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# Effects of silvicultural techniques on the diversity of microorganisms in forest soil and their possible participation in biological control of *Armillaria* and *Heterobasidion*

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**Abstract:** Effects of different pre-planting soil preparations and post-harvest wood debris applications in a clear-cut Scots pine plantation, on the abundance, diversity, and activity of culturable microorganisms were investigated. The investigation was done 9 years after the re-plantings had been done. This formed part of an investigation of silvicultural practices for conservation and the biological control of *Armillaria* and *Heterobasidion* in northern temperate forests (Poland). The treatments being compared, were expected to have altered the soil's physical and chemical properties, and consequently, its biological properties. Only soft-rot microfungi from the Ascomycota and Zygomycota were detected in the soil. Fungi, including those antagonistic to *Armillaria* and *Heterobasidion*, were more abundant after shallow ploughing than after deep ploughing or ridging, and where chipped rather than coarse wood debris was left on the soil surface or incorporated. Scots pine trees had the most biomass and the least mortality after ridging and leaving coarse wood debris on the surface (associated with only a relatively moderate abundance of fungi).

**Key words:** *Armillaria,* bacteria, biological control, fungi, *Heterobasidion, Pinus sylvestris*, silvicultural techniques

## Introduction

*Armillaria* and *Heterobasidion* species cause the most important diseases of conifers in northern temperate forests. Both pathogens cause butt and root rot in conifers and hardwoods. They degrade the lignin and cellulose components of wood (Shaw and Kile 1991; Woodward *et al*. 1998). Annual losses in Europe are estimated at 1.5 x $10^9$ euros (Samils *et al.* 2008). Healthy tree stands become infected with the fungi via rhizomorphs (*Armillaria*), mycelial growth along roots (*Heterobasidion*), and basidiospores, which are produced abundantly and distributed over many kilometers.

Avoiding primary infection in healthy stands is the target for biological control of both pathogens. Following early observations of a strong antagonistic effect of the saprotrophic white rot fungus *Phlebiopsis gigantea* (Fr.:Fr.) Jülich towards *Heterobasidion annosum* (Fr.) Bref. in colonising freshly-cut conifers stumps (Rishbeth 1963), this fungus has been applied for decades to stumps in the field for prevention of *Heterobasidion* infection (Pratt *et al.* 2000). Throughout Europe, *P. gigantea* stump treatment is practised on more than 200,000 ha annually.

Many other microorganisms, mostly fungi, are antagonistic to *Armillaria* and *Heterobasidion*. Antagonism is based on competition for resources [e.g. the white rot fungus *Resinicium bicolor* (Alb. & Schwein.) Parmasto], production of antibiotics, toxins or fungal cell wall degrading enzymes [e.g. the brown rot fungus *Fomitopsis pinicola* (Sw.) P. Karst., the ascomycete *Trichoderma harzianum* Rifai, and the hyphomycetes *Phaeotheca dimorphospora* DesRoch. & Ouell. and *Scytalidium lignicola* Pesante], mycoparasitism [e.g. *Trichoderma polysporum* (Link) Rifai], and/or induction of systemic resistance (e.g. *Trichoderma* spp.) (Harman *et al.* 2004).

The restricted range of conifer species that are protected by *P. gigantea* means that other biological control agents and new mechanisms for biocontrol need to be identified.

Choosing appropriate pre-planting soil preparations and using post-harvest wood debris in forestry, has become a challenge. The standard forestry practice of deep ploughing leads to impeded tree root development, poor stability of trees, late improvement in growth rates, extensive soil disturbance, and water run-off. Deep ploughing is often replaced by a range of alternative pre-planting

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techniques, e.g. shallow ploughing or ridging (planting on built-up soil ridges). Appropriate treatments ensure that a site is favourable for: planting young trees, an improved survival of seedlings, and further tree growth.

Different post-harvest wood debris application techniques are available. Leaving a certain amount of residue in the forest is environmentally beneficial. This practice is generally considered to affect the physical, chemical, and biological properties of the soil (Green *et al.* 2004; Kasel *et al.* 2008). The effects of site preparation on the nutritional status of the soil and plants, and on watersheds, erosion, sediment losses, and water quality, are the effects most often studied. Microbiological studies are rare.

Fungi are important components of the soil microbiota. Fungi typically constitute more of the soil biomass than bacteria. How much more depends on the soil depth and nutrient conditions (Domsch *et al.* 1980). Saprobic fungi represent the largest proportion of fungal species in forest soil. Saprobic fungi perform a crucial role in the decomposition of structural polymers of wood, such as cellulose, hemicelluloses, and lignin, thus contributing to the maintenance of the global carbon cycle. Saprobic fungi are also involved in biotic interactions that can be pathogenic, beneficial or neutral for plants and other organisms (Smith and Read 2008). An understanding of the diversity and dynamics of microbial communities resulting from different management procedures, is essential for understanding the decomposition dynamics of woody debris and soil humus formation, and consequently the quantity and quality of plants.

There has been little research on the effects of different silvicultural techniques on the microbiological status of forest soil and its effect on the health and quality of forest trees. Therefore, the main objectives of this study were to assess the: (i) effects of cultivation methods (pre-planting soil preparation, and post-harvest wood debris application) on the abundance, diversity, and spatial and temporal distributions of bacteria and non-mycorrhizal fungi in non-rhizosphere forest soils; (ii) associations and patterns of co-existence among the dominant fungal taxa; (iii) interactions between occurrence of *Armillaria* and *Heterobasidion* and their fungal antagonists in soil of a *Pinus sylvestris* L. plantation; (iv) interactions between *P. sylvestris* tree quality (height, biomass, shape of crown, mortality) and abundance of saprobic microorganisms, including antagonists of *Armillaria* and *Heterobasidion*. We hypothesised that different management techniques would produce different physical and chemical conditions in the soil, and hence, initiate and stimulate different microbiological changes. Any changes that were effective in conservative biological control (CBC) of *Armillaria* and *Heterobasidion* in *P. sylvestris* plantations might then be identified.

## Materials and Methods

### Site description and treatments

The experiment took place from 1999 to 2008, at Kalisz Pomorski Forest District, Poland (53.2990800 N, 15.9063100 E). Different pre-planting soil preparation treatments, and wood debris application treatments, in 12 combinations,

