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Communities of Mycorrhizal Fungi among Seedlings of Scots Pine (*Pinus sylvestris* L.) Growing on a Clearcut in Microsites Generated by Different Site-Preparation Methods

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Abstract: In European forests, the Scots pine (*Pinus sylvestris* L.) most often regenerates on clearcuts, following mechanical site preparation. Both of these silvicultural treatments (the removal of trees and preparation) have an impact on soil properties, and on the mycorrhizal fungi associated with the roots of seedlings. We therefore compared assemblages of mycorrhizal fungi associating with natural-regeneration pine seedlings growing on a clearcut, in relation to six types of microsite created using three mechanical site-preparation tools, i.e., a double-mouldboard forest plough (creating furrow and ridge), an active single-disc plough (establishing another type of furrow and ridge), and a forest mill—developing strips, as well as a non-mechanical site preparation control. A total of 46 taxa of mycorrhizal fungi were detected, with *Wilcoxina mikolae* being the most abundant species (relative abundance—79.8%), and the one occurring most frequently (96.8%). Other abundant mycorrhizal fungi were *Thelephora terrestris* (3.8%), *Tylospora asterophora* (3.2%), *Hyaloscypha bicolor* (2.2%), and *Cenococcum geophilum* (1.7%). The roots of seedlings growing in the non-mechanical site preparation control were characterised by a significantly greater presence of mycorrhizal root tips, compared with the roots of seedlings growing at other microsites. The highest percentage of non-mycorrhizal root tips was present on pines growing on the two types of ridge: the microsites which characterized the highest levels of mineral nutrients. Communities of mycorrhizal fungi differed between microsites. The five microsites: both types of furrow, forest plough ridge, forest mill strip, and non-mechanical site preparation control, were not found to differ from each other, but did differ from the active plough ridge treatment. The highest diversity of mycorrhizal fungi (Shannon–Wiener and Simpson indexes) was in the non-mechanical site preparation control. Any method of mechanical site preparation in the clearcut decreases the level of root mycorrhization and the biodiversity of mycorrhizal fungi. The least suitable method from the point of view of mycorrhizal fungal communities is the use of an active plough.

Keywords: biodiversity; natural regeneration; ectomycorrhiza; ectendomycorrhiza; mechanical site preparation

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1. Introduction

The Scots pine (*Pinus sylvestris* L.) is a typical Euro-Siberian species, and in fact one of the most important commercially throughout east-central Europe [1]. It is also the main forest-forming tree in Poland, accounting for 58.2% of Poland's forests by area and 61.2% by volume [2]. Scots pines depend critically on ectomycorrhizal (ECM) symbiosis for optimal development under natural conditions [3]. Mycorrhizae increase the root absorptive area,

enhance nutrient acquisition, facilitate water uptake, enhance tolerance to adverse soil conditions, and reduce the effects of soil-borne pathogens on tree roots [4].

A variety of silvicultural options exist to regenerate Scots pine. However, as recruitment among Scots pines is crucially dependent on the factor of light, the clearcut is the main silvicultural system still commonly in use in many European countries, including Poland [5].

Forest clearcut logging entailing complete trunk-removal may induce quantitative and qualitative changes in ECM fungal communities, by changing the age structure and species composition of the forest overstorey trees, as well as the understorey plants serving as hosts. The two major groups of factors contributing to these changes involve a shift in the amount and type of inoculum, as well as a change in the soil environment [6,7].

The main sources of ECM fungal inoculum are pre-senescent mycorrhizae, surviving mycelium, spores, and sclerotia [8]. The time passing from the felling of trees is one of the factors determining the availability of these different forms of inoculum in soil [6]. In the first (usually 2–3) years after felling, there is a rapid loss of activity among the mycorrhizal roots of harvested tree stumps and the associated fungal mycelia [9,10]. The levels of other forms of inoculum also decrease over time [11,12]. Changes of these kinds do not always result in a decline in mycorrhizal colonisation on a clearcut, given the appearance of so-called pioneer species of the genera *Thelephora*, *Hebeloma*, *Inocybe*, *Laccaria*, *Paxillus*, *Geastrum*, *Suillus*, and *Scleroderma* [13,14]. These species owe their abundant mycorrhiza formation to effective spore-mediated dissemination [13,15].

A change in the species composition of communities of ECM on a clearcut may reflect ongoing changes in soil of a physical, chemical, and biological (other than inoculum-related) nature. Soil temperatures on a clearcut may be 2−5 °C higher than in soils under a stand. It is also usual for temperature amplitude to grow greater. Higher soil humidity is a possibility associated with reduced transpiration, but humidity might also fall, as there is greater evaporation from warmer soil [12,16]. Soil temperature has been found to influence ECM fungi, and the associations that form with *Pseudotsuga menziesii* [17]. Populations of soil microorganisms may also change in response to modifications of microclimate and the supply of carbon (with much-reduced litterfall). Depending on their quantitative and qualitative composition, communities of soil microorganisms also exert an influence on the formation of mycorrhizae [18].

Equally, there are authors who contend that the major impact of clearcut logging is in changing the species composition of the ECM fungal assemblage, rather than reducing the percentage of roots colonised. The new communities are better adapted to the new conditions than those in the forest would have been [6].

Mechanical site preparation (MSP) is recommended to create optimum conditions for seed germination and seedling growth in a clearcut area [19], with the proximate aim being to limit competition with other plants for light, water, and nutrients [20,21], but also to mitigate the allelopathic effects of the ground layer of vegetation [22,23]. MSP can modify the physical properties of soil, such as those relating to water content, aeration, temperature, and bulk density; as well as its chemical properties, relating to organic-matter content, nutrient availability, and pH [24–27].

Basic MSP methods include the removal of forest litter to expose mineral soil, inversion of litter and mineral soil, elevation of mineral soil, and the mixing of litter with soil [26,28]. MSP utilises various tools and machines that differ in the degree to which the soil is disturbed, as measured in terms of area and depth [29].

By removing, displacing, or disturbing the organic layers on the forest floor, MSP may reduce the amount of ECM fungal inoculum, as the density of sclerotia and ECM root tips is greater in the soil's organic horizons (the forest floor O and A horizons) than in the mineral layers [30,31]. The numbers of living and dead mycorrhizae are not shown to change to depths of 15 cm below the soil profile. Below that depth it is possible to observe a drastic reduction in the abundance of living mycorrhizae in particular [32–34].

However, there is research indicating how the level of inoculum in the mineral soil of many coniferous forests or clearcuts does not limit mycorrhizal colonisation, even when the density of ECM is much greater in the O horizon of the same soil [34–36]. MSP often tends to ensure a transfer of inoculum, rather than its removal, with a potential consequence for ECM numbers and diversity depending on the method of MSP used [6].

The work detailed here aimed to compare the influence of three methods of MSP, as well as of a non-MSP approach, in relation to the communities of mycorrhizal fungi associated with natural-regeneration Scots pine seedlings on a recently-made clearcut. The tools used in the experiment, i.e., a forest plough (FP), an active plough (AP), and a forest mill (FM) intervene in the soil environment in various ways and to varying extents (as regards both depth and area), but with the overall effect being the generation of five different microsites. The hypothesis to be tested was thus that microsites created by the aforementioned MSP methods affect the numbers of mycorrhizal species, the species composition of the assemblage present, and the degree of mycorrhizal colonisation.

2. Materials and Methods

2.1. Study Area

Field research was conducted in Poland's Spychowo Forest District, located ca. 150 km to the north-east of Warsaw (N 53°28′27″, E 21°28′52″, 132 m a.s.l.). The annual mean temperature in the study area is 7.6 °C. The warmest month there is July (18.3 °C), and the coldest is January (3.1 °C). The growing season (with an average daily temperature higher than 5 °C) lasts 207 days. Long-term mean annual precipitation is at the level of 570.3 mm. The soil in the study area is classified as podsolic, formed on loose sands and with typical mor humus [37].

