes, which may contribute to bi-

ological control of cockchafers. The hypothesis that sawdust amendment would increase the density of insect-associated fungi, including species pathogenic to cockchafers, results from their cellulolytic and xylanolytic abilities and preferences for wood (Bääth & Soderström, 1980; Svarstad et al., 2000; Domsch et al., 2007). Positive effects of pine sawdust as a stimulant of changes in soil microbiology, and its biochemical and phytopathological consequences, have been reported previously (Kwaśna et al., 2000, 2001; Grenni et al., 2012). Pine sawdust as an amendment to fallow soil stimulated the growth of some antagonists of the forest pathogens *Armillaria* and *Heterobasidion* spp., and increased the population of nematodes, including predatory species.

Material and methods

Site description

The studies were carried out in two clear-felled locations (LA and LB) in Lubartów Forest District (south-east Poland, 22°38'E, 51°28'N). The soil of both locations was seriously infested with the common cockchafer (*M. melolontha*). The LA location (division 201c) was not fenced and was foraged by wild boars (*Sus scrofa* L.) and European hare (*Lepus europaeus* Pallas). The area LB (division 159a) was fenced and was non-foraged. In May 2012, in each location, a 640 m^2 area was divided into four blocks of 160 m^2. Each block was sub-divided into three linear plots (40 m × 1.2 m = 48 m^2): T1 plot, T2 plot and control plot. In T1 plots, 7.5 dm^3 of fresh Scots pine sawdust was spread on the surfaces of 1 m × 0.4 m areas and mixed into the topsoil to a depth of 20 cm by rotary cultivator. One-year-old *Pinus sylvestris* L. seedlings were planted in rows with 1.2 m × 0.6 m spacing immediately after treatment. In T2 plots, 0.3 dm^3 of fresh Scots pine sawdust was placed under the roots of each of the 1-year-old *P. sylvestris* seedlings while planting. In control plots there was no sawdust amendment and Scots pine seedlings were planted into non-treated soil.

The soil was sandy-loam (11.8% clay, 13.6% silt, 74.5% sand) with characteristics given in Table 1. Weather conditions are presented in Table 2. In 2012 there was a dry spring, hot summer and wet autumn, and in 2013 a severe winter (–19.2°C above ground in January).

Collection of soil samples

In May 2012 (before treatment) and in May 2013 (12 months after treatment), six 7.5-cm-diam. sub-samples (cores) of non-rhizosphere soil were collected from the A–B horizon (0–20 cm deep) (1) between two rows of pine seedlings in treatment T1, (2) under seedlings in treatment T2, and (3) between two rows of seedlings in the control, in each of the

Table 1. Characteristics of soils at Lubartów before treatment (May 2012) and after treatment (May 2013)

Date	Treatment	pH in H_2O	pH in KCl[1]	C (%)[2]	N (%)[3]	C:N	P (mg 100 g^{-1})[4]	K (mg 100 g^{-1})[5]	Ca (mg 100 g^{-1})[5]	Mg (mg 100 g^{-1})[5]
Foraged by wild boar and hares (LA)										
May 2012	–	4.91b	3.85b	2.31d	0.16a	14.25d	2.46c	7.24c	19.77c	2.93c
May 2013	T1	4.70a	3.70a	2.11c	0.15a	14.10c	1.23a	4.04b	17.0b	2.22b
	T2	5.00b	4.00c	1.36a	0.10a	13.20a	1.69b	3.76a	12.0a	1.53a
	Control	4.84ab	4.80d	1.64b	0.12a	13.30b	5.36d	14.69d	50.0d	5.79d
	F	47.64	2406.25	2331.36	7.58	2906.25	34377.67	289015.58	2949915.58	35010.25
	P	0.0014	0.0000	0.0000	0.0398	0.0000	0.0000	0.0000	0.0000	0.0000
Non-foraged by wild boar and hares (LB)										
May 2012	–	4.68a	3.8a	1.28b	0.09a	14.56b	1.03bc	4.09d	9.1c	1.34c
May 2013	T1	5.00c	3.8a	1.43c	0.10a	14.70c	1.11c	3.60c	10.0d	1.31c
	T2	4.70a	3.9b	1.10a	0.08a	13.80a	0.93a	2.56b	5.0a	0.64a
	Control	4.80b	3.8a	1.29b	0.09a	13.80a	0.95ab	2.28a	8.0b	0.78b
	F	214.33	25.00	183.00	0.96	2329.00	67.67	7299.58	15920.98	1245.58
	P	0.0001	0.0047	0.0001	0.4941	0.0000	0.0007	0.0000	0.0000	0.0000

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
a,b,c – different letters indicate statistically significant difference according to one-way ANOVA at $P \leq 0.01$.
T1 – sawdust mixed into topsoil; T2 – sawdust placed under roots; Control – no sawdust.