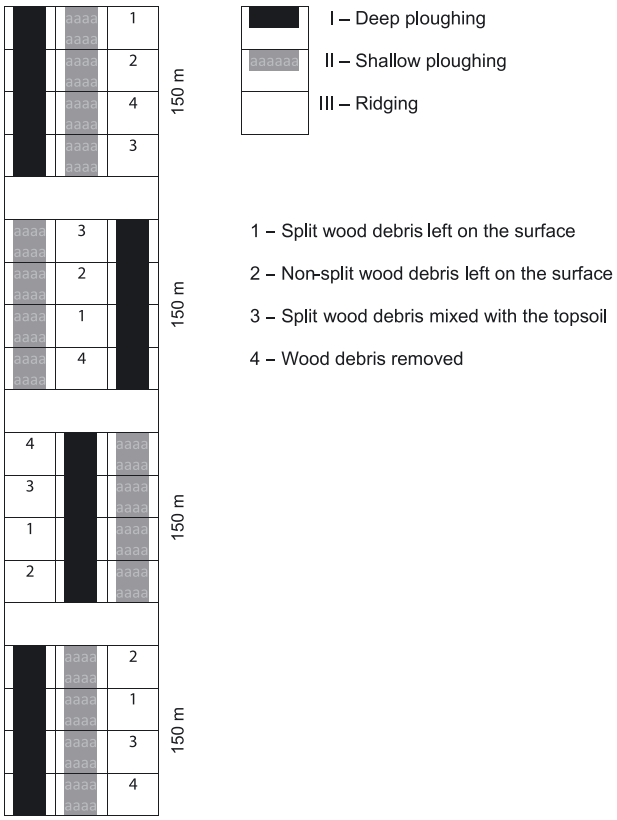


**Fig. 1.** Location of the experimental plots

were applied in a 5 ha area. The treatments were applied in 1999, after clear cutting a 90-year-old *P. sylvestris* stand. Pre-planting soil preparation treatments (in October 1999) were: I – standard deep ploughing to a furrow depth of 45–60 cm, using a forest plough PGŻ-75; II – shallow ploughing to a furrow depth of up to 30 cm, using a disc plough U-162; and III – ridging, i.e. building a 10–50 cm ridge of soil, in which saplings were planted, using a roto-hoe-tiller. Post-harvest wood debris application treatments (in April 1999) were: 1 – wood debris chipped with a cutter and left on the surface; 2 – coarse wood debris left on the surface; 3 – chipped wood debris mixed with the topsoil; and 4 – wood debris removed. One-year-old *P. sylvestris* saplings were planted in April 2000. There were four randomly-located replicate plots of each of the 12 treatment combinations (Fig. 1). The size of a single plot was 1,000 $m^2$ (37 × 27 m). The control was represented by the part of the plantation that surrounded the treatment area. The control area was covered by 9-year-old naturally regenerated *P. sylvestris* with no soil preparation and with wood debris left on the surface after a 1999 clear cutting.

Organic carbon (OC) and total nitrogen (N) were used as indicators of the soil organic status and quantified by dry combustion. Soil pH (1 : 5/soil : KCl), extractable nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) were used as indicators of the acid-base and nutrient status.

### Isolation and identification of fungi

In July 2008, four samples which were 250 g each, of non-rhizosphere soil were collected along a diagonal transect

in each replicate plot of each treatment. In the laboratory, the soil samples were mixed in a flask for 12 h, using a rotating movement. Next, 1 g of soil was mixed with 149 g of sterile quartz sand for 10 min. The sand-soil mixture (27 $mm^3$) was put into a Petri dish and covered with liquid Johnson-Martin's agar [JMA; 10 g · $l^{-1}$ glucose, 5 g · $l^{-1}$ peptone (Sigma-Aldrich), 1 g · $l^{-1}$ $KH_2PO_4$, 0.5 g · $l^{-1}$ $MgSO_4$ · · $7H_2O$, 0.03 g · $l^{-1}$ rose bengal (Sigma-Aldrich), 0.0025 g · · $l^{-1}$ aureomycin (Sigma-Aldrich), 20 g · $l^{-1}$ agar]. For each treatment combination, 10 replicates were made from each of the four sand-soil mixtures. After a 20-day incubation at 25°C, all the colonies on each plate were examined macro- and microscopically. Colonies were distinguished on the basis of colour, growth rate, hyphal characters, and sporulation. Colonies of each species were counted, and representatives of fungi were identified on Potato Dextrose Agar (PDA; 39 g · $l^{-1}$ Difco PDA, pH 5.5), and Synthetic Nutrient Agar (SNA; 1 g · $l^{-1}$ $KH_2PO_4$, 1 g · $l^{-1}$ $KNO_3$, 0.5 g · · $l^{-1}$ $MgSO_4 \cdot 7H_2O$, 0.5 g · $l^{-1}$ KCl, 0.2 g · $l^{-1}$ glucose, 0.2 g · $l^{-1}$ sucrose, 20 g · $l^{-1}$ agar). *Aspergillus* and *Penicillium* species were identified on Czapek Yeast Autolysate agar [CYA; 30 g · $l^{-1}$ sucrose, 5 g · $l^{-1}$ powdered yeast extract (Sigma-Aldrich), 1 g · $l^{-1}$ $KH_2PO_4$, 10 ml · $l^{-1}$ Czapek concentrate, 15 g · $l^{-1}$ agar] and 2% Malt Extract Agar [MEA; 20 g · $l^{-1}$ powdered malt extract (Sigma-Aldrich), 20 g · $l^{-1}$ glucose, 1 g · $l^{-1}$ peptone (Sigma-Aldrich), 20 g · $l^{-1}$ agar]. Mycological keys were used for fungal identification.

Fungi abundance was defined as the number of colony forming units (*cfu*) in a sample. Frequency was defined as the proportion of isolates in the total number of isolates. Diversity was defined as the number of species in a sample. A species, or group of related species, was considered as: eudominant, with a frequency > 10%; dominant, with a frequency of 5–10%; subdominant, with a frequency of 2–5%; recedent, with a frequency of 1–2%; subrecedent, with a frequency of 0–1%.

### Isolation and identification of bacteria

For the isolation of the bacteria, 10 g of soil from each replicate plot was shaken in 90 ml of Ringer's solution for 10 min. The suspension was serially diluted. One ml of suspension from the $10^{-4}$ dilution was poured into an empty Petri dish and covered with 2.5% Standard-Keimzahl agar (MERCK cat. no. 1.01621, pH 7.2). Forty (4 × 10) replicates were made for each treatment. After a 20-day-incubation period, at 25°C, all colonies on each plate were examined macro- and microscopically. Colonies were distinguished on the basis of phenotypic differences (growth rate, colour, shape and character of margins). Colonies of each species were counted and representatives were chosen for identification. The identification of Actinomycetes was based on macro- and microscopic characteristics, i.e. morphology of colonies, spore-bearing aerial hyphae, and presence of pigmentation (Hunter-Cevera and Eveleigh 1990). The identification of the bacteria was based on phenotypic differences, i.e. growth rate, presence of pigmentation, shape and size of cells, and presence of endospores. The identification of the bacteria was also based on biochemical properties, i.e. staining and enzyme tests (Bradbury 1988; Bergey and Holt 1994; Holt 1994).

Molecular identification required extraction and amplification of 16S rRNA from single colonies. Primers used were 968F (5′ AAC GCG AAG AAC CTT AC 3′) and 1401R (5′ CGG TGT GTA CAA GAC CC 3′) (Nübel *et al.* 1996). Each 25 ml PCR mixture consisted of 0.2 mM of each primer, 0.25 U of Taq polymerase (MBI Fermentas, St. Leon-Rot, Germany), buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl, 0.08% Nonidet P-40, 0.1 mg · · $ml^{-1}$ BSA), 25 mM $MgCl_2$, 0.2 mM deoxyribonucleoside triphosphates (dNTPs), and 2 ml (200 ng) diluted total DNA. Cycling conditions were: an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 2 min, and a final extension of 72°C for 10 min. The PCR products were checked by an electrophoresis of 5 ml of product in 1% agarose gel containing ethidium bromide (0.5 μg · $ml^{-1}$) to stain the DNA. Purification of the PCR products was done using MinElute PCR purification Kit (Qiagen, Crawley, UK) and sequencing (at the Centre of DNA Studies, Poznań, Poland). Sequences were queried against the GenBank database using BLAST in July 2011. Abundance and frequency of bacteria were defined as for fungi.