Specifically, trials were run in a clearcut area 600 m long and of width 40 m. Before felling, the stand here had been composed of 135-year-old Scots pine trees (*Pinus sylvestris* L.) (stand volume, 276 m^3 ha^{-1}; stand density, 400 stems/ha; average tree height, 23 m; average dbh, 35 cm). To the east, the felled area borders on to a five-year-old plantation with pines regenerating naturally. On the other side it is surrounded by pine stands aged 120 years. The herb layer is dominated by *Dicranum polysetum*, *D. scoparium*, *Vaccinium myrtillus*, *V. vitis-idaea*, *Calluna vulgaris*, *Luzula pilosa*, *Festuca ovina*, and *Lycopodium annotinum*. The plant community is classified as *Peucedano-Pinetum typicum* [37].

2.2. Experimental Design and Treatments

The established clearcut area was divided into three parts—experimental blocks, each approximately 200 m long. Four different approaches to site preparation were assigned at random to each block. Blocks were subdivided into four plots (of 50 × 40 m), with a different MSP method applied on each, or with the area left without MSP in order to serve as a control (c). MSP was performed using either an LPz OTL double mould-board forest plough (FP), a P1T active single-disc plough (AP), or an FL forest mill (FM) (manufacturer: *Ośrodek Techniki Leśnej*, Jarocin, Poland, http://www.otljarocin.lasy.gov.pl/preparation-of-soil-and-afforestation, accessed on 1 February 2022).

The furrows made by the FP (f1) are rectangular, 70 cm in width and 5–10 cm deep. Leaf litter and the humus layer are cut and placed as ridges (r1) on both sides of the furrow (f1), in which mineral soil lies exposed. Using an AP, the rotating disc forms parabolic furrows (f2), 40 cm in width and up to 10 cm deep. A partial mixing of leaf litter and some of the humus occurs, as these are piled on ridges (r2). A furrow-bottom is scarified and covered with a mixture of humus and mineral soil. The working part of the FM is a horizontal cylinder with cutting blades revolving at a rate of 1000 rpm. This achieves a crushing and mixing of forest vegetation, leaf litter, humus, and mineral soil to a depth of 30 cm in strips 40 cm in width (s). The undisturbed strips between are 110 cm wide. For all of the MSP methods, the distance between the centres of the neighbouring furrows or strips was 1.5 m [38].

The stand was felled in late autumn (October and November 2011), with MSP performed in winter (December 2011). The natural regeneration of Scots pine then commenced in the spring of the next year. The adjacent stands acted as the seed source, with these being located to the west (as young stands are located to the east).

2.3. Sampling of Plant Material

One-year-old pine seedlings with soil surrounding the root system were collected at the end of the growing season (in November 2012). Harvesting of seedlings from each experimental plot was a random process. They were taken from the plots with FP and AP from both furrows and ridges (FP—f1, r1; AP—f2, r2). In the case of FM, seedlings were harvested from the strips (s) only, due to the lack of seedlings on undisturbed strips (os). Forty-two seedlings were in fact collected from each plot, representing the six experimental variants (i.e., microsites: furrows (f1, f2), ridges (r1, r2), strips (s), and without MSP (c)). A total of 756 seedlings were thus picked, placed in labelled plastic bags, transported to the laboratory, and stored at −20 °C for further investigation.

2.4. Mycorrhizal Assessment

Washed root systems were lined on a Petri dish with distilled water and analysed (at 10–40× magnification) using a Delta IPOS-808 dissecting microscope coupled with a camera. Mycorrhizal root-tips were identified by reference to the presence (also colour, shape and surface texture) of the mycelial mantle, as well as the presence of extramatrical mycelium and rhizomorphs. On the basis of the above features and illustrated scientific studies [39], vital mycorrhizal root-tips (VM) were counted and attributed to corresponding morphotypes. Also counted were non-mycorrhizal (NM) root-tips—lacking the characteristics of ectomycorrhizae and having root hairs, and non-vital (NV) root-tips—heavily wrinkled and dead. Each mycorrhizal morphotype was photographed. Three representative mycorrhizal root-tips of each observed morphotype were placed in Eppendorf tubes filled with 70% ethanol, labelled, and stored at −20 °C until further molecular analyses were commenced with. A total of 295 morphotypes were collected. The final grouping and assignment of morphotypes classified previously on the basis of the morphological characteristics of mycorrhizal root-tips was achieved through the analysis of fungal rDNA.

2.5. Molecular Identification of Mycorrhizal Fungi

We amplified the internal transcribed spacer (ITS) region of the ribosomal DNA using the primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) [40,41], before going on to sequence the product of the polymerase chain reaction (PCR). Prior to DNA extraction, individual root-tips were dried, and ground subsequently in an Eppendorf tube, with a plastic mortar in the presence of liquid nitrogen. Genomic DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen, Valencia, CA, USA), in line with the manufacturer's instructions. Reactions were performed in a 25 µL mixture containing 1 ng of genomic DNA, 0.5 µM each primer, 0.2 µM each dNTP, 2.5 µL of 10× PCR reaction buffer, and 1 U of DreamTaq™ DNA Polymerase (Thermo Fisher Scientific, Waltham MA, USA). The amplification reaction was performed in a PTC-200™ Programmable Thermal Controller thermocycler (MJ Research, Inc.) under the following conditions: initial denaturation at 95 °C for 3 min followed by 40 cycles of denaturation, annealing, and elongation for 25 s at 95 °C, 25 s at 56 °C, and 50 s at 72 °C, respectively, with a final extension step at 72 °C for 10 min. Directly after the reaction, 1 µL of PCR product was electrophoresed on a 1% TAE agarose gel with the 1 kb DNA Ladder Plus (Invitrogen, Carlsbad, CA, USA) as a molecular weight marker. The amplified PCR fragments of DNA were purified with the CleanUp Kit (A&A Biotechnology, Gdynia, Poland). Both strands of the PCR products were sequenced with a 3730XL DNA Analyser (Applied Biosystems, Foster City, CA, USA) at the *Genomed* Company (Warsaw, Poland). The nucleotide sequences were read and edited using FinchTV v. 1.4.0 (Geospiza Inc., Seattle, WA, USA) and aligned with sequences available publicly in *GenBank* (http://www.ncbi.nlm.nih.gov,

accessed on 16 November 2021), using the BLASTn algorithm in order to confirm the taxonomy of the fungi analysed. Species-level OTUs were defined based on a 97% sequence similarity level, which is within the range of intraspecific ITS sequence similarity. Taxonomy of the fungi was confirmed in the UNITE database (https://unite.ut.ee, accessed on 16 November 2021).

2.6. Soil Analysis

Soils were sampled at the end of October 2012—a year on from the MSP—using a Kopecki cylinder (volume, 112.3 cm^3; height, 55 mm; inner diameter, 51 mm), and a spade. Combined (0.5 kg) samples were taken by spade, each comprising five sub-samples of around 100 g of soil. Samples were collected from surface soil, down to 10 cm. Each of the plots in the block with MSP variants FP, AP, and FM yielded six core samples and six spade samples. In the FP and AP variant, three sampled each were taken (with a cylinder and a spade) from the furrows and three from the ridges (variants FP f1 and r1, as well as AP f2 and r2). In the FM variant, three samples each were taken from the strips (s), as well as the soil between them (os). The non-MSP plots were sampled by reference to three cylindrical samples and three combined samples. These three samples taken from each microsite came from three different locations across the clearcut, i.e., on the tree-stand side (up to 5 m away from its edge), in the middle (i.e., 17–22 m away from the edge of the stand), and on the plantation side (up to 5 m away from its edge). A total of 63 corer samples were taken, as well as 63 combined samples (7 microsites × 3 main locations across the clearcut × 3 blocks).

