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# Management regime is the most important factor influencing ectomycorrhizal species community in Norway spruce forests after windthrow

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## Abstract
Ectomycorrhizal (ECM) fungi, as symbionts of many tree species in temperate forests, are thought to play an important role in forest regeneration processes after large disturbances. Their reaction to different disturbance and management regimes was studied in spruce forests (*Lariceto-Piceetum*) 10 years after a severe windthrow in the Tatra National Park (Slovak Republic). ECM community structure was compared between different "management types"—cleared area (EXT), area affected by wildfire (FIRE), uncleared area left for natural development (NEX), and mature forest as a control (REF). Based on Illumina sequencing of soil samples, we determined that the percentage of sequences assigned to ECM fungi decreased with increasing disturbance and management intensity (REF → NEX → EXT → FIRE). Similarly, the total number of ECM species per each of ten sampling points per plot (100 ha) differed between managed (EXT-11 species, FIRE-9) and unmanaged (NEX-16, REF-14) treatments. On the other hand, the percentage of sequences belonging to ericoid mycorrhizal fungi increased. Management type significantly influenced the composition of the ECM community, while vegetation and soil characteristics explained less data variation. The ECM species assemblage of the unmanaged site (NEX) was the most similar to the mature forest, while that of the burnt site was the most different. *Thelephora terrestris* dominated in all treatments affected by windthrow, accompanied by *Tylospora fibrillosa* (NEX) and *Tylospora asterophora* (EXT and FIRE). Management regime was also the most important factor affecting ECM species composition on the roots of spruce seedlings assessed by Sanger sequencing.

**Keywords** Ectomycorrhiza · Windthrow · Fire · *Picea abies* · Forest regeneration · Next generation sequencing · Fungi

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## Introduction

Large disturbances like wildfire, insect outbreaks, and windthrow occur naturally in the life cycles of temperate forests (Seidl et al. 2011). Because most tree species of European temperate forests live in symbiosis with ectomycorrhizal (ECM) fungi, these are also expected to be significantly influenced by natural disturbances and are possibly adapted to some extent (Jones et al. 2003; Taudière et al. 2017). Most information about the influence of natural forest disturbance on ECM fungi came from forests affected by wildfire. The destruction of the organic horizon led to decreased fungal diversity and dominance of several possibly fire adapted taxa—*Thelephoraceae*, *Rhizopogon* spp., and *Wilcoxina* (Rincón et al. 2014; Buscardo et al. 2015; Glassman et al. 2016). Insect (e.g., bark beetle) outbreaks proceed more

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slowly, thus not affecting the organic horizon, and it is supposed that there is a higher connectivity between original and new forests (Štursová et al. 2014). Windthrows are similar to wildfires in terms of their abruptness; however, the small effect on the organic horizon is similar to the bark beetle outbreaks that often follow windthrow (Wermelinger 2004).

Forest management after natural disturbances is a hot topic intensively discussed by experts and politicians. It is expected that no intervention results in a more diverse future forest and enhances its resilience to future similar events (Bengtsson et al. 2000). However, conventional management, wood extraction, and its commercial use followed by artificial planting are presented as being more economical. It should also prevent subsequent insect outbreaks or their dispersal, and even reduce the threat of potential wildfires. It is also claimed that such forest restoration is faster and therefore more appreciated by local people (Hußlein et al. 2009).

Surprisingly, there are almost no studies on ECM fungi comparing interventionist and non-interventionist management approaches. Walker et al. (2012) detected the effect of coarse woody debris (CWD) retention on species composition on the roots of *Picea engelmannii* saplings within 15 years after harvest, but no significant changes in species composition in mesh bags. Menkis et al. (2010) studied the influence of stump extraction used as forest biofuel on seedlings. These authors found reduced ECM diversity on *Picea abies* seedling roots in plots with removed stumps but better seedling properties. Hartmann et al. (2014) found negative effects of experimental soil compaction, which often accompanies logging, on ECM communities in soil. On the other hand, Kataja-Aho et al. (2012) using a larger dataset found only minor differences in ECM species composition on seedling roots between treatments.