Table 2. Temperature and rainfall at Lubartów in 2012 and 2013 (until May)

	2012	2013
Air temperature (°C)	minimal –7.5 (February), maximal 20.8 (July)	minimal –4.1 (January), maximal 14.8 (May)
Minimal temperature of air above ground (°C)	–23.9 (February)	–19.2 (January)
Minimal temperature of topsoil at the depth 5 cm (°C)	–7.4 (February)	–2.2 (January)
Rainfall (mm)	minimal 19.3 (February), maximal 87.1 (October)	minimal 34.0 (February), maximal 105.6 (May)

four randomized blocks. The sub-samples from each 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: 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 and 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}, $MgSO_4.7H_2O$ 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 per 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), synthetic nutrient agar (SNA; KH_2PO_4 1 g l^{-1}, KNO_3 1 g l^{-1}, $MgSO_4.7H_2O$ 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}), 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 according to Barron and Peterson (1968), Pitt (1979), Klich and Pitt (1992) and Domsch et al. (2007).

Abundance of fungi was defined as the number of colony forming units (*cfu*) in a sample. Diversity was defined as the number of species in a sample. A species, or group of related species of fungi was considered as: (i) dominant, with frequency >5%, (ii) subdominant, with frequency 1–5%, or (iii) subrecedent, with frequency <1%, at least in one treatment (Tischler, 1949).

Collection of cockchafer larvae

Soil samples were collected before treatment (October 2011) and 5 and 12 months after treatment, in October 2012 (during pupation) and in May 2013 (when adults appeared). Small number of cockchafers collected in 2012 necessitated an increase in sampling locations in 2013 when samples were taken (1) between two rows, (2) under seedlings and (3) between seedlings, in the row. In each combination six samples located 20 m apart were analysed. Each consisted of 0.15 m^3 of topsoil taken from the A–B horizon (0–30 cm deep) in an area 1 m × 0.4 m. Soil was sieved with a 5-mm-diam. sieve and the cockchafer larvae were counted on its surface. Mean values were calculated.

Assessment of *Pinus sylvestris* seedling mortality

One year after treatment (May 2013) all *P. sylvestris* seedlings were assessed for the presence of disease symptoms or mechanical injuries. Dead or dying seedlings, often with grub injuries on roots, were counted. Mortality was defined as the proportion of dead seedlings in the total number of seedlings planted.

Statistics

Differences in soil chemical properties, numbers of cockchafer larvae and numbers of dead *P. sylvestris* seedlings at the two locations after different treatments were analysed using one-way analysis of variance (ANOVA), with treatment or location as the variable, and the two sites being analysed separately, using Statgraphics™ Centurion (Statpoint Technologies, Inc. Warrenton, VA, USA). Abundances and diversity of fungi from two different treatments were analysed by χ^2 – test.

Diversity within and between fungal 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 by Margalef's index (DMg) and by Shannon's diversity index (H'). Evenness and dominance were indicated by Shannon's evenness index (E), Simpson's index (D) and Berger–Parker's index (d). The similarity between fungal communities in two systems was determined by calculating the qualitative Sorensen's similarity index (CN).

Relationships between (1) C (%), N (%), P (mg 100 g^{-1}), K (mg 100 g^{-1}), Ca (mg 100 g^{-1}) or Mg (mg

Table 3. Abundance (number of colony forming units per treatment) of fungal taxa recorded in Lubartów soils