Soil analysis was conducted at the Forest Research Institute's Independent Laboratory for the Chemistry of the Forest Environment (PCA accreditation No. AB 740). The combined soil samples were used to determine soil pH in KCl using the potentiometric method (PN-ISO 10390:1997). Soil organic N and C were determined by high-temperature combustion (500 °C) followed by thermal conductivity detector (TCD) detection (N–PN-ISO 13878:2002, C–PN-ISO 10694:2002). P_2O_5 was quantified using the Egner–Riehm method and exchangeable content of the cations Ca^{2+}, Mg^{2+}, K^+, and Na^+ via ammonium acetate extraction at pH 7.0 using the ICP method.

Exchangeable acidity was determined using Sokołów's method. Granulometric-fraction analysis for the soil was achieved by the sedimentation (pipette) method, in accordance with standard PN ISO 11277:2005. Core samples in turn served bulk-density and actual moisture measurements for the soil, achieved by weighing.

2.7. Data Analysis

Communities of mycorrhizal fungi were described by: species/taxon richness (number of taxa for each variant), relative abundance of mycorrhizal fungal taxa (the ratio of each mycorrhizal species/taxon to all mycorrhizal root-tips expressed as a percentage), degree of mycorrhizal colonisation (ratio of vital mycorrhizal root-tips (VM) to all the root-tips of the root system, including non-mycorrhizal (NM) and non-vital (NV) root-tips, expressed in percentage terms), and frequency of occurrence of species/taxa of mycorrhizal fungi (defined as the percentage of plants colonised by a mycorrhizal species/taxon). To assess mycorrhizal species/taxon diversity, values for the Shannon–Wiener (*H′*), Simpson (1-D), and evenness (*eˆH/S*) indices were determined. True taxon richness (via *Jackknife 1* richness estimator) was calculated using 1000 randomised runs without sample replacement.

Statistical analyses of soil features were carried out using one-way ANOVA, with the Tukey test used in "post hoc" comparisons. This dataset satisfied the assumptions of ANOVA, based on the homogeneity of variances (Levene's test), and the data's normal distribution was checked for (Shapiro–Wilkinson test).

To evaluate possible significant differences in the relative abundances of species between the examined MSP microsites, we used generalised linear models (GLM) with binomial distributions. Tukey's linear contrast was used to make pairwise comparisons between the examined MSP methods for the GLM model.

To explore the potential relationship between the relative abundance of mycorrhizal fungal species and soil parameters, we used canonical correspondence analysis (CCA) and Spearman rank correlation coefficients. Biplot figures were used to present the results of the CCA. By reference to the Bray–Curtis distance matrix, nonmetric multidimensional scaling ordination (NMDS) was used to illustrate the differences between MSP microsites where the abundance and diversity of mycorrhizal fungal species were concerned. The significances of differences between MSP microsites noted with the NMDS analysis was tested for using permutation multivariate *analysis of variance* (perMANOVA).

Statistical analyses were performed using *R* version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria), with the vegan package for multivariate analysis (CCA, NMDS, *perMANOVA*), and diversity indices calculated. The lme4 package was used for the ANOVA and GLM modelling. For means-estimation and the Tukey post-hoc test, lsmeans package in *R* was used. The accepted level of significance was $p < 0.05$.

3. Results

3.1. Soil Properties

Soil in the two types of furrow had a significantly higher pH and lower exchangeable acidity than that located in ridges, and also than soil not subjected to MSP (Table 1). The soil in both types of ridge (r1 and r2) had more C, N, Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+} than was present at the remaining microsites. In the case of r2, the contents of the mineral components (other than Na^{+}) were significantly greater than with the non-MSP variant. Where microsite r1 was concerned, there were similar statistical differences for three of these features, i.e., N, K^{+}, and Na^{+}. However, values for the bulk density of soils in the different microsites did not differ from those noted at sites not subject to MSP. In contrast, differences in values for this parameter, significant at $p < 0.0001$, were noted between the ridges and the furrows generated by the AP and FP methods.

Differences in soil grain-size at different microsites were only noted in relation to the ridges generated by AP (r2). In comparison with that in the non-MSP treatment, the soil in this case contained significantly more silt ($p = 0.0365$) and significantly less sand ($p < 0.0001$). Equally, the microsites generated by MSP or with no MSP did not differ in regard to C/N ratio, content of P_2O_5 and clay, or actual moisture (Table 1).

3.2. Mycorrhizal Colonisation of Roots

Overall values for mycorrhizal colonisation were high at all microsites. The highest recorded level of VM was the 99.2% noted for seedlings growing in the control not subject to mechanical site-preparation. Seedlings growing at the remaining microsites were only mycorrhized to a more limited degree, with the hierarchy for VM level summarisable as f1 > f2 = s > r2 > r1. Significantly higher proportions of NM root tips were observed on seedlings growing on both ridges (r1 10.9% and r2 9.8%), as compared with other microsites. NV tips were observed solely among seedlings in the area not subject to MSP (Figure 1).

3.3. Composition of the Mycorrhizal Assemblage

ITS fungal rDNA provided for the identification of 46 fungal taxa, of which 40 were assigned to the species level, and six only to that of the genus (Table 2, Figure 2). Overall root-tip colonisation among all detected ECM/ectendomycorrhizal (EECM) fungi was 14.1% for Basidiomycetes and 85.9% for Ascomycetes.

The most frequent and abundant fungus was *Wilcoxina mikolae* (relative abundance, 79.8%, frequency, 96.8%). Other abundant species present at all examined microsites were *Thelephora terrestris, Tylospora asterophora*, and *Cenococcum geophilum* (3.8%, 3.2%, and 1.7%, respectively). The mycorrhizal taxa occurring most frequently included: *C. geophilum* (16.0%), *Hyaloscypha bicolor* (8.2%), *T. asterophora* (7.7%), and *T. terrestris* (5.4%), while remaining taxa accounted for colonisations of less than 5% of the seedlings examined (Table 2).

Table 1. Soil characteristics (mean and standard error in parentheses and p value) in relation to MSP methods and microsites. Different letters indicate differences significant at $p < 0.05$ obtained using the Tukey test.