A large number of studies compared ectomycorrhizae on seedlings from nurseries growing in mature forests and clearcuts. In a review, Jones et al. (2003) reported that clearcuts have no influence on the percentage of mycorrhizae, but the species composition of ECM fungi is often changed. However, these changes can be attributed to a modified soil biology and chemistry rather than to ECM inoculum losses or changes. Few studies compared the effect of both clearcuts and wildfire on ECM symbionts. Visser and Parkinson (1999) reported a greater reduction of ECM species colonizing pine roots following wildfire compared to those affected by clearcut. However, Lazaruk (in Jones et al. 2003) found no difference in the number of morphotypes on *Picea glauca* between clearcut and fire plots. Barker et al. (2013) found significantly lower Shannon-Wiener diversity in burnt treatments than in undisturbed forest of *Pseudotsuga menziesii*, while the clearcut treatment had an intermediate Shannon Wiener and Eveness, and the same richness as an undisturbed forest. These authors also showed that the number of fungi with relative abundance > 5% decreased with increasing forest floor disturbance.

A strong windthrow with gusts over 200 km/h seriously damaged ca 12,000 ha of mountain spruce forests (*Lariceto-Piceetum*) in the Tatra National Park in 2004. Fallen trees were extracted from most of the area (EXT). Almost 1 year later, wildfire broke out on a part (220 ha) of this area (FIRE). However, a small area was left for natural development (NEX) (Gömöryová et al. 2011). These events enabled us to compare the structure of the ECM fungal communities in the mature forest and stands affected by different management and disturbance regimes, in addition in natural *Picea abies* stands, where a similar survey has never been conducted.

To analyze fungal community structure, we used next generation sequencing (NGS) of fungal DNA in soil samples. Because of the known biases of this approach (Lindahl et al. 2013; Nguyen et al. 2015; Pickles and Pither 2014), we compared the NGS data with results of fruit body monitoring and the composition of ECM symbionts on spruce seedlings. In agreement with Barker et al. (2013), we expected that management type influences the diversity and species composition of whole ECM fungal communities (mycelia in soil, fruit bodies), as well as their subset associated with spruce seedlings (ectomycorrhizae), with the ECM fungal community in mature forests expected to be the most similar to the community in sites left for natural development (NEX), but the most dissimilar to the ECM community on sites affected by fire (FIRE).

## Methods

### Study site

The study area affected by the windstorm is situated on the south-east slopes of The Tatra Mts. This region has a cold climate with mean annual temperature of 5.3 °C and mean annual precipitation of 833 mm (Fleischer 2008). Soils are characterized as dystric cambisols on glacial moraine deposits. The soil texture is loamy sand with pH values of around 3 (in KCl) in the upper mineral soil (Don et al. 2012). Four permanent research plots in areas with different management regimes, established for long-term observation of ecological changes, were used in our study: (i) the part of the windthrow area in which the fallen trees were extracted (EXT), with the brushwood (slash) left in piles on the site; (ii) the windthrow area left uncleared allowing for natural succession (NEX); (iii) the windthrow area in which partly extracted fallen trees burned (FIRE); and (iv) an intact spruce forest as a reference site (REF). Each plot covers an area of about 100 ha (Table 1, Fig. 1). Before the windstorm event, all plots were covered by spruce forest with an admixture of larch and pine (*Lariceto-Piceetum*). The part subjected to fire burned for several days, resulting in the complete absence of the herb layer

