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Influence of resting and pine sawdust application on chemical changes in post-agricultural soil and the ectomycorrhizal community of growing Scots pine saplings

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Abstract. Changes in chemical compounds and in ectomycorrhizal structure were determined for Scots pine growing on post agricultural soil lying fallow for 3, 6 and 15 years, after amendment with pine sawdust. Soil without any amendments was used as the control treatment. Comparing the ectomycorrhizal structure 15 years after the application of pine sawdust revealed no significant differences in abundance or species richness between soil with and without organic enrichment. The results showed that the ectomycorrhizal status depends on soil conditions (soil pH, nitrogen content), which remain unaffected by saw dust application. In all treatments, the most frequently occurring ectomycorrhizae genera were Dermocybe, Hebeloma, Suillus, Tomentella and Tricholoma. Two species (Paxillus involutus, Amanita muscaria) were specific to the control plots that lay fallow for 15 years.

Keywords: Scots pine, ectomycorrhizal fungi, agricultural lands, pine sawdust

1. Introduction

Afforestation and reforestation programmes, implemented currently in Poland, stipulate high expenditures on the production of appropriate planting stock, establishment of forests and the protection of young plantations. Between 1945 and 2005, 1395 thousand hectares were afforested in Poland (Smykała 1988), and up to 2020, approximately 680 thousand hectares of forests are to be established in line with the Program on the Augmentation of Forest Cover (Zaleski 2003). A part of planting stock produced in open-ground and container forest nurseries is used for afforestation of post-agricultural or set-aside lands. These comprise low-quality and infertile soils, often long-term abandoned or else damaged due to industrial pollution, thus forest planting in such areas can be much less successful when compared to reforestation of forest soils. As a general rule, uncultivated soils have a specific structure, characteristic composition of microbial communities, and they lack mycorrhizal fungi which are antagonistic against pathogens.

Scots pine (Pinus sylvestris) is most represented in planting stock (approximately 27%) produced in Poland's forest nurseries (Zajączkowski 2008). Myccorhizal associations with pine seedling roots are of great importance in adaptation and endurance of trees after planting in new sites, especially on post-agricultural soils with limited supply of mycorrhizal inoculum (e.g. Stenström, Ek 1990; Hilszczańska, Sierota 2006; Iwański et al. 2006; Sierota, Hilszczańska 2009; Hilszczańska et al. 2012).

Inoculation of seedlings with mycorrhizal fungi is one of the means for improving seedling condition and planting efficiency. Other methods used for stimulation of microbial processes in the soil include a variety of cultivation techniques, e.g. mulching (Kowalski 1997) or the so-called extra mycorrhisation, i.e. supplementary application of substrates with mycorrhizal spores (Hilszczańska 2007).

The processes of organic matter decomposition are different in agricultural and forest soils due to their specific physical, chemical and biological properties. In agricultural soils, humus is characteristic of high contents of humic acids, whereas in forest humus, fulvic acids prevail. Hence, agricultural and forest soils are inhabited by different bacterial and fungal populations, and in agricultural soils, bacte-

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rial populations predominate (Paul, Clark 2000). In general, microbial processes and fertility can be enhanced in afforested soils by adding organic substrates, such as composts, sawdust and logging residues (Sobczak 1990; Gorzelak 1998; Sierota, Kwaśna 1999; Oszako, Olejarski 2003; Olejarski 2005). Furthermore, increasing populations of antagonistic microorganisms against root pathogens in the soil has beneficial effects on the growth and health of young forest stands (Kwaśna et al. 2000).

The aim of the present study was to evaluate soil chemistry and the composition of mycorrhizal fungal communities in the soil treated with fresh coniferous sawdust. The evaluation was carried out 15 years after the treatment, which was performed in the area designated for afforestation, in the forest district Głęboki Bród (Forest Division Gulbin, management unit 320a).