Feature	Forest Plough		Active Plough		Forest Mill		Without MSP	p
	Ridge	Furrow	Ridge	Furrow	Strip	Outside the Strip		
pH (KCl)	3.31 (0.14) a	3.86 (0.32) bc	3.31 (0.18) a	3.98 (0.11) c	3.67 (0.17) abc	3.52 (0.18) ab	3.35 (0.24) a	<0.0001
Norg (g kg^{-1} dw)	1.64 (0.31) c	0.46 (0.10) a	2.23 (0.86) d	0.54 (0.12) ab	0.96 (0.28) ab	1.07 (0.24) b	0.96 (0.13) ab	<0.0001
Corg (g kg^{-1} dw)	31.58 (5.34) b	9.32 (2.11) a	46.11 (19.81) c	10.91 (2.41) a	20.20 (5.77) ab	22.37 (5.76) ab	20.71 (3.03) ab	<0.0001
C/N	19.1 (1.10) a	20.8 (2.13) a	20.3 (1.31) a	20.6 (2.13) a	20.6 (1.15) a	20.6 (1.66) a	21.8 (0.64) a	0.1125
P_2O_5 (mg100 g^{-1} dw)	5.76 (1.57) a	7.89 (1.30) a	7.31 (1.27) a	7.73 (1.04) a	7.46 (1.52) a	6.53 (1.9) a	6.81 (1.64) a	0.3225
Ca^{2+} (mg100 g^{-1} dw)	12.78 (5.33) bc	1.74 (0.38) a	20.56 (9.24) c	2.33 (0.98) a	7.19 (3.28) ab	5.41 (1.83) ab	5.70 (1.71) ab	0.0139
K^{+} (mg100 g^{-1} dw)	5.69 (1.60) b	1.06 (0.30) a	8.35 (3.28) c	1.41 (0.26) a	3.05 (0.81) a	2.60 (0.61) a	2.45 (0.50) a	<0.0001
Mg^{2+} (mg100 g^{-1} dw)	1.82 (0.65) b	0.22 (0.05) a	3.13 (1.29) c	0.31 (0.09) a	1.08 (0.44) ab	0.86 (0.27) ab	0.81 (0.22) ab	<0.0001
Na^{+} (mg100 g^{-1} dw)	0.23 (0.11) c	0.12 (0.09) abc	0.21 (0.10) bc	0.04 (0.14) a	0.21 (0.08) bc	0.18 (0.08) bc	0.09 (0.09) ab	<0.0001
Ea (me 100 g^{-1})	2.90 (0.59) bc	1.67 (0.39) a	3.23 (0.51) bc	1.86 (0.28) a	2.66 (0.43) b	3.16 (0.46) bc	3.52 (0.50) c	<0.0001
Bd (g cm^{-3})	0.80 (0.25) a	1.35 (0.07) c	0.79 (0.33) a	1.29 (0.11) bc	1.06 (0.13) abc	0.91 (0.17) ab	1.05 (0.19) abc	<0.0001
Am (g 100 cm^{-3})	14.6 (10.10) a	12.9 (3.71) a	15.2 (7.07) a	13.8 (4.20) a	14.7 (7.18) a	16.7 (10.24) a	20.4 (9.08) a	0.4512
Sand (%)	92.5 (1.01) b	94.9 (0.90) c	90.1 (2.30) a	94.1 (0.80) bc	93.6 (0.87) bc	94.0 (1.11) bc	93.5 (0.84) bc	<0.0001
Silt (%)	2.54 (0.80) a	1.47 (0.59) a	4.94 (1.94) b	1.82 (0.74) a	2.48 (0.84) a	1.79 (1.09) a	2.57 (0.73) a	0.0365
Clay (%)	4.96 (1.40) a	3.66 (0.59) a	4.93 (0.77) a	4.10 (0.47) a	3.94 (0.45) a	4.23 (0.37) a	3.98 (0.37) a	0.411

Ea—exchangeable acidity, Bd—bulk density, Am—actual moisture, dw—dry weight.

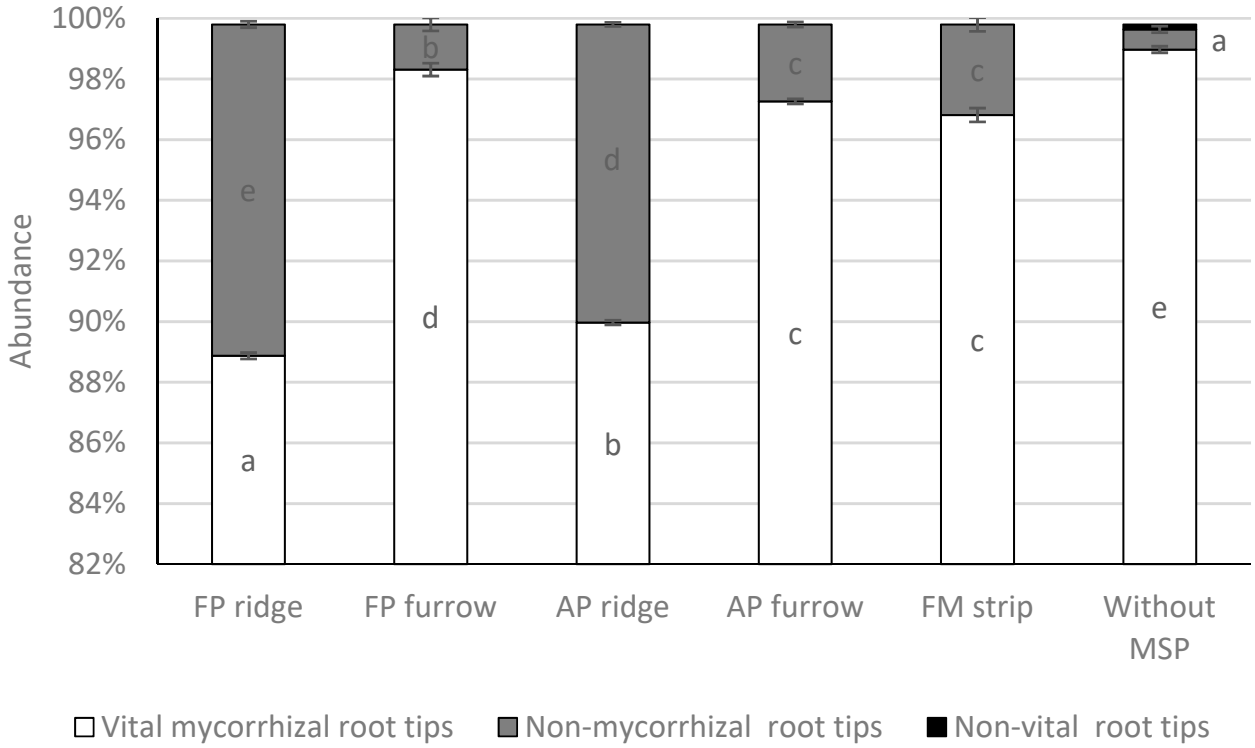

Figure 1. Abundance of vital mycorrhizal, non-mycorrhizal, and non-vital root tips (%) (mean and standard errors bars) of 1-year-old Scots pine seedlings in relation to MSP methods: furrows and ridges made by a forest plough (FP) or an active plough (AP), strips made by a forest mill (FM), and without MSP (the control). Within each classification, different letters indicate significant differences obtained using the Tukey's linear contrast based on the GLM model: $p \leq 0.05$).

Table 2. Molecular identification, relative abundance, and frequency for mycorrhizal fungal taxa on the roots of 1-year-old Scots pine on a clearcut. (Relative abundance—the ratio of each mycorrhizal species/taxon to all mycorrhizal root-tips expressed as a percentage, frequency—the percentage of plants colonised by a mycorrhizal species/taxon.)

Fungal Taxa	Accession Numer	Best Match Sequence/Accession Number	Sequence Similarity (%)	Query Coverage (%)	Relative Abundance	Frequency
Ascomycetes 85.9%						
Acephala macrosclerotiorum	OK042934	HM189696	100	99	0.18	0.46
Cenococcum geophilum	OK042935	MK131421	99	98	1.65	16.00
Helotiales sp.	OK042936	HF947839	100	99	0.28	0.92
Hyaloscypha bicolor	OK042937	MH018932	100	99	2.20	8.15
Hyaloscypha finlandica	OK042938	EU557316	99	97	0.14	2.00
Hyaloscypha variabilis	MK529874	MK131649	99	99	0.89	3.38
Hyaloscypha sp.	OK042939	MH029252	100	99	0.21	1.08
Wilcoxina mikolae	OK042940	JQ310818	100	99	79.76	96.77
Wilcoxina rehmii	MK529903	JX129137	100	100	0.53	0.62
Wilcoxina sp.	MK529904	MT278225	98	99	0.09	0.15
Basidiomycetes 14.1%						
Amanita pantherina	MK529846	AB080775	100	99	0.18	1.08
Amanita spissa	OK042941	KX449404	100	99	<0.01	0.15