### Assessment of tree growth

An evaluation was done of the height (cm) of 30% of the 9-year-old Scots pine trees in each plot. The biomass (kg · · $ha^{-1}$) was estimated from the weight of 10 trees in each plot and the number of trees in 1 ha. Density (number · · $ha^{-1}$) was determined by counting all surviving trees in a plot. The percentage of trees which had a properly shaped crown were estimated visually. The mortality (dead or dying trees) were also estimated visually.

### Assessment of *Armillaria* and/or *Heterobasidion annosum* infection

Dying and recently dead *P. sylvestris* trees were examined in 2009, 2010, and 2012, for the occurrence of *Armillaria* and/or *H. annosum* in their roots. Three primary roots from each tree were excavated. After removing the bark, one wood core sample was taken using a sterile increment borer. The sample was taken from each root at a distance of approximately 20 cm from the stem.

Three pieces (about 1 cm long) of each root core sample were surface sterilised in sodium hypochlorite (active chlorine = 7%) for 30 s and rinsed twice in sterile, demineralised water for 15 s. The pieces were dried between paper towels and placed on malt agar [MA; 20 g · $l^{-1}$ malt extract, 15 g · $l^{-1}$ Bacto agar (Difco, Detroit, MI, USA), 230 mg · $l^{-1}$ thiabendazole (added in 1 ml concentrated lactic acid, 85–90%), 100 mg · $l^{-1}$ streptomycin, 50 mg · $l^{-1}$ polymyxin sulphate, 100 mg · $l^{-1}$ sodic benzylpenicillin (Sigma-Aldrich); modified from Legrand and Guillaumin (1993)]. After incubation for 2–4 weeks in darkness at 25°C, pure cultures were transferred to malt extract agar [20 g · $l^{-1}$ Bacto agar, 20 g · · $l^{-1}$ Diamalt (Hefefabriken AG, Hindelbank, Switzerland)].

Isolations from rhizomorphs were prepared as for root core samples, except that they were surface-sterilised for only 15–30 s. No attempt was made to isolate *Armillaria* spp. from mycelial fans.

The presence of *H. annosum* was confirmed by observation of its *Spiniger meineckellus* (Olson) Stalpers conidial stage in culture.

*Armillaria* isolates were identified by diploid-haploid pairings using, as haploid tester strains, each of *A. borealis* Marxm. & Korhonen, *A. cepistipes* Velen., *A. gallica* Marxm. & Romagn, and *A. ostoyae* (Romagn.) Herink (Korhonen 1978). A tree was considered infected with *H. annosum* and/or *Armillaria*, if either or both pathogens were identified on at least one root core sample.

An additional core was taken just above the soil surface, from the butt of 50 of the 172 trees checked for the incidence of *Armillaria* and *H. annosum*. The methods for isolating these pathogens, to determine the presence of the pathogens in the butts, were the same as for the root samples.

### Statistical analyses

The effects of treatments on abundance and diversity of fungi, and butt and root rot incidence in 9-year-old *P. sylvestris* trees, were tested by two-way analysis of variance (ANOVA). Where interactions (pre-planting soil preparation ´post-harvest wood debris utilizsation) occurred, data were plotted to determine the factors responsible, and subjected to Scheffé's method for the estimation of the significance of the difference between factor averages. Where there were no factorial interactions, the data were evaluated for each factor separately. Statistical significance was assumed at $p \leq 0.05$ or $p \leq 0.001$ using Matlab 7.3.0 with Statistical Toolbox version 5.3 (MathWorks, Inc., Natick, MA, USA).

Species richness and the structure of the fungal communities were determined for each combination-treatment. Isolates of the same species were grouped and the frequency of each species was determined. A number of diversity indices were calculated for each community (Magurran 1988). These indices included three different species richness indicators: (i) the total number of species in the community; (ii) Margalef's index (DMg), which shows richness from the ratio between the number of species and their ln function; (iii) and Shannon's diversity index (H'), a general diversity index that considers both species richness and evenness. Three different indices were also calculated for evenness and dominance: (i) Shannon's evenness index (E), which is the ratio of Shannon's diversity index to the maximum possible value with the observed number of species; (ii) Simpson's index (D), which gives the probability that two isolates chosen at random will be from the same species; (iii) Berger-Parker's index (d), which is the relative abundance of the most abundant species. The similarity between fungal communities on roots of chosen treatments was determined by calculating Sorensen's quantitative similarity index (CN) from the number of co-occurring species.

## Results

### Soil characterisation

The soil was sandy loam. In the Ah horizon (3–20 cm), the soil consisted of sand (64.4%), silt (24.7%), and clay (10.9%). Data on the nutritional status of the topsoil are given in table 1. Analyses of the soil profiles showed that topsoil layers contained much more organic matter and organic carbon than the subsoil layers (data not shown).

### Abundance and diversity of fungal communities

Nine years after different treatments (pre-planting soil preparation, and post-harvest wood debris utilisation), and 8 years after the 1-year-old *P. sylvestris* saplings were planted, fungi were significantly more abundant in the soil of most treatments than in the control soil (Table 2). Fungal abundance ranged from 246 to 2,514 (*cfu*) per treatment sample, and diversity ranged from 13 to 23 species. Culturable fungi (71 species in total) were mostly Ascomycota and rarely Zygomycota. The diversity of fungal communities measured as the number of species was similar in the treatments and the control. The greatest abundance of fungi (ANOVA $p \leq 0.05$ or $p \leq 0.001$) occurred after shallow ploughing and leaving chipped wood debris or no debris on the soil surface. The least abundance occurred after deep ploughing or ridging and leaving coarse wood debris or no debris on the soil surface (Tables 2–4).

Eudominants in at least one treatment combination included three species of *Penicillium*. Four species of fungi, including *Tolypocladium geodes* and *Trichoderma viride*, were dominants. Other species or groups of species were subdominants, recedents or subrecedents (Table 2). The main emphasis was placed on known antagonists of *Armillaria* and *Heterobasidion*, including Mortierellales and Mucorales, *Penicillium citrinum, P. adametzii, P. janczewskii, P. spinulosum, T. geodes*, and *Trichoderma*. The greatest recorded populations of these were: (i) Mortierellales and Mucorales, and *P. spinulosum* after ridging; (ii) *P. adametzii, P. citrinum, P. janczewskii*, and *T. geodes* after shallow ploughing; (iii) *Trichoderma* spp. after the soil was the most disturbed; (iv) *P. adametzii* and *P. janczewskii* after leaving chipped or coarse wood debris on the surface; (v) *P. spinulosum* after mixing chipped wood debris with the topsoil (Tables 3, 4).

The greatest abundance of fungi was often associated with the least diversity of the fungal communities. A high frequency of *P. adametzii* was usually associated with a low frequency of *P. janczewskii* and *P. spinulosum*. The greatest average abundance of known *Armillaria* and *Heterobasidion* antagonists (listed above) occurred after shallow ploughing and after leaving chipped wood debris on the soil surface, or to a slightly lesser extent, mixing chipped debris with the topsoil (Tables 3, 4). *Beauveria brongniartii* (Sacc.) Petch, an entomopathogenic species, was isolated mainly after ridging (Table 2).