2. Materials and Methods

The experimental plot (5 acres) was established in 1995 on post-agricultural land designated for afforestation. Natural spatial arrangement of the area comprised three land sections, which differed in terms of the duration of the set -aside period. At the start of observations, no land section was under agricultural cultivation (for 3, 6 and 15 years, as per information obtained from the Forest District Głęboki Bród), and only herbaceous vegetation was recorded in this area. The treatment was performed in the fall of 1995, when 5-8 cm layer of pine sawdust (5-8 m³/a) was applied onto the soil and then ploughed down to the depth 35 cm. In the spring of 1996, after soil ploughing and harrowing, one-year-old Scots pine seedlings (produced in a nearby nursery) were planted (planting density: 1.0 × 1.5 m). Taking into account experiment determinants at that time, only one study strip of land could be set out. The strip included three rows of trees growing in sawdust-treated soil and two adjacent control strips with three rows of trees each (six control rows) (Fig. 1). The area of plots (3) on the soil fallow for 3 years (K3 and T3) was 20 m × 4.5 m each, whereas experimental plots (6) on 6-year and 15-year fallows were half as large (10 × 4.5 m each). Consequently, in 1996, the opening number of the control trees was two times higher than that of trees growing in soil treated with sawdust (246 control specimens versus 123 treated specimens). Furthermore, the spatial arrangement of the study area had a decisive effect on the number of trees observed on different categories of fallow land (in terms of the land set-aside duration) (Fig. 1).

In 2011 (15 years after sawdust treatment), soil chemical analyses were carried out along with quantitative and qualitative evaluations of mycorrhizal associations with the roots of treated and untreated trees.

Soil samples for chemical analyses were taken at the depth 20 cm, after removal of the litter layer. Soil chemical analyses were performed following the methodology by ICP Forests (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests). Soil reaction (pH-H₂O and pH-KCl) was measured potentiometrically in accordance with PN-ISO 103390:1997 standard; organic carbon content was determined using PN-ISO10694:2002 elementary analysis and total nitrogen content - with PN-13878:2002 elementary analysis; potassium, calcium, magnesium contents in the presence of ammonium acetate - in line with PB-05 ed. 2 procedure, and soluble phosphorous (P2O5) was determined with the use of the Egner-Riehm method. All the analyses were performed by the Laboratory of Environmental Chemistry, Forest Research Institute, Poland.

Soil samples with mycorrhizal roots (0.7 dm³, without the litter layer) were collected at the depth 20 cm with the use of a soil drill (7 cm diameter), around the trees observed, at three points within each study plot. The samples were transported to the laboratory and stored in the cold room at 4°C. Mycorhizas were separated from the soil (sieved and rinsed with tap water) and stored in distilled water in the cold room for further analyses. Mycorrhiza examinations were carried out using a stereomicroscope (10–50 × magnification). The identification was based on the presence and

	Control plot	Sawdust application plot	Control plot	
Fallow 3 years	K3 (62)	T3 (61)	K3 (62)	
Fallow 6 years	K6 (31)	T6 (32)	K6 (31)	
Fallow 15 years	K15 (30)	T15 (30)	K15 (30)	

Figure 1. Study plot design in Głęboki Bród Forest District. Descriptions: K – control plot; T – plots with pine sawdust application; 3, 6, 15 – fallow period (years); (...) – number of trees on each plot.

appearance of hyphal mantle, extramatrical mycelium and hyphae (Agerer 1987–2006; Agerer, Rambold 2004–2007, Ingelby et al. 1990). There were determined fungal species (or genera) as well as the total number of mycorrhizas observed in each experimental treatment. Next, the mean mycorrhiza number per treatment and species richness per 1 dm³ were appraised.

Differences in abundance of mycorrhizal associations in the treatments examined was tested with ANOVA (StatgraphicsTM Centurion software). The differences between the means obtained were assessed with the RIR Tukey's test at (p < 0.05). Correspondence analysis (Statistica 2008, StatSoft) was used for determination of the structure of relationships between mycorrhizal fungi and the treatment applied. Data for analyses were arranged in the code matrix, and the treatments were treated as experimental variables.

3. Results

In the experimental land sections (fallow for 3, 6 or 15 years), initial values of soil reaction (measured in 1995, before sawdust application) differed from each other (Table 1). The highest soil reaction value (pH = 7) was observed in 6-year fallow, and the lowest in 3-year fallow (pH = 4.4). Fifteen years after the treatment with pine sawdust and tree planting, soil reaction values evened out and ranged from 4.7 to 5.8. At the same time, higher pH values were observed in sawdust-treated soils when compared to the control. On the other hand, soil carbon contents (C%) increased twofold while soil nitrogen contents stayed at almost unchanged levels (N% = 0.6–0.9). In all the fallow land variants tested, C% values were slightly higher in sawdust treated soils. In

1995, the initial C/N ratio was typical for post-agricultural soils and ranged from 13.2 to 14.7, whereas 15 years after the treatment, it increased in all the fallow lands tested. This increase was the smallest in 3-year fallow (16.85) and the biggest in 15-year fallow (28.7), and each of these values was obtained in sawdust-treated soils. Generally, soil P contents assessed in 2011 were higher when compared to 1995, except for 3-year fallow, where initial P content was considerably high (10.7 mg/100 g P₂O₅) and it decreased by almost half. Fifteen years after the treatment, soil potassium content increased only in 3-year fallow, whereas in other two types of fallows observed, K contents in the soil were somewhat lower. Magnesium and calcium contents decreased when compared to the initial values, except for sawdust treated soil in 3-year fallow.