Table 2. *Cont.*

Fungal Taxa	Accession Numer	Best Match Sequence/Accession Number	Sequence Similarity (%)	Query Coverage (%)	Relative Abundance	Frequency
Boletus edulis	MK529848	AF438565	100	100	0.04	0.46
Boletus ferrugineus	MK529849	AJ889934	100	99	0.01	0.15
Cantharellus cibarius	OK042942	MT644929	100	100	0.53	0.31
Cortinarius bataillei	OK042943	MN751017	100	100	<0.01	0.15
Cortinarius cinnamomeus	MK529853	HQ604649	100	100	0.39	1.54
Cortinarius croceus	MK529854 MK529860	MT908274	100	100	0.07	0.77
Inocybe lacera	MK529863	GQ267473	100	100	0.58	1.23
Inocybe umbrina	MK529864	HQ604524	98	99	0.06	0.31
Laccaria proxima	MK529866	JQ310816	100	99	0.25	0.46
Lactarius rufus	OK042944	MN992614	100	99	0.15	0.15
Paxillus involutus	MK529876	HQ604826	100	99	0.04	0.62
Piloderma olivaceum	MK529879	KP814428	99	99	<0.01	0.15
Piloderma sphaerosporum	MK529880	JQ711875	100	99	0.46	0.62
Rhizopogon evadens	MK529883	KT968587	99	99	0.06	1.08
Rhizopogon roseolus	MK529884	KX449430	100	98	<0.01	0.15
Rhizopogon rubescens	OK042945	LC198723	99	100	0.21	0.31
Russula adusta	OK042946	JQ888194	100	99	0.07	0.15
Russula aeruginea	OK042947	MK028882	100	99	0.04	0.15
Russula decolorans	MK529886	JX029947	100	99	0.88	4.92
Russula emetica	OK042948	KX579814	100	99	0.04	0.15
Russula paludosa	MK529887	JQ888199	100	99	1.02	2.46
Russula vesca	OK042949	KX655856	100	99	0.24	0.15
Russula vinosa	MK529888	KM517240	100	99	0.07	0.77
Russula sp.	OK042950	MK537200	100	99	0.01	0.15
Suillus bovinus	MK529889	KF482482	100	99	0.26	2.15
Suillus granulatus	OK042951	MK402134	100	99	0.10	0.31
Suillus luteus	MK529890	KR673431	100	99	0.36	2.00
Suillus variagatus	MK529891	JQ888209	100	99	0.07	0.46
Thelephora terrestris	MK529894	HM189965	100	99	3.84	5.38
Tomentella badia	MK529895	MT908290	100	99	0.05	0.31
Tomentella sp.	OK042952	MT229604	100	99	0.06	0.15
Tomentelopsis sp.	MK529896	HM190011	99	99	0.26	0.62
Tylospora asterophora	MK529898	KR019865	100	99	3.21	7.69
Xerocomus badius	MK529905	HQ207697	100	100	0.46	1.38

The relative abundances and frequencies of *H. bicolor* and *T. asterophora* were significantly greater on furrows of the two types (f1 and f2) than at other microsites, while *T. terrestris* was significantly less abundant. No such relationships were found for *W. mikolae*. *C. geophilum and Russula decolorans* were most abundant on the non-MSP control (Tables 3 and S1).

Mean numbers of ECM/EECM taxa per seedling ranged from 1.4 species for r1 to 2.0 for the control, with no significant differences between variants found. Observed ECM/EECM species richness ranged from 16 for seedlings growing on the FP ridge and the AP furrow to 23 for those in strips, the FP furrow, and the control. The Shannon–Wiener and Simpson indices assumed their highest values in the case of the variant without MSP.

In turn, the evenness index assumed its highest values in the f1 variant, the control, and the r2 variant, these being higher than in the f2, s, and r1 variants. The *Jackknife1* richness estimator was in the range of 19.0–32.0, with the index significantly greater for the non-MSP "c" microsite, the FP furrow (f1), and the "s" strip generated by FM site preparation (Table 4).

Figure 2. Morphological features of mycorrhizae observed on 1-year-old Scots pine seedlings from natural regeneration on a clearcut.

Table 3. Relative abundance of mycorrhizal fungal taxa of 1-year-old Scots pine seedlings growing in different microsites created by MSP methods. Different letters indicate differences significant at $p < 0.05$ obtained using the Tukey test.

Fungal Taxa	Forest Plough		Active Plough		Forest Mill	Without MSP	*p*
	Ridge	Furrow	Ridge	Furrow	Strip		
Ascomycetes							
Acephala macrosclerotiorum		0.09			0.57	0.12	
Cenococcum geophilum	0.08 a	0.79 ab	3.73 c	0.56 a	1.19 b	6.37 d	<0.0001
Helotiales sp.		0.50	0.12	0.78			
Hyaloscypha bicolor	1.39 a	2.08 b	1.85 a	3.01 c	2.95 c		0.0033
Hyaloscypha finlandica	0.01 a	0.34 a		0.09 a	0.14 a	0.12 a	0.4212
Hyaloscypha variabilis	0.42 a	1.38 a	0.25 a		0.47 a	2.19 a	0.0867
Hyaloscypha sp.	0.90					0.79	
Wilcoxins mikolae	81.48 b	73.69 a	73.17 a	85.04 c	80.18 b	75.30 a	0.0061
Wilcoxina rehmii		0.94			0.86		
Wilcoxina sp.						0.85	
Basidiomycetes							
Amanita pantherina		2.07				0.20	
Amanita spissa		0.01					
Boletus edulis				0.18			
Boletus ferrugineus				0.05			
Cantharellus cibarius		1.41					
Cortinarius bataillei			0.01				

Table 3. *Cont.*

Fungal Taxa	Forest Plough		Active Plough		Forest Mill	Without MSP	*p*
	Ridge	Furrow	Ridge	Furrow	Strip		
Cortinarius cinnamomeus			0.01		1.65		
Cortinarius croceus	0.06				0.41		
Inocybe lacera		1.72 c	0.17 a	0.64 b	0.05 a	0.55 b	0.0178
Inocybe umbrina		0.29					
Laccaria proxima					0.98		
Lactarius rufus			0.45				
Paxillus involutus			0.17		0.21		
Piloderma olivaceum					0.03		
Piloderma sphaerosporum		1.26	0.08			0.39	
Rhizopogon evadens					0.20		
Rhizopogon roseolus				0.02			
Rhizopogon rubescens						1.15	
Russula adusta		0.25					
Russula aeruginea		0.16					
Russula decolorans	0.45 a	0.10 a	1.54 b	0.03 a	0.32 a	3.39 c	0.0002
Russula emetica						0.45	
Russula paludosa	0.76 a	0.26 a	1.17 a	1.56 a		0.76 a	0.2341
Russula vesca						0.88	
Russula vinosa	0.23		0.02			0.11	
Russula sp.	0.05						
Suillus bovinus	0.01 a	0.34 a	0.04 a	0.31 a	0.50 a	0.38 a	0.3388
Suillus granulatus					0.32		
Suillus luteus	0.13 a	0.16 a		1.31 b	0.35 a	0.04 a	0.0343
Suillus variagatus					0.52		
Thelephora terrestris	1.74 ab	0.95 a	4.62 c	0.32 a	2.32 b	2.58 b	<0.0001
Tomentella badia		0.05				0.28	
Tomentella sp.				0.34			
Tomentelopsis sp.					0.82	0.40	
Tylospora asterophora	0.42 a	9.38 d	2.17 b	3.36 c	1.77 b	0.45 a	<0.0001
Xerocomus badius	0.44				0.31	1.30	

Table 4. Diversity parameters describing mycorrhizal fungal communities of Scots pine seedlings in relation to microsites created by MSP methods. Different letters indicate differences significant at $p < 0.05$ obtained using the Tukey test.