The relatively small number of fungal taxa and the infrequent occurrence of many taxa resulted in relatively small diversity indices based on species richness (DMg) and proportional abundance of species (H') (Table 5). Mixing chipped wood debris with the topsoil usually resulted in the least species richness, and removing wood debris often resulted in low evenness indices. The dominance of single taxon or a few taxa in communities, resulted in small values for Shannon's evenness index (E) and high values for dominance indices (D and d). Evenness

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**Table 1.** Characteristics of topsoil (3–20 cm layer) of the 9-year-old *Pinus sylvestris* plantation in Kalisz Pomorski, in 2008 – after different pre-planting soil preparations, and the characteristics of the 1999 post-harvest wood debris utilisations

| Soil characteristics | I – Deep ploughing | | | II – Shallow ploughing | | | III – Ridging | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 – coarse wood debris left on the surface | 3 – chipped wood debris mixed with the topsoil | 4 – wood debris removed | 2 – coarse wood debris left on the surface | 3 – chipped wood debris mixed with the topsoil | 4 – wood debris removed | 2 – coarse wood debris left on the surface | 3 – chipped wood debris mixed with the topsoil | 4 – wood debris removed |
| pH in KCl | 3.22 | 3.56 | 3.68 | 3.45 | 3.32 | 3.54 | 3.25 | 3.56 | 3.64 |
| Organic carbon [%] | 1.098 | 0.815 | 1.250 | 1.143 | 0.815 | 1.311 | 1.069 | 1.041 | 0.899 |
| Organic carbon [an average] | | 1.054 | | | 1.089 | | | 1.003 | |
| Total nitrogen [%][a] | 0.07 | 0.07 | 0.06 | 0.08 | 0.08 | 0.07 | 0.08 | 0.08 | 0.07 |
| Extractable phosphorus [mg · kg$^{-1}$][b] | 40 | 42 | 25 | 52 | 40 | 38 | 45 | 38 | 37 |
| Extractable potassium [mg · kg$^{-1}$][c] | 20 | 30 | 30 | 30 | 40 | 30 | 20 | 20 | 20 |
| Extractable magnesium [mg · kg$^{-1}$][d] | 10 | 15 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Extractable calcium [mg · kg$^{-1}$][c] | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a]analysed with the Kjeldahl method; [b]analysed with the Egner-Riehm method; [c]analysed with flame atomic emission spectrometry; [d]analysed with the Schachtschabel method

**Table 2.** Frequency of fungi in soils of the 9-year old *Pinus sylvestris* plantation after different pre-planting soil preparations (I–III), and post-harvest wood debris utilisations (1–4) (Kalisz Pomorski, July 2008): 1 – wood debris chipped with a cutter and left on the surface; 2 – coarse wood debris left on the surface; 3 – chipped wood debris mixed with the topsoil; 4 – wood debris removed

| Taxon | I – Deep ploughing | | | | II – Shallow ploughing | | | | III – Ridging | | | | The control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | |
| Eudominants – with a frequency of 10–100%, at least in one treatment combination | | | | | | | | | | | | | |
| *Penicillium adametzii* Zaleski | 21.6 | 13.7[a] | 77.5[b] | 24.0 | 71.4[c] | 47.8[d] | 67.1[e] | 74.7[f] | 30.4 | 45.2[g] | 4.9[h] | 17.6 | 23.7[a,b,c,d,e,f,g,h] |
| *P. janczewskii* Zaleski | 58.6[k] | 62.1[a,l] | 8.5[b,k,l,m] | 45.9[m] | 22.5[c] | 35.4[d,n,o] | 22.7[e,n,p] | 13.3[f,o,p] | 33.6[g,r] | 27.3[h,s] | 13.9[i,r,s,t] | 27.3[j,t] | 49.3[a,b,c, d,e,f,g,h,i,j] |
| *P. spinulosum* Thom | 5.6[a] | 9.2[a] | 3.4[c] | 0.4[d] | 1.7[e] | 4.4[f] | 4.5[g] | 3.8[h] | 17.1 | 10.3[i] | 58.9[j] | 27.9[k] | 18.9[a,b,c,d,e,f,g,h,I,j,k] |
| Dominants – with a frequency of 5–10%, at least in one treatment combination | | | | | | | | | | | | | |
| *Beauveria brongniartii* (Sacc.) Petch | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 7.4 | 0.6 | 0 |
| *Penicillium minioluteum* Dierckx | 0 | 0 | 0 | 5.3 | 0.1 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Tolypocladium geodes* W. Gams | 3.9 | 1.7 | 2.2 | 6.5 | 1.1 | 6.0 | 1.7 | 1.4 | 0.5 | 2.1 | 0.5 | 6.1 | 1.4 |
| *Trichoderma viride* Pers. | 1.8 | 1.3 | 0.1 | 5.7 | 0.5 | 0.6 | 0.3 | 1.0 | 2.7 | 1.5 | 1.3 | 2.8 | 0 |
| Subdominants – with a frequency of 2–5%, at least in one treatment combination | | | | | | | | | | | | | |
| *Clonostachys candelabrum* (Bonord.) Schroers + *Gliocladium virens* J.H. Mill., Giddens & A.A. Foster | 0.2 | 0 | 2.4 | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 2.** Frequency of fungi in soils of the 9-year old *Pinus sylvestris* plantation after different pre-planting soil preparations (I–III), and post-harvest wood debris utilisations (1–4) (Kalisz Pomorski, July 2008): 1 – wood debris chipped with a cutter and left on the surface; 2 – coarse wood debris left on the surface; 3 – chipped wood debris mixed with the topsoil; 4 – wood debris removed – *continuation*