**Table 1** Characteristics of the management treatments

| | NEX | EXT | FIRE | REF |
|---|---|---|---|---|
| Altitude (m s.l.) | 1100 | 1260 | 1065 | 1210 |
| Coordinates (WGS 84) | N 49.180 E 20.251 | N 49.121 E 20.164 | N 49.136 E 20.199 | N 49.121 E 20.121 |
| Slope (%) | 5 | 5–10 | 5–10 | 5–10 |
| Exposition | SE | S | SE | SE |
| Area (ha) | 113 | 93 | 90 | 110 |
| Bedrocks | moraine würm | moraine donau-mindel | moraine | moraine mindel-riss |
| % tree composition before 2004 | PA 70, LD 30 | PA 90, LD 10 | PA 70, LD 20, PS 10 | PA 80, LD 20 |
| Age of forest in r. 2004 | 125/60/25 | 80 | 80 | 120/25 |
| Soil | dystric cambisol | dystric cambisol | dystric cambisol | dystric cambisol |
| pH $H_2O$ [a] | $3.8 \pm 0.3$ | $4.0 \pm 0.2$ | $4.1 \pm 0.2$ | $3.7 \pm 0.3$ |
| %C [a] | $16.0 \pm 4.2$ | $11.1 \pm 2.6$ | $11.5 \pm 4.0$ | $14.9 \pm 5.4$ |
| %N [a] | $0.8 \pm 0.2$ | $0.5 \pm 0.1$ | $0.5 \pm 0.2$ | $0.7 \pm 0.2$ |
| cover dominant trees (more than 10%) [a] | *Picea abies* (15.4) | *Larix decidua* (29.5) *Picea abies* (12.2), *Betula pendula* (9.2) | *Larix decidua* (17.2), *Salix* sp. (14.3) | *Picea abies* (30.9), *Larix decidua* (11.9) |
| cover dominant herbs (more than 10%) [a] | *Calamagrostis villosa* (34), *Vaccinum myrtillus* (14.4) | *Calamagrostis villosa* (20.6), *Rubus idaeus* (12.5), *Avenella flexuosa* (10.8) | *Calamagrostis villosa* (54.5), *Avenella flexuosa* (15.2), *Epilobium angustifolium* (10.1), *Calluna vulgaris* (10.7) | mosses (61.5) *Vaccinum myrtillus* (30.4), *Calamagrostis villosa* (20.9) |
| wood (more than 10%) [a] | lying logs (25.5) | piles of branches (14.5) | burnt snags (13.5) | piles of branches (17), lying logs (15.5), roots (10.5) |

[a] Based on our observation around sampling points, soil properties relate to the organic horizon

Treatment abbreviations: *NEX* uncleared area left for natural development, *EXT* cleared area, *FIRE* area affected by wildfire, *REF* mature forest

immediately after the fire, and the soil being burned to the mineral horizon (Gömöryová et al. 2011).

# ECM community in soil

## Soil sample collection

We used an existing net of spruce seedling establishment monitoring points (established in the frame of post-windstorm research, Fleischer 2008) and selected 10 points per treatment (plot ca 100 ha, Table 1) with spruce seedlings and the absence of other tree species in the close surroundings. Five soil cores were collected in a circle (d = 10 m) around the spruce seedling(s), and dominant herbs and types of dead wood items in the area of 500 m$^2$ were recorded (9th–12th September 2014). After litter removal, the soil material from each sampling point (combined sample of the five soil cores) was passed through a 5-mm sterile mesh, mixed, and stored at − 20 °C. Approximately 30 g of soil material was used for the soil analysis. C and N contents were



**Fig. 1** Map of the studied area. Orange line—border of area affected by windstorm

measured using a Flash 2000 CHN analyzer (Thermo Scientific, USA). The pH$_{(H_2O)}$ of the soil suspension was determined by the potentiometric method on an OP-208 pH-meter (Radelkis, Hungary) equipped with a combination glass electrode (Electrochemical Detectors, Czech Republic) (Online Resource 1).