Diversity Parameter	Forest Plough		Active Plough		Forest Mill Strip	Without MSP	*p*
	Ridge	Furrow	Ridge	Furrow			
Richness per microsite (count)	16	23	17	16	23	23	-
Richness per seedling (mean)	1.4 a	1.7 a	1.7 a	1.6 a	1.9 a	2.0 a	0.5647
Shannon–Wiener *H′* (mean)	0.55 a	1.16 c	0.93 b	0.67 a	1.01 b	1.38 d	0.0021
Simpson 1-D (mean)	0.19 a	0.43 c	0.39 b	0.25 a	0.35 b	0.52 c	<0.0001
Evenness *e^H/S* (mean)	0.11 a	0.18 b	0.15 b	0.12 a	0.12 a	0.17 b	0.0188
Jackknife 1 (mean)	22.9 a	29.0 b	26.9 a	19.0 a	27.0 b	32.0 b	<0.0001

According to the NMDS analysis, a significant difference was observed between MSP microsites ($p = 0.0323$, iterations = 999). Reference to the results of perMANOVA confirmed the existence of differences between the mycorrhizal fungal communities present at different microsites. The five microsites (f1, f2, r1, s, and control) did not differ from each other, but were different from the r2 (AP ridge) treatment (Table 5, Figure 3).

T. asterophora, *W. mikolae*, and *R. vinosa* differed from other species in that their relative abundances were correlated more strongly with soil parameters. In these species, there were, respectively, 13, 11, or 10 out of 15 features of the soil that displayed significant correlations. In the cases of *T. asterophora* and *W. mikolae*, positive correlations were noted with pH, sand content, and bulk density, while the relationship was inverse when it came to

the contents of N, C, Ca^{2+}, K^{+}, Mg^{2+}, and silt, clay, and exchangeable acidity. Additionally, the relative abundance of *T. asterophora* correlated positively with P_2 O_5, and negatively with actual moisture. The reverse situation applied to *R. vinosa*, with correlations proving positive with contents of N, C, Ca^{2+}, Mg^{2+}, exchangeable acidity, and clay, as well as inverse when it came to pH, P_2O_5, sand content, and bulk density. In contrast, the relative abundances of *T. terestrris* and *C. geophilum* correlated solely with pH and exchangeable acidity (Table S2, Figure 4).

Table 5. Results of perMANOVA comparing mycorrhizal fungal communities among 6 microsites: forest plough ridge (r1) and furrow (f1), active plough ridge (r2) and furrow (f2) and forest mill strip (s), as well as non-MSP as the control (c). Bold values indicate differences significant at $p \leq 0.05$.

	s	f1	r1	f2	r2
C	0.8952	0.3556	0.1016	0.4160	**0.0004**
s		0.5819	0.2455	0.5471	**0.0231**
f1			0.4563	0.2099	**0.0040**
r1				0.0690	**0.0016**
f2					**0.0132**

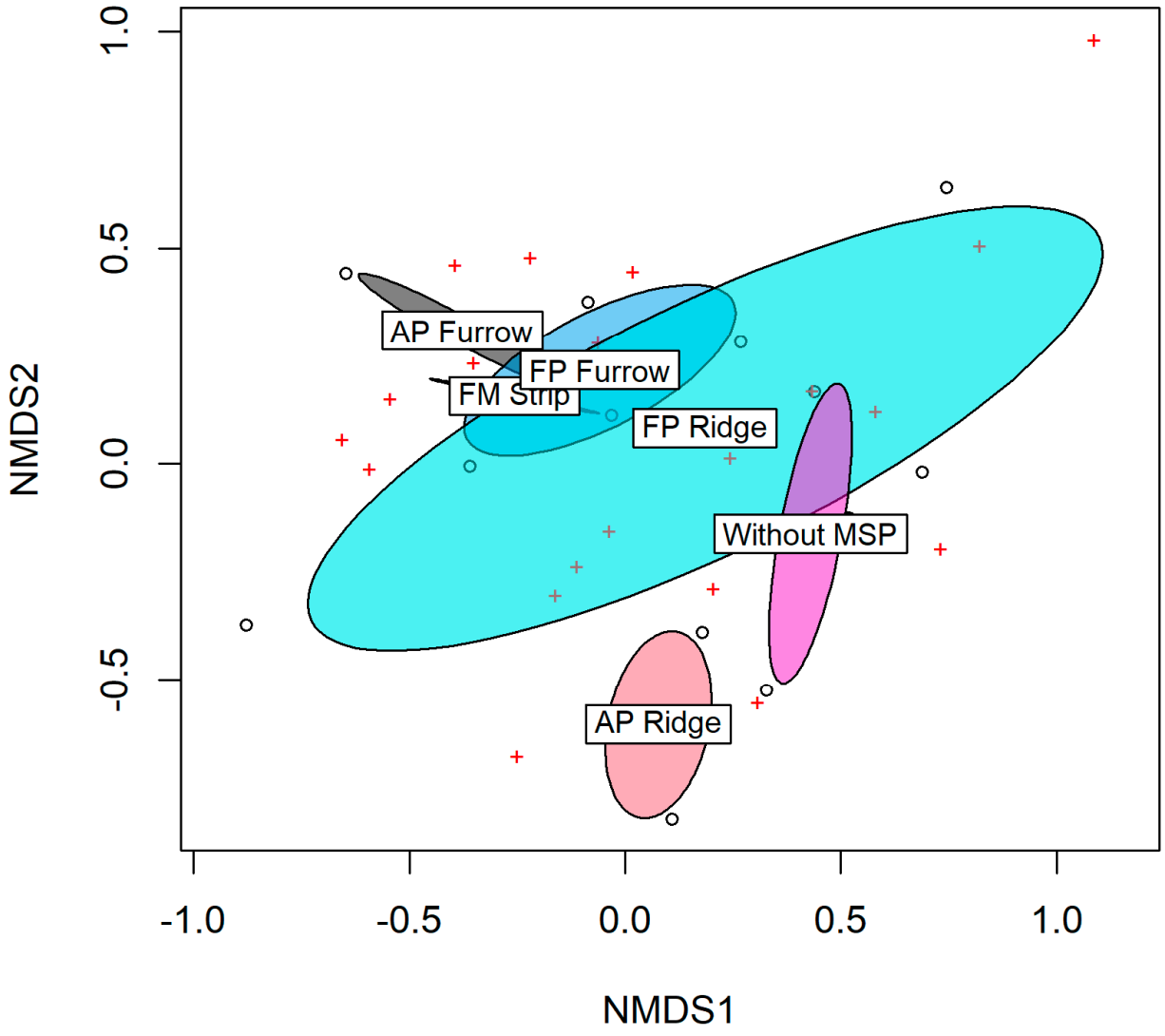

Figure 3. Results from non-metric dimensional scaling (NMDS) analysis of MSP microsites.