| Taxon | I – Deep ploughing | | | | II – Shallow ploughing | | | | III – Ridging | | | | The control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | |
| Eudominants – with a frequency of 10–100%, at least in one treatment combination | | | | | | | | | | | | | |
| *Chrysosporium merdarium* (Ehrenb.) J.W. Carmich. + *Geomyces pannorum* (Link) Sigler & J.W. Carmich. + *G. sulphureus* Traaen | 0.5 | 3.8 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0.5 | 0.2 | 3.7 | 0.2 | 0.2 |
| *Mortierella alpina* Peyronel + *M. gemmifera* M. Ellis + *M. parvispora* Linnem. + *M. verticillata* Linnem. | 0 | 0.8 | 0.1 | 0 | 0.1 | 0.6 | 0.2 | 0 | 0.5 | 0 | 0.2 | 2.5 | 0 |
| *Myxotrichum* sp. | 0.1 | 0 | 0 | 0 | 0.1 | 0.3 | 0 | 0.7 | 0 | 0 | 1.6 | 3.4 | 0 |
| *Oidiodendron griseum* Robak + *O. tenuissimum* (Peck) S. Hughes | 1.8 | 0.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.0 | 0 | 0 | 0 |
| *Penicillium citrinum* Thom | 1.1 | 1.1 | 2.9 | 0.8 | 2.0 | 0.8 | 0.7 | 3.8 | 1.2 | 0 | 2.9 | 5.1 | 0.2 |
| *P. commune* Thom | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 2.1 | 0.2 | 0 | 0.2 |
| *P. daleae* Zaleski | 1.3 | 2.5 | 0.8 | 0 | 0 | 2.7 | 1.5 | 0.3 | 4.5 | 2.3 | 0.3 | 2.2 | 3.6 |
| *P. simplicissimum* (Oudem.) Thom | 0.2 | 0.7 | 1.5 | 4.9 | 0 | 0 | 0 | 0 | 0 | 0 | 3.0 | 0.8 | 0.4 |
| Recedents – with a frequency of 1–2%, at least in one treatment combination | | | | | | | | | | | | | |
| *Absidia californica* J.J. Ellis & Hesselt. + *A. cylindrospora* Hagem + *A. glauca* Hagem | 0 | 0.2 | 0 | 0 | 0.1 | 0 | 0 | 0.1 | 1.2 | 0 | 0 | 0 | 0 |
| *Aspergillus ochraceus* G. Wilh. + *A. repens* (Corda) Sacc. + *A. ruber* Thom & Church + *A. tardus* Bissett & Widden | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0.1 | 0 | 0.2 | 0.3 | 1.3 | 0.2 |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | 0.2 | 0 | 0.3 | 2.0 | 0.1 | 0.1 | 0.1 | 0.2 | 1.2 | 0.2 | 0.3 | 1.3 | 0.2 |
| *Penicillium canescens* Sopp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.5 | 0 | 0.1 | 0 | 0 |
| *Trichocladium opacum* (Corda) S. Hughes | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.2 | 0 | 0 | 0 | 0 |
| *Trichoderma koningii* Oudem. | 0.2 | 0.2 | 0 | 0 | 0 | 0 | 0 | 0.1 | 1.1 | 0.6 | 0 | 0 | 0 |
| Frequency of *Penicillium* species | 90.5 | 89.8 | 94.7 | 82.9 | 97.9 | 91.7 | 97.3 | 96.1 | 89.6 | 88.0 | 84.4 | 81.0 | 96.9 |
| Frequency of *Trichoderma* species | 2.0 | 1.5 | 0.1 | 6.5 | 0.5 | 0.6 | 0.3 | 1.2 | 3.7 | 2.1 | 1.3 | 2.8 | 0.5 |
| Abundance – number of colony forming units (*cfu*) in a sample | 909[a] | 531 | 715[b] | 246[c] | 2514[d] | 933[e] | 1604[f] | 2314[g] | 405[h] | 476 | 1152[i] | 473 | 497[a,b,c,d,e,f,g,h,i] |
| Diversity – number of species | 21 | 19 | 13 | 16 | 14 | 18 | 15 | 17 | 19 | 23 | 20 | 18 | 15 |

[a]The same letter in a row in two columns indicates a statistically significant difference, according to two-way ANOVA at $p \leq 0.001$ or $p \leq 0.05$

Subrecedents – with a frequency of 0–1%, included *Acremonium ochraceum* (Onions & G.L. Barron) W. Gams, *Alternaria alternata* (Fr.) Keissl., *Epicoccum nigrum* Link, *Fusarium javanicum* Koord., *F. sporotrichioides* Scherb., *Lecanicillium lecanii* (Zimm.) Zare & W. Gams, *Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons, *Memnoniella echinata* (Rivolta) Galloway, *Monocillium indicum* S.B. Saksena, *Mycelium radicis atrovirens* Melin, *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm., *Penicillium aurantiogriseum* Dierckx, *P. citreonigrum* Dierckx, *P. corylophilum* Dierckx, *P. dierckxii* Biourge, *P. funiculosum* Thom, *P. glabrum* (Wehmer) Westling, *P. islandicum* Sopp, *P. jensenii* Zaleski, *P. pinophilum* Thom, *P. purpurogenum* Stoll, *P. raistrickii* G. Sm., *P. thomii* Maire, *P. verruculosum* Peyronel, *P. vinaceum* J.C. Gilman & E.V. Abbott, *P. waksmanii* Zaleski, *Penicillium* spp., *Periconia britannica* M.B. Ellis, *Phoma epicoccina* Punith., M.C. Tulloch & C.M. Leach, *Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis, *Pseudogymnoascus roseus* Raillo, *Trichoderma aureoviride* Rifai, *T. harzianum* Rifai, *Trichoderma* spp., *Ulocladium oudemansii* E.G. Simmons, Non-sporulating, white, Non-sporulating, dark

**Table 3.** Association of an average abundance and frequency of fungi antagonistic to *Armillaria* and *Heterobasidion* in soil with *Pinus sylvestris* tree parameters

| Category | I – Deep ploughing | II – Shallow ploughing | III – Ridging | The control – no soil preparation |
|---|---|---|---|---|
| | Abundance – average number of colony forming units (*cfu*) in a sample | | | |
| Mortierellales + Mucorales | 2 | 3 | $6^{a}$ | $0^{a}$ |
| *Penicillium adametzii* | $220^{a,d,f}$ | $1{,}262^{b,d,e}$ | $120^{e,f}$ | $118^{a,b}$ |
| *P. citrinum* | $10^{a,d}$ | $39^{b,d,e}$ | $15^{c,e}$ | $1^{a,b,c}$ |
| *P. janczewskii* | $259^{c,e}$ | $392^{a,c,d}$ | $138^{b,d,e}$ | $245^{a,b}$ |
| *P. spinulosum* | $31^{a,d,f}$ | $60^{b,d,e}$ | $233^{c,e,f}$ | $94^{a,b,c}$ |
| *Tolypocladium geodes* | $19^{a,d}$ | $37^{b,d,e}$ | $12^{e}$ | $7^{a,b}$ |
| *Trichoderma* spp. | $11^{a}$ | $13^{b}$ | $13^{c}$ | $2^{a,b,c}$ |
| Number of antagonists *cfu* | $553^{a,d}$ | $1{,}806^{b,d,e}$ | $537^{c,e}$ | $467^{a,b,c}$ |
| Number of all fungi *cfu* | $600^{a,d}$ | $1{,}841^{b,d,e}$ | $626^{c,e}$ | $497^{a,b,c}$ |
| Diversity – number of species | 17 | 16 | 20 | 15 |
| | Frequency – average percentage of isolates in the total number of isolates | | | |
| Mortierellales + Mucorales | 0.3 | 0.2 | 0.9 | 0 |
| *Penicillium adametzii* | 36.7 | 68.5 | 19.2 | 23.7 |
| *P. citrinum* | 1.7 | 2.1 | 2.4 | 0.2 |
| *P. janczewskii* | 43.2 | 21.3 | 22.0 | 49.3 |
| *P. spinulosum* | 5.2 | 3.3 | 37.2 | 18.9 |
| *Tolypocladium geodes* | 3.2 | 2.0 | 1.9 | 1.4 |
| *Trichoderma* spp. | 1.8 | 0.7 | 2.1 | 0.4 |
| Frequency of antagonists | 92.1 | 98.1 | 85.7 | 93.9 |
| | *Pinus sylvestris* trees parameters | | | |
| Height [cm] | 284.7 | 275.3 | 274.3 | 283.1 |
| Biomass [kg · ha$^{-1}$] | $32{,}598.77^{a,b}$ | $35{,}568.53^{a,c,d}$ | $41{,}550.78^{b,c,e}$ | $32{,}432.77^{d,e}$ |
| Density [number · ha$^{-1}$] | $10{,}856^{a,b}$ | $11{,}844^{a,c,d}$ | $12{,}313^{b,c,e}$ | $10{,}243^{d,e}$ |
| With properly shaped crown [%] | 92.7 | 90.8 | 85.5 | 92.2 |
| Mortality [%] | $56.7^{a,c,e}$ | $39.3^{b,c,d}$ | $3.8^{d,e}$ | $2.1^{a,b}$ |

The same letter in a row in two columns indicates a statistically significant difference, according to two-way ANOVA at $p \leq 0.001$ or $p \leq 0.05$