## Amplicon sequencing of fungal communities

Total DNA was extracted from ~ 300 mg soil material using the NucleoSpin Soil Kit (Machery–Nagel Gmbh & Co., Germany) in three replicates per sample (sampling point). The ITS2 region was amplified using a gITS7/ITS4 primer combination, extended by code- and spacer sequences specific for each sample. Before the PCR reaction, three DNA replicates were pooled and used as the matrix for PCR amplification. The PCR reaction was performed in three replicates in a 25-μl reaction mixture, containing 1× reaction buffer (Bioline, USA), 1.5 μl BSA (10 mg/ml), 1 μl of each primer (0.01 mM), 0.75 μl of a polymerase mix (4:100 of Pfu DNA polymerase: MyTaq polymerase), and 1 μl of template DNA. The amplification conditions: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s, and the final extension of 72 °C for 7 min. The three PCR products from one sample were pooled and purified using the MinElute PCR purification Kit (Qiagen, Germany). The concentration of purified PCR amplicons was determined using a QUBIT 2.0 Fluorometer (Life Technologies, USA). The purified amplicons were equimollary pooled into one library, and then after adaptors ligation was applied on the flow cell in a concentration of 1000 ng of PCR products. The library was quantified using the KAPA Library Quantification Kit Illumina platforms (Kapa Biosysytems, USA) followed by the NGS run on MiSeq (Illumina, USA).

## Molecular data analysis

The NGS sequence data were processed using SEED 2.0 (Větrovský and Baldrian 2013) according to the authors' workflow. Chimeric sequences were detected using the Uchime implementation in USEARCH v 7.0.1090 (Edgar et al. 2011) and deleted. The ITS regions of fungal DNA were extracted using Qiime 1.9.0 (Caporaso et al. 2010). The ITS2 region was used for further processing. Sequences were clustered into operational taxonomic units (OTU) using Usearch 7.0, the UPARSE-OUT algorithm (Edgar 2013) at a 97% similarity level. Consensus sequences of each OTU were constructed from MAFFT alignment (Katoh et al. 2009) based on the most abundant nucleotide at each position. The closest hits were identified using BLASTn against the database of ITS sequences of specimens identified at the species level retrieved from Genbank, and those with sequence similarity <

97% subsequently against the database of ITS sequences with identification at least at the genus level. Sequence data were deposited in the MG-RAST public database (http://metagenomics.anl.gov/, dataset number 316996).

A total of 411,939 reads remained after discarding non-fungal sequences. Because the number of sequences assigned to ECM fungi (Tedersoo and Smith 2013) significantly differed between samples, we prepared two datasets. The first dataset (I) was based on fungal sequences resampled to the depth of 3367 reads per sampling point (total of 134,680 reads). It was composed of 2790 OTU, including 760 singletons. Due to the non-reproducibility of low abundant OTUs (Lindahl et al. 2013), only OTUs with an abundance more than 0.5% per plot (319) were used for other processing. Dividing the dataset according to trophic status resulted in 66 OTUs assigned to ECM fungi. The second dataset (II) was based on sequences assigned to ECM species only (94,350 reads) resampled to a depth of 500 reads per sampling point (total of 18,675 reads) to get comparable data on ECM species composition. In five cases, the total number of ECM sequences was lower than 500; therefore, all ECM reads were used and recalculated as percentages (FIR4–400 reads, NEX 14–271 reads, FIR 12–209 reads, EXT 19–201 reads, EXT 15–94 reads). Only OTUs with an abundance of more than 0.5% per plot (108 OTUs from the total number of 193 OTUs including 43 singletons) were used for further analysis. Meliniomyces, Sebacina, and Endogone were excluded from the ECM datasets because their mycorrhizal status is not always clear and depends on the host (Tedersoo and Smith 2013; Vohník et al. 2013; Yamamoto et al. 2015). We assigned OTU to the species level if the sequence similarity was higher than 97% and coverage higher than 70%. Sequence similarity higher than 85% was the criterion for OTUs assigned to the genus level with the number corresponding to the original OTU number. Several OTUs assigned to the same species were then grouped together to obtain data that would be comparable with the data on fruit bodies and ECM root tips. For this purpose, sequences belonging to the same genera or species group sequences were aligned together with the best hit sequences (Unite, Genbank), reference sequences (Unite), and our own sequences from identified fruit bodies by ClustalW, implemented in Mega6. Phylogenetic trees were constructed using the maximum likelihood method, partial deletion gaps (Mega6, Online Resource 7). The occurrences of the ten most abundant species (dataset II) were mapped onto sampling points to show their spatial distribution and possible co-occurrence.