Considerably more mycorrhizas (on average 239.3) were formed in Scots pine trees growing in sawdust treated soils in comparison with the control trees (on average 181). The differences were not statistically significant (Fig. 2a).

The highest numbers of living mycorrhizas (app. 300) were found on the roots of trees growing in the soils fallow for 3 and 15 years and treated with pine sawdust (Fig. 2b). The control trees growing on analogous set-aside lands showed considerably fewer micorrhizas (on average: 213 in 3-year fallow and 123 in 15-year fallow). However, in 6-year fallow, higher numbers of mycorrhizas were observed in the control trees when compared to those growing in sawdust treated soils. On the whole, when compared with all the treatment tested, the lowest mycorrhiza number (101) was observed in pine trees growing in sawdust-treated soil in 6-year fallow. Nonetheless, no statistical significance was proved for the differences observed.

Table 1. Soil parameters for the treatments in two terms of evaluation

Soil parameters	Fallow 3 years			Fallow 6 years			Fallow 15 years		
	1995*	2011		1005*	2011		1005*	2011	
		Sawdust	Control	- 1995*	Sawdust	Control	- 1995*	Sawdust	Control
pH H ₂ O	4,4	5,8	5,2	7,0	4,9	4,7	5,1	4,9	4,7
pH KCl	3,8	5,23	4,3	6,0	4,3	4,0	4,1	4,1	4,1
C (%)	0,87	1,525	1,163	0,98	2,030	2,015	1,2	2,385	2,040
N (%)	0,059	0,066	0,069	0,071	0,083	0,098	0,091	0,083	0,088
C/N	14,7	23,11	16,85	13,8	24,46	20,56	13,2	28,7	23,18
P ₂ O ₅ (mg/100g)	10,7	6,4	5,1	2,4	6,7	5,5	1,1	3,2	3,3
K (mg/100g)	1,2	4,8	3,7	5,0	4,3	4,3	4,0	3,7	2,9
Ca (mg/100g)	18,5	40,1	16,3	143,5	13,8	5,1	32,5	10,6	6,7
Mg (mg/100g)	2,5	1,9	0,7	9,2	1,2	0,6	2,5	0,5	0,3

Source: Sierota, Kwaśna (1998)

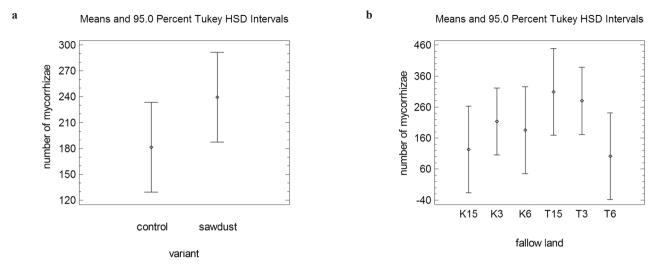


Figure 2. Number of mycorrhizae associated with Scots pine roots growing in: a – different treatments (p = 0.2594), b – soils at different fallow period (p = 0.1290). Descriptions as in the fig. 1.

Table 2. Mycorrhizal fungal taxa associated with Scots pine roots and their presence in the studied treatments

			Sawdust		Control			
No.	Fungal taxa	fallow 3 years (T3)	fallow 6 years (T6)	fallow 15 years (T15)	fallow 3 years (K3)	fallow 6 years (K6)	fallow 15 years (K15)	
1	Amanita muscaria						+	
2	Cenococcum geophilum	+		+				
3	Cortinarius odorifer			+	+	+		
4	Cortinarius sp.	+	+	+	+			
5	Dermocybe palustris	+			+		+	
6	Dermocybe semisanguinea	+	+	+	+	+	+	
7	Dermocybe sp.					+		
8	Elaphomyces sp.		+		+			
9	Hebeloma crustuliniforme			+	+			
10	Hebeloma edurum						+	
11	Hebeloma sacchariolens			+	+			
12	Hebeloma sp.	+	+	+	+	+	+	
13	Paxillus involutus						+	
14	Rhizopogon sp.				+			
15	Russula sp.		+					
16	Suillus luletus	+	+			+	+	
17	Suillus sp.	+	+	+	+		+	
18	Thelephora terrestris	+		+	+	+	+	
19	Tomentella ferruginea		+	+	+	+	+	
20	Tomentella sp.	+	+	+	+		+	
21	Tricholoma sp	+	+	+	+	+	+	
22	Wilcoxina sp.	+			+	+		
Total	(for fallows)	11	10	12	15	9	12	
Total (for treatments)			17			20		