**Table 4.** Association of an average abundance and frequency of fungi antagonistic to *Armillaria* and *Heterobasidion* in soil with *Pinus sylvestris* tree parameters

| Category | 1 – Chipped wood debris left on the surface | 2 – Coarse wood debris left on the surface | 3 – Chipped wood debris mixed with the topsoil | 4 – Wood debris removed | The control – coarse wood debris left on the surface |
|---|---|---|---|---|---|
| | Abundance – average number of colony forming units (*cfu*) in a sample | | | | |
| Mortierellales + Mucorales | 4 | 4 | 2 | 4 | 0 |
| *Penicillium adametzii* | $704^{a,e,g,h}$ | $245^{b,e,f,i}$ | $562^{c,f,g}$ | $624^{d,h,i}$ | $118^{a,b,c,d}$ |
| *P. citrinum* | $22^{a,d,g}$ | $4^{d,e}$ | $22^{b,e,f}$ | $37^{b,f,g}$ | $1^{a,b,c}$ |
| *P. janczewskii* | $412^{a,d,f,g}$ | $264^{d,e,h}$ | $195^{b,e,f}$ | $183^{c,g,h}$ | $245^{a,b,c}$ |
| *P. spinulosum* | $54^{a,g,h}$ | $46^{b,e}$ | $258^{c,e,f,g}$ | $73^{d,f,h}$ | $94^{a,b,c,d}$ |
| *Tolypocladium geodes* | $22^{a}$ | $25^{b}$ | $16^{c}$ | $27^{d}$ | $7^{a,b,c,d}$ |
| *Trichoderma* spp. | $20^{a,c,e}$ | $8^{c}$ | $6^{d,e}$ | $19^{b,d}$ | $2^{a,b}$ |
| Number of antagonists *cfu* | $1{,}239^{a,e,h,i}$ | $596^{b,e,f,j}$ | $1061^{c,f,g,h}$ | $967^{d,g,I,j}$ | $467^{a,b,c,d}$ |
| Number of all fungi *cfu* | $1{,}276^{a,e,h,i}$ | $647^{b,e,f,j}$ | $1157^{c,f,g,h}$ | $1{,}011^{d,g,I,j}$ | $497^{a,b,c,d}$ |
| Diversity – number of species | 18 | 20 | 16 | 17 | 15 |
| | Frequency – average percentage of isolates in the total number of isolates | | | | |
| Mortierellales + Mucorales | 0.3 | 0.6 | 0.2 | 0.4 | 0 |
| *Penicillium adametzii* | 55.2 | 37.9 | 48.6 | 61.7 | 23.7 |
| *P. citrinum* | 1.7 | 0.6 | 1.9 | 3.7 | 0.2 |
| *P. janczewskii* | 32.3 | 40.8 | 16.8 | 18.1 | 49.3 |
| *P. spinulosum* | 4.3 | 7.1 | 22.3 | 7.2 | 18.9 |
| *Tolypocladium geodes* | 1.7 | 3.9 | 1.4 | 2.7 | 1.4 |
| *Trichoderma* spp. | 1.6 | 1.2 | 0.5 | 1.9 | 0.4 |
| Frequency of antagonists | 97.1 | 92.1 | 91.7 | 95.7 | 93.9 |
| | *Pinus sylvestris* tree parameters | | | | |
| Height [cm] | 279.4 | 286.7 | ND[1] | 268.2 | 283.1 |
| Biomass [kg · ha$^{-1}$] | $34{,}364.9^{a,c,e}$ | $42{,}667.0^{b,c,d}$ | ND | $32{,}685.9^{d,e}$ | $32{,}432.77^{a,b}$ |
| Density [number · ha$^{-1}$] | $11{,}783^{a,d}$ | $12{,}221^{b,d,e}$ | ND | $11{,}009^{c,e}$ | $10{,}243^{a,b,c}$ |
| With properly shaped crown [%] | 89.1 | 90.5 | ND | 89.3 | 92.2 |
| Mortality [%] | $59.0^{a,d,e}$ | $20.0^{b,d}$ | ND | $25.0^{c,e}$ | $2.1^{a,b,c}$ |

The same letter in a row in two columns indicates a statistically significant difference, according to two-way ANOVA at $p \leq 0.001$ or $p \leq 0.05$; [1]not determined

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**Table 5.** Diversity indices for fungi from soil after different pre-planting soil preparations, and post-harvest wood debris utilisations

| Index | I – Deep ploughing | | | | II – Shallow ploughing | | | | III – Ridging | | | | The control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 – chipped wood debris left on the surface | 2 – coarse wood debris left on the surface | 3 – chipped wood debris mixed with the topsoil | 4 – wood debris removed | 1 – chipped wood debris left on the surface | 2 – coarse wood debris left on the surface | 3 – chipped wood debris mixed with the topsoil | 4 – wood debris removed | 1 – chipped wood debris left on the surface | 2 – coarse wood debris left on the surfaces | 3 – chipped wood debris mixed with the topsoil | 4 – wood debris removed | |
| Species richness indices | | | | | | | | | | | | | |
| Margalef's index (DMg) | 2.93 | 2.86 | 1.82 | 2.72 | 1.66 | 2.48 | 1.89 | 2.06 | 2.99 | 3.56 | 2.69 | 2.76 | 2.25 |
| Shannon's diversity index (H') | 1.40 | 1.42 | 0.95 | 1.70 | 0.84 | 1.33 | 1.01 | 0.95 | 1.81 | 1.68 | 1.52 | 1.99 | 1.35 |
| Evenness or dominance indices | | | | | | | | | | | | | |
| Shannon's evenness index (E) | 0.45 | 0.48 | 0.37 | 0.61 | 0.31 | 0.46 | 0.37 | 0.33 | 0.61 | 0.53 | 0.50 | 0.68 | 0.48 |
| Simpson's index (D) | 0.39 | 0.41 | 0.61 | 0.28 | 0.56 | 0.36 | 0.50 | 0.57 | 0.23 | 0.29 | 0.37 | 0.19 | 0.33 |
| Berger-Parker's index (d) | 0.58 | 0.62 | 0.77 | 0.45 | 0.71 | 0.47 | 0.67 | 0.74 | 0.33 | 0.45 | 0.58 | 0.27 | 0.49 |
| Sorensen qualitative similarity index (CN) | 1.1 | | | | 0.62 | | | | 0.57 | | | | |
| | | 0.56 | | | | 0.66 | | | | 0.51 | | | |
| | | | 0.55 | | | | 0.56 | | | | 0.68 | | |