## Fruit bodies of ectomycorrhizal fungi

Inside each permanent research plot (100 ha), one permanent plot (50 × 50 m) was established for monitoring ECM fungal fruit bodies. Fruit bodies were recorded once a year

(September 2013, 2014, 2015). Specimens that were not identified directly in the field were dried and studied under the microscope using several identification keys (Bernicchia and Gorjón 2010; Knudsen and Vesterholt 2012; Kõljalg 1995). The plot in the mature forest was destroyed by windthrow in 2015 and therefore moved to another place (ca 400 m distance, vertical difference 40 m) and reduced to 20 × 20 m to maintain the plot in a homogeneous environment.

## Ectomycorrhizae on seedlings

In total, 15–17 Norway spruce (*Picea abies*) seedlings aged 4 to 9 years old were randomly selected in each treatment in the area 200 × 200 m (around each permanent plot where fungal fruit bodies were observed) in September 2013. Each seedling was carefully removed so that the root system was not damaged. The growth environment (presence of dominant plant species, mosses, litter, wood) was noted and seedlings (1) age (according to the number of nodes), (2) height of the aboveground part, and (3) length of the root system were measured. Subsequently, seedlings were dried at 50 °C (12 h, drier ULE 400) and biomass of the aboveground part and approximate biomass of the belowground (without roots for morphotyping) were calculated (Table 2, Online Resource 2).

Each seedling root system was carefully washed under tap water, and three sections (ca 3 × 10 cm) of root system (from the upper, middle, and lower parts) were randomly chosen and cleaned using tweezers to remove any residual soil particles. Roots were then observed under a stereomicroscope (Motic SMZ 168 TL) and documented (Canon 1000D). All ECM tips were preliminarily assigned to morphotypes and at least three root tips per morphotype and treatment were selected for molecular identification and preserved in 70% ethanol.

## Sanger sequencing of ectomycorrhizal root tips

DNA was extracted from the root tips using the Plant Dneasy Extraction kit (Qiagen). Prior to the protocol, ethanol was removed from the sample by heating at 40 °C for 10 min. PCR reactions were set up using standard protocols. DNA fragments spanning the rDNA ITS region were amplified using the primers ITS1F and ITS4, while low-quality products were reamplified using ITS1 and ITS4 (White et al. 1990). In case that double products occurred, the ITS region was amplified using specific primers for basidiomycetes (ITS1F, ITS4 Basidio or ITS1F, ITS4B; Gardes and Bruns 1993; Nikolcheva and Bärlocher 2004) and ascomycetes (ITS5, ITS4Asco; Nikolcheva and Bärlocher 2004) in order to separate the products from fungi belonging to different taxonomic groups. If product separation failed, the products were cut from a gel and purified by Gel and PCR Clean-up (Machery Nagel). PCR condition used an initial cycle of 2:30 min at 94 °C, followed by 37 cycles of 30 s at 94 °C, 40 s at 50 °C, 30 s at 74 °C, and a 4:30 min final extension at 72 °C. Annealing temperature for primer ITS5 and ITS4 Asco was 55 °C. Sequencing was done by Macrogen Inc., Seoul, Korea, utilizing an ABI 3730 XL automated sequencer (Applied Biosystems, Foster City, CA, USA).

## Molecular data analysis

Sequence chromatograms were analyzed using FinchTV (Geospiza, Inc.) and aligned in ClustalX (Larkin et al. 2007) with sequences of the best BLAST matches and related sequences from the Unite (Abarenkov et al. 2010) and GenBank (Benson et al. 2007) public databases. A value of 97% ITS region sequence identity was used as a molecular species criterion. The sequences were deposited in GenBank with accession no: MF162185-268 (Online Resource 5).