No.	Taxon	Foraged by wild boars and hares				Non-foraged by wild boars and hares			
		May 2012	May 2013			May 2012	May 2013		
		–	T1	T2	Control	–	T1	T2	Control
		Dominants (with frequency >5%)							
	Zygomycota								
1.	*Mortierella parvispora* Linnem. + *M. verticillata* Linnem. + *Mortierella* sp. + *Mucor luteus* Linnem. ex Wrzosek	2	1a	2	6b	0a	28b	19c	7d
	Ascomycota								
2.	*Cephalotrichum nanum* (Ehrenb.) S. Hughes + *C. stemonitis* (Pers.) Nees	0a	3b	103c	36d	0	0	0	0
3.	*Chrysosporium merdarium* (Ehrenb.) J.W. Carmich	30a	5b	4c	102d	7a	10b	0c	64d
4.	*Geomyces pannorum* (Link) Sigler & J.W. Carmich.	34a	227b	191c	41a	60a	244b	42c	60ac
5.	*Penicillium aculeatum* Raper & Fennell + *P. chrysogenum* Thom + *P. citreonigrum* Dierckx + *P. citrinum* Thom + *P. commune* Thom + *P. corylophilum* Dierckx + *P. daleae* Zaleski + *P. expansum* Link + *P. granulatum* Bainier + *P. janczewskii* Zaleski + *P. janthinellum* Biourge + *P. raistrickii* G. Sm. + *P. spinulosum* Thom + *P. waksmanii* Zaleski + *Talaromyces islandicus* (Sopp) Samson, Yilmaz, Frisvad & Seifert + *T. pinophilus* (Hedgc.) Samson, Yilmaz, Frisvad & Seifert + *T. purpurogenus* (Stoll) Samson, Yilmaz, Frisvad & Seifert + *T. verruculosus* (Peyronel) Samson, Yilmaz, Frisvad & Seifert	286a	11b	91c	97c	126a	49b	99c	188d
6.	*Phoma eupyrena* Sacc. + *P. leveillei* Boerema & G.J. Bollen + *Pyrenochaeta unguis-hominis* Punith. & M.P. English	0a	1a	14b	4a	0a	0a	10b	0a
7.	*Pseudogymnoascus roseus* Raillo	0a	1251b	455c	74d	4a	402b	131c	200d
8.	*Sporothrix schenckii* Hektoen & C.F. Perkins	15a	2b	16c	118d	8a	2ac	12bd	2ae
9.	*Trichoderma atroviride* P. Karst. + *T. citrinoviride* Bissett + *T. harzianum* Rifai +*T. koningii* Oudem. + *T. longipilis* Bissett + *T. polysporum* (Link) Rifai + *T. pubescens* Bissett +*T. strigosum* Bissett + *T. viride* Pers.	55a	0b	51c	15d	59a	17b	14c	36d
		Subdominant (with frequency 1–5%)							
10.	*Candida albicans* (C.P. Robin) Berkhout	0	1	0	8	0	3	1	0
11.	*Chaetosphaeria vermicularioides* (Sacc. & Roum.) W. Gams & Hol.-Jech.	0	0	0	0	0	0	0	10
12.	*Cladosporium cladosporioides* (Fresen.) G.A. de Vries	0a	1a	0a	18b	0	0	0	1
13.	*Clonostachys candelabrum* (Bonord.) Schroers	6	0	0	0	3	0	0	0
14.	*Exophiala jeanselmei* (Langeron) McGinnis & A.A. Padhye	0	0	7	0	0	0	5	0
15.	*Hormiactis candida* Höhn.	0	2	5	0	0a	17b	0a	11b
16.	*Humicola grisea* Traaen	1	0	0	7	0	0	0	1
17.	*Leptosphaeria coniothyrium* (Fuckel) Sacc.	0	0	0	0	5	0	0	0
18.	*Myrmecridium schulzeri* (Sacc.) Arzanlou, W. Gams & Crous	0	0	2	1	0	0	5	0
20.	*Oidiodendron echinulatum* G.L. Barron + *O. chlamydosporicum* Morrall + *O. griseum* Robak + *O. tenuissimum* (Peck) S. Hughes	2	0	0	6	4	2	4	0
21.	*Paraconiothyrium fuckelii* (Sacc.) Verkley & Gruyter	2	0	0	1	6	1	9	15
22.	*Tolypocladium inflatum* W. Gams	0	1	0	0	3	10a	0	0b
23.	*Truncatella truncata* (Lév.) Steyaert	2	0	0	0	4	0	0	0
	Total abundance (including subrecedents) – number of colony forming units (*cfu*) in a sample	441a	1512b	969c	556d	295a	787b	364c	604d
	Diversity – number of species in a sample	25	19a	30	38b	26	22	29	27