On tree roots collected with soil samples, altogether 22 my-corrhiza morphotypes were identified. Fungal species were determined in 12 morphotypes and the fungal genera were determined in 10 morphotypes (Table 2). Morphotype numbers were similar in all the treatments tested and ranged from 9 to 15. Outlier values were observed in Scots pine trees growing on the control fallows (3- and 6-year fallows). The highest species richness (12 morphotypes) was observed in 15-year fallow treated with sawdust (as well as in 15-year control fallow land). Notwithstanding the duration of the set-aside period, in general, the richness mycorrhizal fungal species was slightly lower in sawdust treatments than that observed in untreated areas (in sum 17 and 20 morphotypes, respectively).

The analyses carried out with reference to species richness of ectomycorrhizal fungi indicated that fungi from *Dermocybe*, *Hebeloma*, *Suillus*, *Tomentella* and *Tricholoma* genera were omnipresent in trees growing under the conditions of all six treatments tested. At the same time, mycorrhizas formed by *Cenococcum geophilum*, *Elaphomyces* sp., *Rhizopogon* sp., *Russula* sp., *Paxillus involutus*, *Amanita muscaria* were hardly ever observed, and the latter two fungal species were found only in tree roots collected from the control area of 15-year fallow.

Correspondence analysis of ectomycorrhizal fungi communities showed the structure of mycorhiza–treatment relationships (Fig. 3). The quality of data representation was high, i.e. on average about 0.72 (the maximum value = 1). The first dimension (X-axis) explained 42.07% of the variability, and the second dimension (Y-axis) 29.7%. Fungal species positioned at

the longest distance from 0.0 axis, namely *A. muscaria*, *P. involutus*, *Elaphomyces* sp. and *Russula* sp. showed the highest effects on the inertia of rows (here fungal species in mycorrhizas) (Fig. 3). The inertia of columns (here experimental treatments) were affected the most by sawdust treatment in 6-year fallow (T6 plot) as well as no treatment applied in 15-year fallow (K15 plot). Scots pine seedlings grown under the conditions of other treatments tested (K3, K6, T3 and T15) showed similar mycorrhiza structures.

4. Discussion

Fifteen years after sawdust application into fallow soils, the richness of mycorrhizal fungal species was analogous in all the treatments tested. Several reasons could be attributed to no evident differences in the qualitative status of mycorrhizas in Scots pine trees grown on soils enriched with sawdust when compared to the control trees. Similar soil reaction and nitrogen contents observed in all the treatments seem to be one of the explanations. Significant differences in mycorrhizal species abundance can be expected in microhabitats, which differ in terms of soil reaction and chemical properties or humidity and temperature conditions (Last et al. 1987; Blasius, Oberwinkler 1989; Jumpponen et al. 1999; Buee et al. 2005). Another factor involved could be competition between organisms sharing the same ecological niche with mycorrhizal fungi (e.g. saprophytic fungi, soil invertebrates) (Kwaśna et al. 2000).

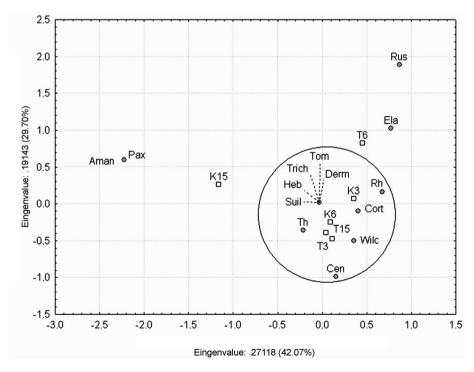


Figure 3. Correspondence analysis (CA) comparing mycorrhizal community of Scots pine in different treatments: Aman – Amanita muscaria, Cen – Cenococcum geophilum, Cort – Cortinarius sp., Derm – Dermocybe sp., Ela – Elaphomyces sp., Heb – Hebeloma sp., Pax – Paxillus involutus, Rh – Rhizopogon sp., Rus–Russula sp., Suil–Suillus sp., Th–Thelephora terrestris, Tom–Tomentella sp., Trich–Tricholoma sp., Wilc – Wilcoxina sp.