Because of difficulties with proper ECM species identification of each ectomycorrhizal root tips, we prepared a semi-quantitative dataset based on the number of root parts (1–3 per seedling), where the species was present. Species not known to form ectomycorrhizae, e.g., possible endophytes, were excluded from the dataset. The identified morphotypes were assigned to an exploration type according to Agerer and

**Table 2** Characteristics of seedlings

| | Number | Height | Root length | Age | Shoot biomass | Root biomass | Surroundings |
|---|---|---|---|---|---|---|---|
| NEX | 17 | 27 ± 4.5 | 24 ± 9.2 | 5.8 ± 0.9 | 5.3 ± 2.7 | 0.8 ± 0.5 | *Calamagrostis villosa, Vaccinum myrtillus* |
| FIRE | 16 | 28.6 ± 6.2 | 26 ± 23.7 | 5.4 ± 0.7 | 6.7 ± 4.1 | 1.0 ± 0.8 | *Calamagrostis villosa* |
| REF | 16 | 22.3 ± 4.9 | 21 ± 6.5 | 4.6 ± 0.6 | 3.4 ± 1.9 | 0.9 ± 0.7 | *Avenella flexuosa, mosses* |
| EXT | 15 | 25.7 ± 6.2 | 36 ± 26.7 | 6.5 ± 1.3 | 6.2 ± 4.2 | 1.5 ± 1.3 | *Vaccinum myrtillus, Calamagrostis villosa* |

Treatment abbreviations: *NEX* uncleared area left for natural development, *EXT* cleared area, *FIRE* area affected by wildfire, *REF* mature forest

Rambold (2004), Agerer (2006) and on basis of our own observations.

## Statistics

Detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) in Canoco 5 (Ter Braak and Šmilauer 2012) were used to analyze the multivariate data of ECM species abundance (number of sequences, percentage of sequences) in soil, the data of ECM species occurrence on roots of seedlings, and exploration type occurrence on seedling roots. Logarithmic transformation of the data was used in all cases. Management, position, vegetation, dead wood, and soil properties were used as explanatory variables in the CCA analyses of ECM species abundance in soil. Management, seedling characteristic (height, root length, aboveground biomass, root biomass, age), and characteristics of the surroundings were used as explanatory variables in the CCA analysis of ECM species and exploration type occurrence on seedling roots. The effect of the explanatory variables was tested by Monte Carlo permutation test, and only significant variables were selected using the forward selection procedure.

Analysis of variance in R 3.4.0 (R Core Team 2017) was used to test the differences among treatments in the percentage of ECM sequences, percentage of sequences assigned to fungi forming ericoid mycorrhizae, the number of ECM species among treatments, the number of ECM species per seedling forming ectomycorrhizae sequence abundance of dominant species. To identify if there was a spatial correlation structure, we first made variograms of the residuals for a visual assessment. Then, we included a spatial correlation structure to a GLS model and compared it with the model without the spatial correlation. We tried five types of correlation structure available in R package nlme: exponential, gaussian, linear, rational, and spherical correlation, and the model without correlation structure. The best model was selected by using the AIC values. The approach is described in detail in Zuur et al. (2009). Since we did not find spatial autocorrelation in any model with significant differences among treatments, we then used analysis of variance and Tukey test to detect the differences among treatments.