Subrecedents (with frequency <1%) included *Aphanocladium album* (Preuss) W. Gams, *Aspergillus kanagawaensis* Nehira, *A. repens* (Corda) Sacc., *A. tardus* Bissett & Widden, *Aspergillus* sp., *Beauveria bassiana* (Bals.-Criv.) Vuill., *Chaetomium globosum* Kunze, *Cylindrocarpon didymum* (Harting) Wollenw., *C. obtusisporum* (Cooke & Harkn.) Wollenw., *Davidiella macrocarpa* Crous, K. Schub. & U. Braun, *Dicoccum asperum* (Corda) Sacc., *Epicoccum nigrum* Link, *Fusicolla merismoides* (Corda) Gräfenhan, Seifert & Schroers, *Humicola fuscoatra* Traaen, *Mammaria echinobotryoides* Ces., *Metarhizium anisopliae* (Metschn.) Sorokīn, *Myceliophthora thermophila* (Apinis) Oorschot, *Paecilomyces carneus* (Duché & R. Heim) A.H.S. Br. & G. Sm., *Papulaspora nishigaharanus* Ts. Watan, *Pochonia bulbillosa* (W. Gams & Malla) Zare & W. Gams, *Sarocladium kiliense* (Grütz) Summerb., *Scolecobasidium constrictum* E.V. Abbott, *Trichocladium opacum* (Corda) S. Hughes, *Verticillium chlamydosporium* Goddard, *Wardomyces humicola* Hennebert & G.L. Barron.

a,b,c,d – different letters indicate statistically significant difference according to χ^2 test at $P \leq 0.001$or $P \leq 0.05$.

T1 – sawdust mixed into topsoil; T2 – sawdust placed under roots; Control – no sawdust.

100 g^{-1}) content in foraged or non-foraged soil and total abundance (number) of fungi, abundance of the frequent taxa, i.e. *P. roseus*, *Penicillium* and *Trichoderma*, number of larvae or number of dead pine seedlings in May 2013 and (2) abundance of fungi and number of larvae were estimated with Pearson's correlation coefficient. There were 73 tests in total.

Results

Changes in chemical properties of soils after sawdust amendment

Application of sawdust amendment changed the chemical properties of forest soils. Spreading sawdust on the surface of the soil and mixing it with the topsoil (T1) resulted in: (1) increase in C and N content and C:N ratio, and decrease in pH and P, K, Ca and Mg content in foraged soil; (2) increase in pH and in C, N, P, K, Ca and Mg content and C:N ratio in non-foraged soil (Table 1). Placing sawdust under roots of seedlings (T2) resulted in: (1) change in pH; (2) decrease in C, N, P, Ca and Mg content in foraged and non-foraged soils.

Fungal community structure

A total of 81 fungal species was recorded in non-rhizosphere soil, in two locations (foraged-LA, and non-foraged-LB), before and after amendment with Scots pine sawdust. Forty-one species occurred at both locations. Twenty-six species occurred only in LA soil and 14 species occurred only in LB soil. There were nine dominant species or groups of related species, 13 subdominant and 25 subrecedent species (Table 3). There was a significantly greater total abundance of fungi (in LA and partially in LB plots) and decreased diversity of fungi (in LA, T1 plot) one year after sawdust amendment.

Two coprophilous and keratinophilic species (*Cephalotrichum nanum* and *C. stemonitis*), three entomopathogenic species (*B. bassiana*, *M. anisopliae* and *Tolypocladium inflatum*) and some insect-associated species (*Aspergillus* spp., *Chaetomium globosum, Cladosporium cladosporoides, Geomyces pannorum, Mortierella* spp., *Mucor luteus, Paecilomyces carneus, Penicillium chrysogenum, P. citrinum, Pseudogymnoascus roseus* and *Trichoderma koningii*) were recorded (Petch, 1937; Visser et al., 1987; Marshall, 1998; Del Frate & Caretta, 1990; Deshmukh, 2002; Ali et al., 2013; Domsch et al., 2007). *Beauveria brongniartii*, which is the most common and important natural enemy of *M. melolontha*, was not detected (Dolci et al., 2006).

Only *C. nanum* + *C. stemonitis, G. pannorum, Mortierella* spp. + *Mucor luteus*, *P. roseus* and *T. koningii* were among the dominants (frequency >5%). *Tolypocladium inflatum* was subdominant (frequency 1–5%) and *B. bassiana*, *M. anisopliae* and *P. carneus* were subrecedent (frequency <1%).