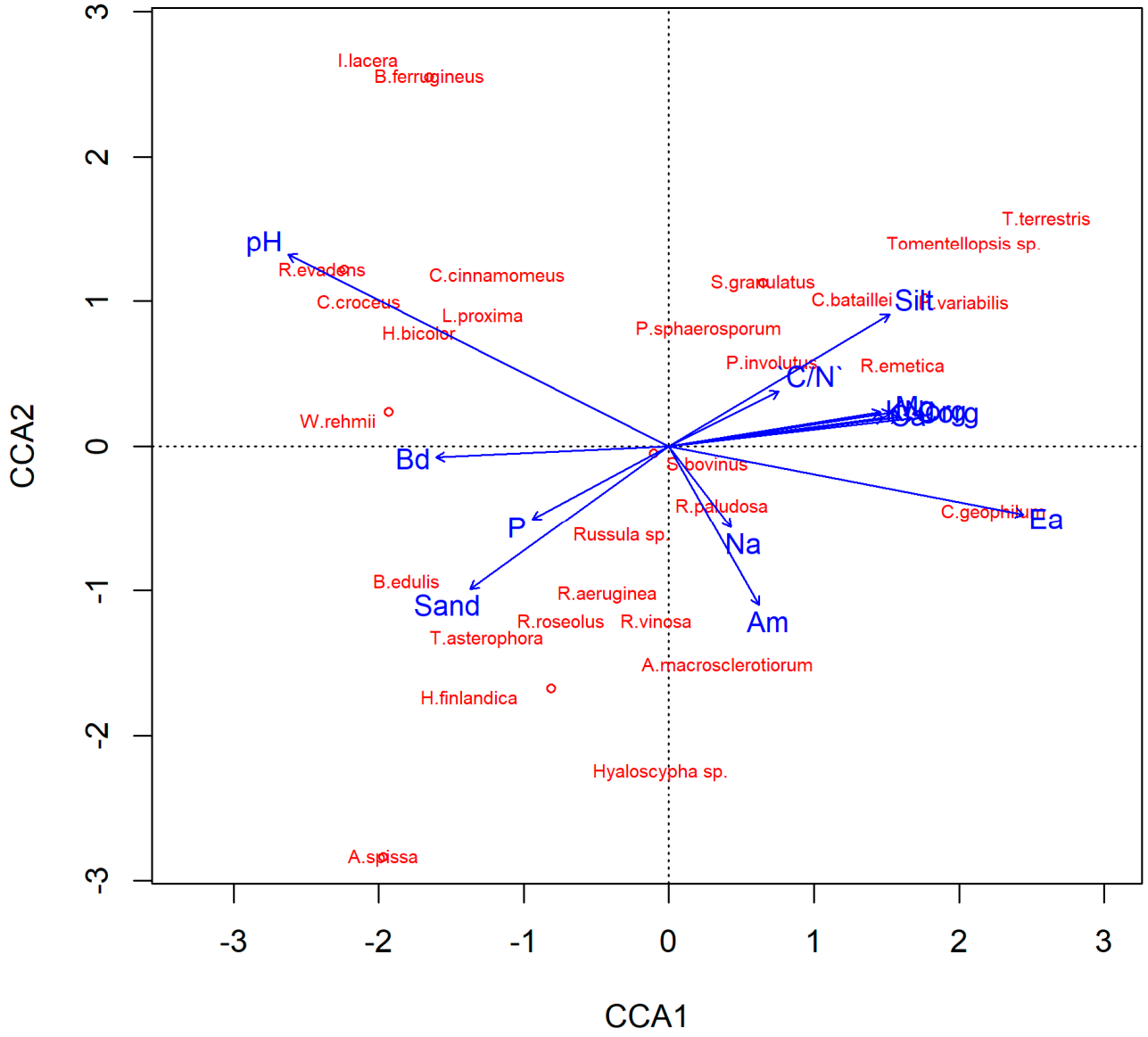

Figure 4. CCA graphs for mycorrhizal fungal communities underlined by soil parameters in relation to Scots pine seedlings growing on a clearcut. Ea—exchangeable acidity, Bd—bulk density, Am—actual moisture, overlapping arrows: N, C, Ca, K, Mg, Clay.

4. Discussion

The degree of mycorrhizal colonisation of Scots pine seedlings studied in our experiment proved to be high, irrespective of the microsite on which they were growing. Trees on the clearcut that was researched had been removed almost half a year prior to seed-sowing and the appearance of seedlings. It may therefore be presumed that the soil at this site retained inoculum of all types [10,42–44].

Comparisons of the mycorrhizal colonisation of seedlings growing at the microsites generated by MSP revealed a significantly greater share of VM root-tips in seedlings growing in the furrows of the two types (f1 and f2). This confirms the results obtained by other authors that the amount of inoculum in mineral soil proves not to be a factor limiting mycorrhizal colonisation [34,35,43,45]. Equally, removal of litter and humus leads to a lowering of contents of micro- and macro-elements, especially nitrogen, as well as other components of humus, such as phenolics [46], while pH increases. Moisture conditions are more favourable in furrows than at other microsites, in line, not only with precipitation totals, but also capillary water transport to the soil surface [47,48]. Such factors may exert a

favourable influence on the generation of mycorrhizae [49]. Similar results were obtained by Baar [50], who found greater numbers of ECM root-tips among Scots pines growing in mineral soil following the removal of litter and humus.

In turn, and quite significantly, the highest share of NM root-tips was found for the roots of seedlings growing in ridges of the two kinds (r1 and r2), as opposed to at the other microsites. These sites also had the lowest reported species richness (16 and 17 species/taxa, respectively). Although several studies have shown that ECM are most abundant in the organic layer of forest soils [30,31], specific microenvironmental conditions in these microsites may influence the results obtained. The raising of ridges ensures that these microsites have a higher substratum temperature, such that—where moisture is adequate [26]—microclimatic conditions favourable to the development of soil microorganisms take shape, with organic matter then undergoing decomposition [51]. A consequence of this is an increase in the amounts of readily plant-available macro- and microelements [38,52], as a rule associated with a decline in numbers of ECM species [53], and potentially capable of explaining a higher share of NM root-tips. Equally, the fact that ridges are elevated above the surroundings while containing a relatively large amount of humus also ensures that these substrata tend to have low levels of moisture [54]. This is likely to be an issue even where rainfall is abundant (if irregular) [55], and the impact on the formation of mycorrhizal symbioses will be an unfavourable one [56]. While it is true that our actual-moisture measurements made at the end of the growing season did not reveal statistically-significant differences between microsites, moisture conditions through that growing season as a whole are likely to be differentiated. Indeed, such microsites have been found to be characterised by major fluctuations in soil moisture content [51].

The 46 mycorrhizal fungal taxa observed on the roots of Scots pine correspond to 75.3% (*Jackknife 1*) of the potential estimated species richness, which is of 61 taxa. Leaving aside the possible effects of our sampling strategy (1 year only, with a single, autumnal sampling session), such a level of fungal species richness encountered in comparison with estimated richness may point to the lack of a homogeneous spatial distribution of fungal symbionts. Moreover, our goal was less to learn about diversity as a whole, and more to compare communities of mycorrhizal fungi between microsites generated by MSP, as well as the relevant implications for forestry practice.

The fungal communities noted in our work would seem to be relatively rich when set against those focused on by other authors. Mah et al. [57] found 12 ECM species present on their Norway spruce seedlings regenerating naturally on a clearcut; as well as 20 on such seedlings regenerating from a mature stand. In turn, while Heinonsalo et al. [58] classified a total of 34 different morphotypes on Scots pine seedlings, they did so over a five-year sampling period. What distinguishes the assemblage of fungi we studied is the abundance breakdown noted for the different taxa. All species, barring *W. mikolae*, were in fact at relative abundance levels below 5%. This seems quite a contrast with the aforementioned Heinonsalo et al. [58], 16 of whose studied species were present at abundances above 5%.

The composition of ECM fungal communities is under the strong influence of abiotic conditions [59,60]. Among the key factors determining distribution are moisture, temperature, pH, and nitrogen concentration [61,62]. It emerges that the microsites generated by MSP do differ in relation to these kinds of features (Table 1) [24–27], hence our assumption underpinning the research hypothesis that there would be significant differences among the communities of mycorrhizal fungi associated with Scots pine seedlings growing at different microsites has been validated. However, this hypothesis gained only partial confirmation. The assemblage of fungi present where MSP was not engaged proved to be similar to that in both furrows (FP and AP), FP ridge, and the strips made by FM. These five communities were not found to differ between themselves. The PA ridge supported a quite different assemblage of fungi. Our results show that, on the "fresh" clearcut, environmental conditions such as the availability of nutrients, substrate moisture, and temperature have a greater impact on the formation of mycorrhizal fungus communities than the availability of inoculum. While there is a great deal of research showing that microsites shape ECM

fungal communities, our results resemble those of Lazaruk et al. [30], as well as Walker and Jones [63], in indicating how niche-specific communities will not always develop on clearcuts, notwithstanding significant differences between microsites as regards their physical and chemical features.