**Table 8.** Abundance of bacteria in the soil of the 9-year-old *Pinus sylvestris* plantation after different pre-planting soil preparations, and post-harvest wood debris utilisations (Kalisz Pomorski, July 2008): 1 – wood debris chipped with a cutter and left on the surface; 2 – coarse wood debris left on the surface; 3 – chipped wood debris mixed with the topsoil; 4 – wood debris removed

| Taxon | | I – Deep ploughing | | | | II – Shallow ploughing | | | | III – Ridging | | | | The control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| based on phenotypic and biochemical properties | based on sequencing of 16S rRNA[1] | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | |
| – | *Actinomyces* sp. | – | 40 | – | – | – | 40 | 10 | – | – | – | – | – | – |
| *Arthrobacter* sp. | *Arthrobacter ramosus* 99% | 10 | – | – | 450 | – | – | – | – | 115 | 330 | 5 | 25 | 65 |
| *Arthrobacter* sp. | *Arthrobacter* sp. 98% | – | 40 | 10 | 400 | – | – | 45 | – | 10 | 10 | 120 | 25 | 35 |
| *Alcaligenes* sp. | *Collimonas fungivorans* 99% | – | 1 | – | 1 | – | – | 1 | – | – | 1 | 1 | 1 | 1 |
| *Bacillus cereus* group | – | 230 | 81 | 10 | – | – | 10 | 50 | 40 | 5 | 20 | – | 10 | – |
| *Bacillus* sp.<br>*Clostridium* sp. | *Paenibacillus* sp. 99% | 70 | 100 | 30 | 55 | 10 | 5 | 35 | – | 5 | – | 35 | 5 | 45 |
| *Pseudomonas putida* | *Pseudomonas putida* 99% | 1 | – | – | 1 | – | – | – | – | 1 | – | 1 | – | 1 |
| *Serratia* sp.<br>*Aeromonas* sp. | *Serratia* sp. 99% | 10 | 40 | 7 | 50 | – | – | 5 | 20 | 5 | – | – | – | – |
| – | *Bacteria* sp. 98% | 10 | 365 | 142 | – | 45 | 30 | 5 | 60 | – | – | – | 35 | 30 |
| Abundance – number of colony forming units (*cfu*) in a sample | | 331[a] | 667[b] | 199 | 957[c] | 55[d] | 85[e] | 151 | 120[f] | 141 | 361[g] | 162 | 101[h] | 177[a,b,c,d,e,f,g,h] |
| Diversity – number of species | | 6 | 8 | 6 | 6 | 2 | 4 | 7 | 3 | 6 | 4 | 5 | 6 | 6 |

The same letter in a row in two columns indicates a statistically significant difference, according to two-way ANOVA at $p \leq 0.001$ or $p \leq 0.05$

[1]Blast search was done in July 2011

**Table 6.** Sorensen quantitative similarity index (CN) for fungal communities from different treatments

| Combination | CN |
|---|---|
| **Chipped wood debris left on the surface** | |
| Deep ploughing and shallow ploughing | 0.46 |
| Deep ploughing and ridging | 0.50 |
| Shallow ploughing and ridging | 0.48 |
| **Coarse wood debris left on the surface** | – |
| Deep ploughing and shallow ploughing | 0.54 |
| Deep ploughing and ridging | 0.57 |
| Shallow ploughing and ridging | 0.38 |
| **Chipped wood debris mixed with the topsoil** | – |
| Deep ploughing and shallow ploughing | 0.57 |
| Deep ploughing and ridging | 0.54 |
| Shallow ploughing and ridging | 0.57 |
| **Wood debris removed** | – |
| Deep ploughing and shallow ploughing | 0.42 |
| Deep ploughing and ridging | 0.52 |
| Shallow ploughing and ridging | 0.51 |

**Table 7.** An average abundance of bacteria in the soil of the 9-year-old *Pinus sylvestris* plantation after different pre-planting soil preparations, and post-harvest wood debris utilisations (Kalisz Pomorski, July 2008)

| Pre-planting soil preparations | An average abundance of bacteria | Post-harvest wood debris utilisations | An average abundance of bacteria |
|---|---|---|---|
| I – Deep ploughing | $539^{a,d,f}$ | 1 – Chipped wood debris left on the surface | $176^{c,g}$ |
| II – Shallow ploughing | $103^{b,d,e}$ | 2 – Coarse wood debris left on the surface | $371^{a,c,d}$ |
| III – Ridging | $192^{b,e,f}$ | 3 – Chipped wood debris mixed with the topsoil | $171^{d,e}$ |
| The control – no soil preparation | $177^{a,b,c}$ | 4 – Wood debris removed | $393^{b,e,g}$ |
| | | The control – coarse wood debris left on the surface | $177^{a,b}$ |

The same letter in a row in two columns indicates a statistically significant difference, according to two-way ANOVA at $p \leq 0.001$ or $p \leq 0.05$

**Table 9.** An average fungal *cfu* number : bacterial *cfu* number ratio in soil of the 9-year-old *Pinus sylvestris* plantation after different pre-planting soil preparations, and post-harvest wood debris utilisations (Kalisz Pomorski, July 2008)

| Pre-planting soil preparations | *cfu* | Post-harvest wood debris utilisations | *cfu* |
|---|---|---|---|
| I – Deep ploughing | 1.1 | 1 – Chipped wood debris left on the surface | 7.3 |
| II – Shallow ploughing | 17.9 | 2 – Coarse wood debris left on the surface | 1.8 |
| III – Ridging | 3.3 | 3 – Chipped wood debris mixed with the topsoil | 6.8 |
| The control – no soil preparation | 2.8 | 4 – Wood debris removed | 2.6 |
| | | The control – coarse wood debris left on the surface | 2.8 |

The same letter in a row in two columns indicates a statistically significant difference, according to two-way ANOVA at $p \leq 0.001$ or $p \leq 0.05$

tended to be least, and dominance most, immediately after mixing wood debris with the topsoil. Sorensen's quantitative similarity index (CN), used for comparing fungal communities in two treatments, suggests that the greatest similarity occurred between deep ploughing and shallow ploughing or ridging. This was particularly true when coarse wood debris was left on the surface or chipped wood debris was mixed with the topsoil (Table 6).

### Abundance and diversity of bacterial communities

Bacterial populations were greatest after deep ploughing and leaving coarse wood debris on the surface or removing it (Tables 7, 8). At least nine species of culturable bacteria were recorded (Table 8). The most common were species of *Arthrobacter* and *Bacillus*. Other species, of the genera *Collimonas*, *Paenibacillus*, *Pseudomonas*, and *Serratia*,

were rarely recorded. Larger populations of fungi were often associated with smaller populations of bacteria.

### Assessment of tree growth

Biomass (kg · ha$^{-1}$) and tree density (number · ha$^{-1}$) were significantly greatest, and mortality was significantly least, after ridging, and after leaving coarse wood debris on the surface (Tables 3, 4). These effects were not associated with the greatest abundance of fungi. Biomass and density of trees were significantly least and mortality significantly highest after deep ploughing and leaving chipped wood debris on the surface or removing wood debris. These effects were associated with only a moderate, or high abundance of fungi. The diversity of fungal communities tended to be associated, but not significantly, with lower mortality of the Scots pines.

### Assessment of *Armillaria* and/or *Heterobasidion annosum* infection

The mortality of Scots pine trees was 40% in 2009, 43% in 2010, and 57% in 2012, and was caused mostly by *H. annosum*. The fungus species, *Armillaria ostoyae,* occurred only sporadically (1% of trees).

## Discussion

Different methods of pre-planting soil preparation and post-harvest wood debris application, in a clear-cut forest, were compared. The methods were compared by assessing their effects on the abundance, diversity, and possible activity of microbiota – including two main butt and root rot pathogens, *Armillaria* and *Heterobasidion*. Different management practices were expected to affect the physical and chemical properties of the soil, which in turn would affect the biological properties. After the *P. sylvestris* plantation had been growing for 9 years, the microbial communities in the topsoil were analysed. Analysis was done according to the greatest: (i) agglomeration of roots of young trees due to availability of nutrients and access to precipitation; (ii) concentration of organic matter; (iii) biological activity due to the concentration of microorganisms; (iv) incidence of infection by butt and root rot pathogens (Shaw *et al.* 1991; Woodward *et al.* 1998).