Pine sawdust amendment usually increased significantly the abundance of *G. pannorum* and *P. roseus*, and decreased the abundance of *Ch. merdarium* and *Penicilium* spp. In foraged plots, pine sawdust amendment significantly increased the abundance of *Cephalotrichum* spp. and *Trichoderma* spp. (in T2) and decreased the abundance of *S. schenckii*. In non-foraged plots, sawdust amendment significantly increased the abundance of *Mortierella* spp. and decreased the abundance of *Trichoderma*. Application of pine sawdust under roots of 1-year-old Scots pine seedlings significantly increased the abundance of three *Phoma* and *Pyrenochaeta* species. Foraging by wild boars and hares locally increased the abundance of *C. nanum* + *C. stemonitis*, *Chrysosporium merdarium* and *Sporothrix schenckii*.

The most abundant *Penicillium* and *Trichoderma* species were *P. citrinum*, *P. daleae, P. janczewskii* and *T. strigosum*. *Cephalotrichum nanum* was more abundant than *C. stemonitis*.

Table 4. Diversity indices for fungi from soil foraged and non-foraged by wild boars and hares

Index	Foraged (LA)				Non-foraged (LB)			
	May 2012 –	May 2013 T1	May 2013 T2	May 2013 Control	May 2012 –	May 2013 T1	May 2013 T2	May 2013 Control
	Species richness indices							
Margalef's index (DMg)	3.94	2.45	4.21	5.85	4.39	3.14	4.74	4.06
Shannon's diversity index (H')	2.25	0.57	1.83	2.54	2.44	1.35	2.25	2.07
	Evenness or dominance indices							
Shannon's evenness index (E)	0.70	0.19	0.54	0.69	0.75	0.43	0.66	0.62
Simpson's index (D)	0.16	0.71	0.27	0.28	0.14	0.36	0.18	0.17
Berger–Parker's index (d)	0.33	0.83	0.47	0.21	0.26	0.51	0.36	0.33
Sorensen qualitative similarity index (CN)	0.27	0.51	0.53		0.46	0.55	0.42	

T1 – sawdust mixed into topsoil; T2 – sawdust placed under roots; Control – no sawdust.

Table 5. Number of cockchafer larvae in soils at Lubartów in October 2011 (before treatment) and in October 2012 and May 2013 (after treatment)

Date	Treatment	Mean number of cockchafer larvae in 0.15 m³ of topsoil			
		Mean	Between rows	Under seedlings	Between seedlings, in the row
Foraged by wild boars and hares (LA)					
October 2011		16c			
October 2012		9ab			
May 2013	T1	4.1a	4.1a	4.1a	4.1a
	T2	9.0ab	8.4b	8.3b	10.4b
	Control	11.8bc	10.4c	20.9c	4.1a
	F	46.83	1036.33	7644.0	1323.0
	P	0.0004	0.0001	0.0000	0.0000
Non-foraged by wild boars and hares (LB)					
October 2011		35d			
October 2012		9a			
May 2013	T1	13.3ab	14.7b	20.9a	6.3a
	T2	20.1c	16.6c	31.3b	12.5b
	Control	17.4bc	10.4a	35.4c	6.3a
	F	341.53	1009.0	5587.0	1281.33
	P	0.0000	0.0001	0.0000	0.0000

Different letters indicate a statistically significant difference according to one-way ANOVA at $P \leq 0.01$.
T1 – sawdust mixed into topsoil; T2 – sawdust placed under roots; Control – no sawdust.

No correlation was observed between C, N, P, K, Ca or Mg content in soil and total abundance of fungi. There were positive correlations between C and N content and abundance of *P. roseus* ($r = 0.80$ and 0.78, $P \leq 0.001$), and weak negative correlations between C and N content and abundance of *Penicillium* spp. ($r = -0.65$ and -0.66, $P \leq 0.001$) and *Trichoderma* spp. ($r = -0.53$ and -0.55, $P \leq 0.001$).