Description of fungi as above, descriptions of treatments as in Table 2.

The results of the studies on mycorrhizal status, conducted in several-year-old Scots pine plantations on post-agricultural lands, showed considerable quantitative and qualitative resemblance in the communities of ectomyccorrhizal fungi observed (Hilszczańska et al. 2008; Sierota, Hilszczańska 2009; Małecka, Hilszczańska 2014). This seems to be a result of a significant effect of myccorrhizal inoculum availability as well as changes ongoing in the organisms inhabiting the rhizosphere. In forest ecosystems, an increase of myccorrhizal diversity in Douglas fir (*Pseudotsuga menziesii*) was observed in 5- to 26-year-old stands (Twieg et al. 2007). Analogous patterns could also occur in Scots pine (*P. sylvestris*); however, in forest tree stands growing on post-agricultural lands, the processes might be slower due to, among others, specific soil biological characteristics.

In view of the results of a study on ectomycorrhizal symbiosis effects on tree growth and nutrition determined by the type of extramatrical (soil) mycelium of mycorrhizal fungi (Agerer 2001), the presence of Cortinarius spp. mycorrhizas observed in Scots pine trees growing in the soil treated with sawdust favored arguments of the necessity of such treatment. Cortinarius spp. mycelia represent the so-called medium-distance exploration type (Agerer 2001). Tree seedlings with such mycorrhizas are able to take more nitrogen, phosphorus and potassium from soil organic layers (humus) when compared to those with mycorrhizas formed by short-distance exploration mycelia (Bendig, Read 1995). In the present study, mycorrhizas formed by fungal species, typical of early growth stages of Scots pine (Suillus spp., Hebeloma spp., Wilcoxina sp.) and universal fungi, i.e. these present regardless of host-plant age (Thelephora terrestris, Tomentella sp.), were observed. The presence of mycorrhizas with various functions and survival strategies looks promising for seedling development. For instance, Suillus spp. fungi represent long-distance exploration type (Agerer 2001) and they are hydrophobic (Unestam, Sun 1995), whereas T. terrestris is a hydrophilic species. No statistically significant differences observed with regard to mycorrhiza abundance found in sawdust treated Scots pine seedlings when compared with untreated trees are probably due to the lack of differences between chemical characteristics of treated and untreated soils. Soil reaction values were initially different depending on the fallow land observed, and then they changed in the first year after sawdust treatments (Sierota, Kwaśna 1998b) as well as in the second year after the treatment and land afforestation (Sierota, Kwaśna 1999; Kwaśna et al. 2000). Afterwards, soil reaction values observed in the area stayed at similar levels during subsequent years of the growth of planted trees. Soil enrichment with sawdust and turf plowing resulted in more than twofold increase of soil organic carbon (SOC) contents. Since soil N content stayed at a stable level, a rapid increase of the C/N ratio (depending on the type of fallow land tested C/N ratio ranged from 28 to 38) (Sierota, Kwaśna 1998b) was observed. Soil analyses, carried out 15 years after sawdust treatment, showed that C/N values in sawdust treated soil ranged from 23 to 28 (Table 1). In other words, the C/N ratio pointed to the level characteristic for soils under Scots pine stands (Wawrzoniak et al. 2004; Małecka et al. 2014). In the conditions of afforested fallow land, the patterns observed could be explained by longterm equilibrium between soil carbon input via organic residues and its loss caused by decomposition of organic matter. When assessed 15 years after sawdust treatment, notwithstanding the duration of the set-aside period, carbon contents in sawdust treated soils were higher than those in the control. This suggests continuing effects of sawdust wood decomposition as carbon constitutes about 40% of Scots pine wood dry mass (Kollmann 1951). The role of the aggregate of organisms that live in the soil (edaphon) cannot be neglected, since edaphon activity is associated both with soil mineralisation to simple inorganic compounds (carbon immobilisation) as well as carbon consumption (secondary immobilisation). Numerous studies on fungal communities in the soil (Sierota, Kwaśna 1998a,b; Kwaśna, Sierota 1999; Sierota, Kwaśna 1999; Kwaśna et al. 2000) showed a changing soil chemistry as well as qualitative and quantitative changes in soil fungal community composition after sawdust treatment. The most important element of these alterations was the emergence of fungal species antagonistic against root pathogens (mostly from the genus Trichoderma) together with other species involved in sawdust substrate colonisation and decomposition. Furthermore, soil treatment with sawdust activated soil nematodes, and especially those feeding on bacteria, whereas the proportion of nematode plant parasites decreased many times (Kwaśna et al. 2001).