Biotic and abiotic elements of the soil environment contribute to suppressiveness, although biological elements have been described as primary in most defined systems (Mazzola 2002; Garbeva *et al*. 2004). Bacteria were included in the present study because they facilitate wood degradation, provide the initial enzymes or nutrients, and remove chemical preservatives before colonisation by fungi (Blanchette and Show 1978). The emphasis, though, was placed on the fungi. It is the fungi which dominate the microbial communities in forest soils because of the soil acidity and fungal preferences for wood constituents. The fungi are also very sensitive to management practices and can be useful bio-indicators of soil quality and soil health (Avidano *et al.* 2005; Bossio *et al.* 2005; Epelde *et al.* 2008).

This study involved bacteria and fungi amenable to culturing. Phenotypic and biochemical identification of bacteria was, however, supported by 16S rRNA sequencing. The classical methods were preferred for studies of the functional diversity of microbiota in soil. Profiling based on DNA sequences would detect mostly the slow-growing, non-sporulating components of communities with unknown activity and relevance in microbiological interactions (Kwaśna *et al.* 2008).

Increases in fungal abundance and diversity in the topsoil of the *P. sylvestris* plantation 9 years after ploughing or ridging, and leaving or removing chipped or coarse wood debris on the soil surface, were significant and non-significant, respectively. The fungal communities included more than 70 species of Ascomycota and a few Zygomycota. Ascomycota are holocellulose-degrading fungi categorised as soft-rot fungi. Zygomycota are secondary sugar fungi (Osono and Takeda 2001). In fungal successions, both phyla occur in the later stages of wood decomposition (Lumley *et al.* 2001; Fukasawa *et al.* 2005, 2009a, b, 2010). No Basidiomycota were recorded although they may dominate in forest soil (Buee *et al*. 2009). Basidiomycota are present more often in the earlier stages of wood decomposition.

Studies have shown that a few species, i.e. *Cladosporium*, *Penicillium, Tolypocladium*, and *Trichoderma*, may co-exist because of their relative immobility and limited competition. The spatial separation of other, rarer species, e.g. *Beauveria*, does occur. Intrinsic population processes such as reproduction and dispersal capabilities, and limited resources lead to their spatial patterning and agglomeration.

The recorded fungal communities included species which are considered to be antagonistic towards *Armillaria* and *Heterobasidion,* e.g. Mortierellales and Mucorales, *P. adametzii, P. citrinum*, *P. janczewskii, P. spinulosum, T. geodes*, and *Trichoderma* (Kwaśna 1997a, b, c, 2001, 2002; Kwaśna *et al.* 2001, 2004; Mańka *et al.* 2006; Arhipova *et al.* 2008; Szwajkowska-Michałek *et al*. 2012). The species often colonise living or dead wood and soil, increasing the suppressiveness of these species to butt and root rot pathogens. *Penicillium adametzii* was often the most frequent. Szwajkowska-Michałek *et al.* (2012) showed that mycelium and metabolites of *P. adametzii* may: (i) inhibit growth of *Armillaria* and *Heterobasidion* in paired cultures *in vitro*; (ii) inhibit growth of *Armillaria* rhizomorphs on/in oak *in vivo*; (iii) decrease the extent of root rot caused by *Armillaria* and of necrosis caused by *H. annosum* on tree seedlings; (iv) stimulate the growth of Scots pine seedlings infected with *Armillaria.* Their antagonistic activity results mostly from the production of fungistatic antibiotics (Raper and Thom 1949; Kozlovsky *et al*. 2013).

*Trichoderma* species have proven antagonistic properties towards *Armillaria* and *Heterobasidion*. For this reason, the *Trichoderma* species have been suggested as possible biocontrol agents (Sierota 1976; Nelson *et al.* 1989; Reaves *et al.* 1990; Dumas and Boyonoski 1992; Fox *et al.* 1994; Onsando and Waudo 1994). Successful control may be achieved at moderate moisture levels, temperatures of 15–32°C, when there is high organic matter content and when the pH 5.5–8.5. The effectiveness of the *Trichoderma*

species depends on their age, availability of amino acids, and prior substrate decomposition. According to Harman *et al*. (2004) *Trichoderma* is supposed to be eliminated after soil disturbance. The present results do not confirm this, since the *Trichoderma* population was greater in disturbed soil. Effects of wood incorporated into the soil were unclear.

Bacteria form the most diverse and adaptable group of microbiota colonising wood, but only nine species were recorded in soil which had wood debris incorporated in it. Some of the bacteria, e.g. *Bacillus* and *Pseudomonas*, can degrade wood constituents and increase wood porosity and permeability. *Pseudomonas putida*, recorded in this study, is a stimulant of Basidiomycota (Rainey 1991), while Actinomycetes, *Alcaligenes, Bacillus cereus, B. subtilis, Collimonas*, and *Pseudomonas* have been described as parasites of Ascomycota and Basidiomycota (Leveau and Preston 2008; Wichmann *et al*. 2008).

The highest average ratio of fungal *cfu* number : bacterial *cfu* number was recorded after shallow ploughing and where chipped wood debris was left on the soil surface (Table 9). This agrees with the generally reported tendency in colonisation of organic matter, whereby depositing organic residues on the soil surface results in microbial activity dominated by fungi (Hendrix *et al*. 1986; Sá *et al*. 2001).

The abundance of fungi, including *Armillaria* and *Heterobasidion* antagonists, increased with higher concentrations of potassium (particularly after shallow ploughing) and decreased with higher concentrations of magnesium (after deep ploughing). A similar effect of magnesium was observed by Grantina-Ievina *et al.* (2013). Potassium and magnesium are necessary for biological activity because they are water content regulators and enzyme cofactors (Miles and Chang 1997).

Low incidence of *Armillaria* and *Heterobasidion* butt and root rot are said to correlate generally with increased populations of antagonistic fungi, particularly of *Trichoderma* and *Penicillium* species (Korhonen and Stenlid 1998; Stenlid and Redfern 1998). In the present study, however, the greatest biomass and density and the least tree mortality on the Scots pine plantation were not associated with the greatest fungal abundance, but rather with only moderate abundance of fungi, including antagonists. Some reasons for our results may include: (i) inter- and intraspecific variation among *Armillaria* and *Heterobasidion* in reaction to antagonist metabolites; (ii) intraspecific variation among antagonists in production of metabolites; (iii) the quantity and quality of metabolites produced; (iv) character of the active compound; (v) phytotoxicity of fungal metabolites. Similar reasons were cause of inconsistencies in reactions of plants and pathogens to *P. adametzii* extracts and mycelium found in the studies of Szwajkowska-Michałek *et al.* (2012).

It is relevant that the microbiological analysis was done only once, which was 9 years after the treatment, whereas the health of the trees also resulted from natural and uncontrolled plant-fungus-bacteria interactions occurring during earlier years. These interactions are highly dynamic in nature and based on co-evolutionary pressures. Information on the temporal development of microbial community structure by analyses at intervals may be necessary to provide a microbiological explanation for the benefits of the procedures that gave the best tree growth.

## Conclusions

The implications of different pre-planting soil preparations and post-harvest wood debris utilisation for plant protection should be considered in forest management. Since biomass and density of *P. sylvestris* trees were highest and mortality was least after ridging and leaving coarse wood debris on the surface, this treatment should be recommended when re-planting clear-cut forest.

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