Found in our research, the ascomycete fungi of the genus *Hyaloscypha* form the *Rhizoscyphus ericae* aggregate [64,65]. Members of this are able to form mycorrhizal associations with both coniferous trees and ericoid plants [64]. While this is true of *H. bicolor* and *H. finlandica* [65], *Hyaloscypha variabilis* cannot form ectomycorrhizae [66]. Vohník et al. [67] and Sietiö et al. [68] claim that *H. variabilis* is an obligate biotroph and may be an endophyte in ectomycorrhizae established by other species [66]. Research to date has shown either a neutral or a positive influence of the colonisation of roots by *H. variabilis* on the growth of ectomycorrhizal plants [66]. The share of taxa coming within the *Rhizoscyphus ericae* aggregate was 3.4%. This may be presumed to reflect the presence on the clearcut of species such as *V. myrtillus*, *V. vitis-idaea*, and *C. vulgaris* from among the *Ericaceae*, whose roots form ericoidal mycorrhizae with species belonging to the *Rhizoscyphus ericae* aggregate and are characterised by abundant inoculum [67].

In our study, the ascomycete *W. mikolae* was the most abundant and most frequently occurring species at all microsites. *Wilcoxina* species form ectendomycorrhiza and are the most common colonisers of young conifers. They are often the dominant fungi at forest nurseries, as well as disturbed sites such as those present post-fire, or following forest clearance [69]. Our results are not surprising and are in line with those of other studies [70].

T. terrestris is a pioneer, multi-host mycobiont, present in nursery, young, and old forest [71]. Our study found it at all the different kinds of microsite, albeit only far more rarely in the two kinds of furrow. These results confirm what had gone earlier, indicating that *T. terrestris* is present in all of the soil horizons, even if it creates abundant ECMs in the organic layer [72,73]. In turn, significantly the largest share accounted for on roots by this species (if still at only 4.6%) characterises seedlings prepared for with PA r2, with this confirming the adaptation of *T. terrestris* to high-N conditions [53,74]. The N-content of r2 soil was 2.5 times as high as in the non-MSP variant, and 5 times as high as in the soil of the two kinds of furrow (f1 and f2).

T. asterophora is one of the most abundant species forming ECMs among conifers [75,76]. It was present on the roots of seedlings growing at all of the microsites studied, though the share was significantly higher in the two kinds of furrow, f1 and f2 (at 16.8% and 12.4%, respectively). These are of course the most nutrient-poor microsites, also displaying higher values for pH and bulk density. The species preferences are confirmed by statistically-significant Spearman rank correlation coefficients, which are inverse where mineral components in the soil are concerned, but positive in respect of the aforementioned pH and bulk density (Table S2). This could point to the more-limited abundance of the species in question, where contents of mineral components in the soil are greater, even though such an interpretation would not coincide with the results obtained by such other authors as Lilleskov and Bruns [77] and Toljander et al. [78]. The higher share taken by *T. asterophora* in mineral soil might reflect the differential availability of inoculum in the different soil layers. While Rosling et al. [45] found *T. asterophora* in all soil horizons, the species accounted for its highest share in the lowest (the C horizon of parent material). In turn, Vašutova et al. [73] found that the species in question predominated in a windthrow area in which extracted fallen trees had been burned. Both our results and those of the authors cited above suggest that inoculum of *T. asterophora* is present in abundance in deeper soil layers, i.e., in mineral soil.

Practical Implications for Silviculture

Comparing the MSP methods with no division into microsites, it is possible to arrive at the hierarchy C > FM = FP > AP, on the basis of mycorrhization as well as indicators of biodiversity. While seedlings growing in soil without MSP are characterised by a significantly higher level of mycorrhization and the highest observed values for the diversity parameters

(Shannon–Wiener and Simpson Indices), doubts would need to be expressed regarding recommending this kind of procedure in the circumstances of natural regeneration. Success with seedling recruitment among Scots pines depends largely on soil-surface properties, such as moisture content and the depth of the humus layer. A litter or humus layer without scarification may have a moisture level too low to sustain germination. The organic soil layer dries out quickly, and so has a highly variable moisture regime [79]. A thick layer of litter and humus may delay or abort seedling emergence by preventing contact between the seminal root and mineral soil. Mechanical restrictions to hypocotyl elongation and the emergence of cotyledons also arise out of the presence of litter and humus [80,81]. Thus, in the absence of MSP, natural regeneration will either not be obtained at all, or will be sporadic at best [38].

The intensive MSP methods considered (FP and AP) give rise to differential conditions for the founding of mycorrhizae, given the creation of furrows and ridges (with the share of VM root-tips being greatest in the former, and the share of NM root-tips in the latter). However, the biodiversity is lower. On the other hand, the methods in question can guarantee effective and abundant natural regeneration of high quality [82].

MSP gives rise to environmental conditions influencing features of natural regeneration in various different directions. Limited interference in the soil environment (as with FM) favours the establishment of mycorrhizal symbioses and a diversity of mycorrhizal fungal communities, even as this method may have an unfavourable influence on both seed germination and seedling survival. Conversely, more intensive interference in the soil environment (as with the FP and AP methods of site preparation) engenders favourable conditions for germination and seedling growth [82], albeit with variable levels of mycorrhization of seedlings, in line with the characteristics of the microsites put in place. There is, moreover, a shift in both composition and diversity among communities of mycorrhizal fungi.

From the point of view of forestry practice, there would also seem to be justification of the selection of methods intervening more markedly in the soil environment (as with FP and AP), should this be appropriate silviculturally. While seedlings are mycorrhized more weakly, there is a comparable wealth of fungal species present, while the state of mycorrhizae continues to suffice for the proper growth of seedlings.

5. Conclusions

Through this study, the microsites put in place as different methods of mechanical site preparation (MSP), pursued over a clearcut, are found to differ in terms of both physical and chemical features of the soil—a circumstance impacting on the level of mycorrhization and assemblage species diversity characterising ECM/EECM fungi on the roots of seedlings of Scots pine growing there. While values for both are high, the parameters nevertheless differ significantly in terms of the values characterising one microsite or another, and ultimately as a reflection of the different MSP methods. Pine seedlings growing in the control not subject to mechanical site preparation were characterised by the highest level of mycorrhization, high species richness, and high diversity of mycorrhizal fungi. The poorest and more acidic microsites—of the two kinds of furrow generated by FP and AP—also support pine seedlings mycorrhized to a high degree. In turn, seedlings growing on the two kinds of ridge (also generated by FP and AP)—as the microsites which report the highest levels of mineral nutrients—are mycorrhized most weakly. The mycorrhizal fungal communities of the five microsites, i.e., with the two types of furrow (FP and AP), the FP ridge, the forest mill strip, and the non-mechanical site preparation control did not differ from each other, but were different from those present on AP ridges. When set against the non-preparation control, methods of MSP interfering in the soil environment are seen to link up with lower overall mycorrhization of pine roots, and with a shift noted in the assemblage of mycorrhizal fungi, in terms of both composition and diversity. Nevertheless, the disturbances arising with MSP are not great in general, whatever the method involved.

The results of the work detailed here can prove valuable to forestry practitioners as they seek an optimal choice of site-preparation method, taking into consideration, not only silviculture premises and local climatic conditions (mainly as regards precipitation), but also the level of mycorrhization and the biological diversity of mycorrhizal fungi.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f13020353/s1, Table S1: Frequency of mycorrhizal fungal taxa of 1-year old Scots pine seedlings growing in different microsites created by MSP methods. Different letters indicate significant differences according to the Tukey test, $p < 0.05$., Table S2: Values of Spearman rank coefficients for the correlation between soil features and the relative abundance of mycorrhizal fungal species. Bold values indicate differences significant at $p \leq 0.05$.

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