A fungal community that shapes mycorrhizal systems with tree roots is an indispensable element of soil microorganisms' habitat, and its qualitative and quantitative composition is closely related to microbial and chemical processes taking place in the soil. The rhozosphere of the trees tested was shaped by dominant species of soil fungi. i.e. Geomyces pannorum (Link) Sigler & J.W. Carmich (common fungal species in soils, including those degraded and post-agricultural) and Pseudogymnoascus roseus Raiłło (soil fungus associated with tree roots and wood) as well as diminutive amounts of fungi from the Trichoderma genus, the absence of pathogenic fungi (Pythium, Phytophthora, Heterobasidion annosum) (Małecka 2012) and the aforementioned chemical soil properties. Even though no statistical differences in mycorrhizal abundance were found among the treatments studied, the higher mycorrhiza numbers were observed in two variants of sawdust treatment. Sawdust application probably enhanced soil aeration (Kwaśna et al. 2000), and therefore it stimulated mycorrhiza formation. There is no explanation for the fact that the lowest abundance of mycorrhizas was observed in Scots pine growing under the conditions of 6-year fallow land treated with sawdust. It is possible that position of pine trees in this area (Fig. 1) could somehow

hinder the development of seedling roots, and thus mycorrhiza numbers relatively decreased. It would be very helpful to analyse historical data on the area studied to better understand the results obtained but these are unavailable.

Application of sawdust to the soil had a positive effect on the average value of DBH in treated trees, which was higher than that in the control trees (not statistically significant differences) (Małecka 2012). Supposedly, quantitative and qualitative mycorrhiza composition in the first years of the growth of planted trees positively influenced their height increment. This was confirmed by historical data concerning the differences observed in tree height and height increment between treated and the control trees during the first years of the growth of planted Scots pine seedlings for the benefit of those growing on sawdust-treated soils (Sierota et al. 2002). Similar relationships were shown in the study on electrical resistance in vascular bundle tissues (phloem, xylem, sapwood) assessed in sawdust treated and untreated trees (Małecka 2012). The parameter measured is an indirect indicator of tree health condition (Ubvsz 2001). The interaction of the obtained values proved significantly lower electrical resistance (higher tissue conductivity) in sawdust-treated trees in comparison with the control (notwithstanding the duration of the set-aside period). The lowest electrical resistance values (best health condition) were observed in Scots pine trees growing on sawdust-treated soils, earlier set-aside for 3 years. The best health condition of these trees was confirmed by the results of the survival analysis performed on the planted trees growing under the conditions of all the treatments tested (Małecka 2012), as well as by the number of mycorrhizas observed in tree roots (Fig. 2b). Comparable relations were shown when tree electrical resistance index was analysed. This parameter values were higher in trees with less vitality (here: the control trees) (Małecka 2012).

5. Conclusions

The results of the present study showed differentiated effects of sawdust amendments added to fallow post-agricultural soils on mycorrhiza abundance. The highest mycorrhiza numbers were observed in 15-year-old Scots pine trees growing on sawdust treated soils, however, no statistical differences were found among the mean values obtained in all the treatments tested. The mycorrhizas observed were similar in terms of their quality and no statistically significant differences in the numbers of mycorrhiza morphotypes were found with reference to the duration of the land set-aside period. Omnipresent fungi prevailed in the composition of the mycorrhizas examined. The content of available nitrogen (evaluated based on the C/N ratio) in the soil decreased with the duration of the set-aside period (especially in 6- and 15-year fallow lands). This resulted in increased values of the C/N ratio obtained in sawdust-treated soils. A decreased content of soil nitrogen in 15-year fallow land could have an effect on formation of more mycorrhizas in Scots pine trees growing on sawdust treated soils in 15-year fallow land.

Conflict of interest

The authors declare no potential conflicts.

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Author's contribution

M.M. – research conception, field works, statistical tests, literature review, manuscript preparation and edition; D.H. – evaluations of mycorrhizas, literature review, manuscript preparation and edition.