Diversity of Fungi in microbial communities, measured as number of species, was mostly similar among treatments (Table 3). In the foraged locations there was significantly less diversity immediately after mixing sawdust with topsoil than in the control. The relatively small number of fungal taxa and the infrequent occurrence of many of these taxa resulted in relatively small diversity indices based on species richness (DMg) and proportional abundance of species (H', Table 4). Sawdust amendment, particularly when it was mixed with the topsoil, decreased species richness and species abundance. The dominance of single taxa in communities resulted in small values for Shannon's evenness index (E) and high values for dominance indices (D and d). Evenness tended to be least, and dominance most, immediately after mixing sawdust with topsoil (May 2013, T1). Sorensen's qualitative similarity index (CN), used for comparing fungal communities in two treatments, suggests greater similarity (1) between sawdust treatments (May 2013, T1, T2) and control (C) in foraged than in non-foraged soil, (2) before sawdust treatment (May 2012) and after treatment (May 2013) in non-foraged than in foraged soil.

Number of cockchafer larvae

Before treatment (October 2011) the mean numbers of cockchafer larvae in foraged locations and non-foraged locations were 16 and 35 (Table 5). Application of pine sawdust usually significantly decreased the number of cockchafer larvae in both locations. One year after treatment the number of cockchafer larvae decreased to 4.1 and 9.0 in foraged locations and to 13.3 and 20.1 in non-foraged locations. Only in non-foraged locations after application of sawdust under roots of seedlings was the mean number of cockchafer larvae higher than in the

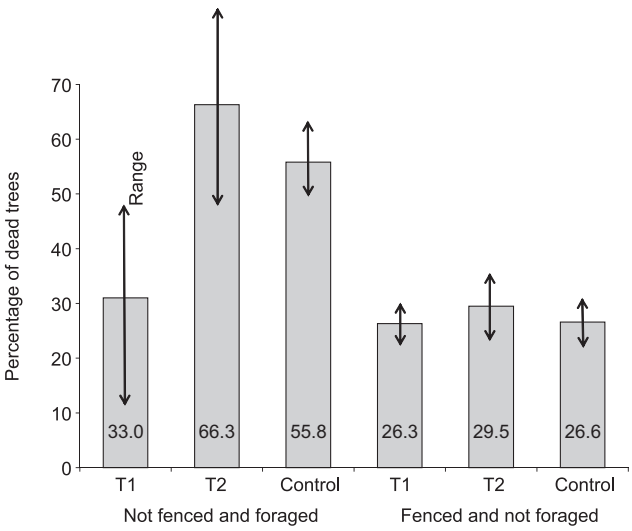

Fig. 1. Mortality (%) of Scots pine seedlings in soils at Lubartów that were foraged by wild boars and hares or not foraged and amended with pine sawdust mixed into topsoil (T1) or under seedling roots (T2)

control. Numbers of larvae were strongly negatively correlated with C and N content (r –0.88 and –0.90, $P<0.0001$) and total abundance of fungi (r –0.91, $P<0.0001$).

Pinus sylvestris seedling mortality

One year after treatment (May 2013), the proportion of dead or dying *P. sylvestris* seedlings in foraged and non-foraged locations was 31.0–66.3% and 26.3–29.5%, respectively (Fig. 1). Mortality of *P. sylvestris* seedlings on a single plot ranged from 11.1% to 81.7%. There was a significant effect of location (LA > LB, one-way ANOVA, F=5.62, P=0.013) but not of treatment (T1=T2=C, one-way ANOVA, F= 1.03, P= 0.378). There was a weak negative correlation between P, K, Ca and Mg content and number of dead seedlings (r = –0.50 to –0.59, $P<0.0001$).

Discussion

Conservation biological control is a biological control strategy in which farming practices and environmental manipulations are adopted to enhance the living conditions for specific natural enemies of pests.

Pine sawdust amendment was used to alter the functional diversity of microorganisms in topsoil of a Scots pine plantation infested with cockchafers. We hypothesized that pine-wood amendment of soil would stimulate the growth of entomopathogenic fungi effective in biological control of cockchafer larvae. In agriculture, such an effect, i.e. increased population of entomopathogenic fungi, resulting from biological inputs, has been observed in soils on organic farms (Hummel et al., 2002; Klingen et al., 2002; Jabbour & Barbercheck, 2009).

The topsoil (A–B horizon – 0–20 cm deep) was chosen for study because it is abundantly colonized by microorganisms, which find here nutrients present in root exudates, litter and amendment inputs, and because cockchafer larvae occur here.

The classical method, based on dilution plating and morphotyping, was chosen for evaluation of soil function based on abundance and known activity of viable components of the fungal communities. Profiling based on DNA sequences often tends to detect only the slow-growing, non-sporulating organisms whose activity is less recognized and understood and which have less obvious relevance in microbiological interactions (Kwaśna et al., 2008).

Application of pine sawdust (1) more often increased than decreased the total abundance of fungi as well as of some insect-associated taxa, dermatophytes and keratinophilic species, and (2) decreased the number of cockchafer larvae in soil. Abundance of *G. pannorum*, *Mortierella* spp. + *M. luteus, P. roseus, T. inflatum* and *T. koningii* increased, at least locally. These fungi are common soft-rot wood decomposers with strong cellulolytic and xylanolytic activity, often with preferences for pine wood (Bääth & Soderström, 1980; Blanchette et al., 1990; Eriksson et al., 1990; Rodriguez et al., 1996; Sierota & Kwaśna, 1988, 1998; Kwaśna et al., 2000; Svarstad et al., 2000; Lumley et al., 2001; Domsch et al., 2007). The greater abundance of *G. pannorum* and *P. roseus* in foraged plots suggests their preferences also for animal-associated debris.

Abundance of *Beauveria* spp., *M. anisopliae* or *P. carneus* did not increase after sawdust amendment, probably because of their preferences for proteins rather than carbohydrates, slow growth and poor competitiveness (Hajek & Lager, 1994; Hajek, 1997; Keller & Zimmermann, 1989; Sun et al., 2008; Jabbour & Barbercheck, 2009; Vega, 2009). The environmental conditions seemed to be irrelevant since they behaved similarly despite different habitat preferences (Fargues & Robert, 1985; Bidochka et al., 1998; Klingen et al., 2002; Keller et al., 2003).

A strong decrease in abundance of *Penicillium* was observed in both locations, particularly after spread of sawdust and mixing it into the topsoil. This effect is commonly observed after incorporating wood into soil (authors, unpublished). It may result from antibiosis by *Trichoderma* (Christensen, 1969). However, since *Trichoderma* itself was not very abundant or was decreasing it could have resulted from successful competition from basidiomycetous wood-rot fungi, which can easily colonize the small particles of pine sawdust. Basidiomycota were not recorded with the dilution plate method used here because of their association with organic matter particles that are eliminated in the isolation procedure.

Coprophilous, dermatophytic and keratinophilic species, i.e. *Cephalotrichum nanum, C. stemonitis, Ch. merdarium* and *S. schenckii*, occurred only or mostly in plots foraged by wild boars and hares. The primary habitat of *Cephalotrichum* is dung. Records of *C. nanum* and *C. stemonitis* have so far been from dung of coyote, deer, rabbit and mice (Ellis & Ellis, 1998; Chlebicki, 2008). Only *Cephalotrichum microsporum* (Sacc.) P.M. Kirk and *C. purpureofuscum* (Schwein.) S. Hughes have so far been recorded in/on dung of domestic pig (Ellis & Ellis, 1998). *Cephalotrichum nanum* produces tall, slender, sharply pointed synnemata and characteristic large, distinctly rough conidia, and so its misidentification can be discounted. *Chrysosporium merdarium* has been observed on dung of mice, coyotes, rats and domestic pigs and their skin (Domsch et al., 2007). *Sporothrix schenckii* has been recorded on humans and animals as a cause of cutaneous sporotrichosis (de Hoog et al., 2000). However, the natural habitats of these fungi also include soil, com-

post, wood, herbaceous stems, seeds, sawdust and decaying plant material (Abbott, 2000; Domsch et al., 2007; Chlebicki, 2008).

Increased populations of certain species after amendment with sawdust may increase the risk of some plant disorders and diseases. An increased population of *T. inflatum* increases the risk of damage to mycorrhizae (Summerbell, 1987). An increased population of species of *Phoma* + *Pyrenochaeta* under roots of seedlings increases the possibility of *Phoma* blight (Kliejunas et al., 1985). This disease (with symptoms of defoliation and dieback) may have contributed to the greater mortality of seedlings in foraged plots.

The presence of animal tissues and dung in Lubartów A soil increased the variety of the substrates and this increased, non-significantly, fungal diversity measured as number of species. The greater abundance but lower diversity of fungi in the sawdust-amended plots seems to have resulted from antibiosis exerted by dominants.

Whilst sawdust treatments and foraging by animals made potentially beneficial alterations to the fungal community structure in soil infested by cockchafers, there is insufficient evidence to suggest that the altered communities would contribute to significant biological control of the insects.

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