## Results

### ECM community in soil

Dataset I resulted in 57 ECM species, dataset II (same number of ECM sequences per sampling point—500) in 92 ECM species (Online Resource 3). Based on dataset I, the percentage of sequences assigned to ECM fungi decreased with management and disturbance intensity, whereas the percentage of ericoid sequences increased (Table 3, Fig. 2a, b). The total

number of ECM species per treatment as well as the number of ECM species per sampling point was higher in the mature and unmanaged plots than in the clear-cut treatment and the site affected by fire (Table 3, Fig. 2c). However, if we compare the same number of ECM sequences per sampling point (dataset II), there is no difference between the species number in the managed (FIRE, EXT) and unmanaged (REF, NEX) treatments (Table 3). Both datasets had a higher number of subordinate species (with abundances of 2–12% of ECM sequences ($p < 0.01$) in the expected order REF–NEX–EXT–FIRE (Tables 3 and 4, Fig. 2d)). No significant differences were found among the diversity indices.

The proportion of higher taxonomical units based on ECM sequences is shown in Fig. 3. The differences among treatments were statistically significant only for Thelephorales ($p < 0.05$), which occurred on all plots affected by windthrow. Agaricales and Russulales were the most abundant in the mature forest, while Pezizales were abundant on plots affected by windthrow but not by fire. *Amanita fulva* and *Piloderma* sp. were the most abundant ECM species in the mature forest. *Thelephora terrestris* ($p < 0.01$) dominated in all other plots, accompanied by *Tylospora fibrillosa* (NEX) and *Tylospora asterophora* (EXT and FIRE). Fungi forming hypogeous fruit bodies, *Hydnotrya* sp. 079 ($p < 0.05$) and *Elaphomyces* sp. 048 were present mainly in NEX. Mapping the occurrence of the most abundant species showed the similarity of adjoining sampling points (Online Resource 6).

Detrended canonical correspondence analysis of ECM species composition showed that the main variability followed the management and disturbance intensity gradients (Table 4, Fig. 4a), which explained 12% of the variation. Based on the ordination diagrams (Fig. 4b), it is clear that the environmental data were correlated. Sampling points in the mature forest were surrounded by more mosses and hanging logs (not fully lying on the ground). The unmanaged sampling points (NEX) had the slightly lower C/N ratio and were the most distant from the mature forest. Sampling points affected by fire possessed the most willow shrubs, *Calluna vulgaris* and the highest pH value. However, if we used management as a co-variate, the other environmental data explained only 9% of the variability, with the most variation explained by the presence of mosses and willows.

### Fruit bodies of ectomycorrhizal fungi

Fruit bodies of 58 ECM species were found during three monitoring terms. Forty-one ECM species known to form ectomycorrhizae with Norway spruce were recorded in the mature forest, whereas only 7 ECM species produced fruit bodies in NEX and 3 ECM species in both EXT and FIRE. Two species known to form ectomycorrhizae with

**Table 3** Percentage of sequences and number of ECM species

|  | NEX | EXT | FIRE | REF |
|---|---|---|---|---|
| Dataset I-3367 fungal sequences |  |  |  |  |
| Average percetage of ECM sequences per plot | 29.9 ± 18.4 | 23.1 ± 18.1 | 12.5 ± 6.6 | 45.9 ± 21.9 |
| Averge percentage of ericoid sequences | 12.5 ± 5.2 | 14.2 ± 7.4 | 15.9 ± 7.2 | 6.2 ± 3.9 |
| Average percentage of no hit sequences | 14 ± 4.9 | 16.7 ± 5.8 | 23.9 ± 5.1 | 12.1 ± 6.7 |
| Total number of ECM species (57) | 47 | 35 | 37 | 46 |
| Average number of ECM species per subplot | 16 | 11 | 9 | 14 |
| Average number of subordinate ECM species (abundance 2–12%) | 3.5 | 2.7 | 1.6 | 4.1 |
| Dataset II—500 ECM sequences |  |  |  |  |
| Total number of ECM species (92) | 61 | 53 | 58 | 59 |
| Average number of ECM species per subplot | 19 | 15 | 16 | 16 |
| Average number of subordinate ECM species (abundance 2–12%) | 3.2 | 2 | 1.5 | 4.4 |

Treatment abbreviations: *NEX* uncleared area left for natural development, *EXT* cleared area, *FIRE* area affected by wildfire, *REF* mature forest
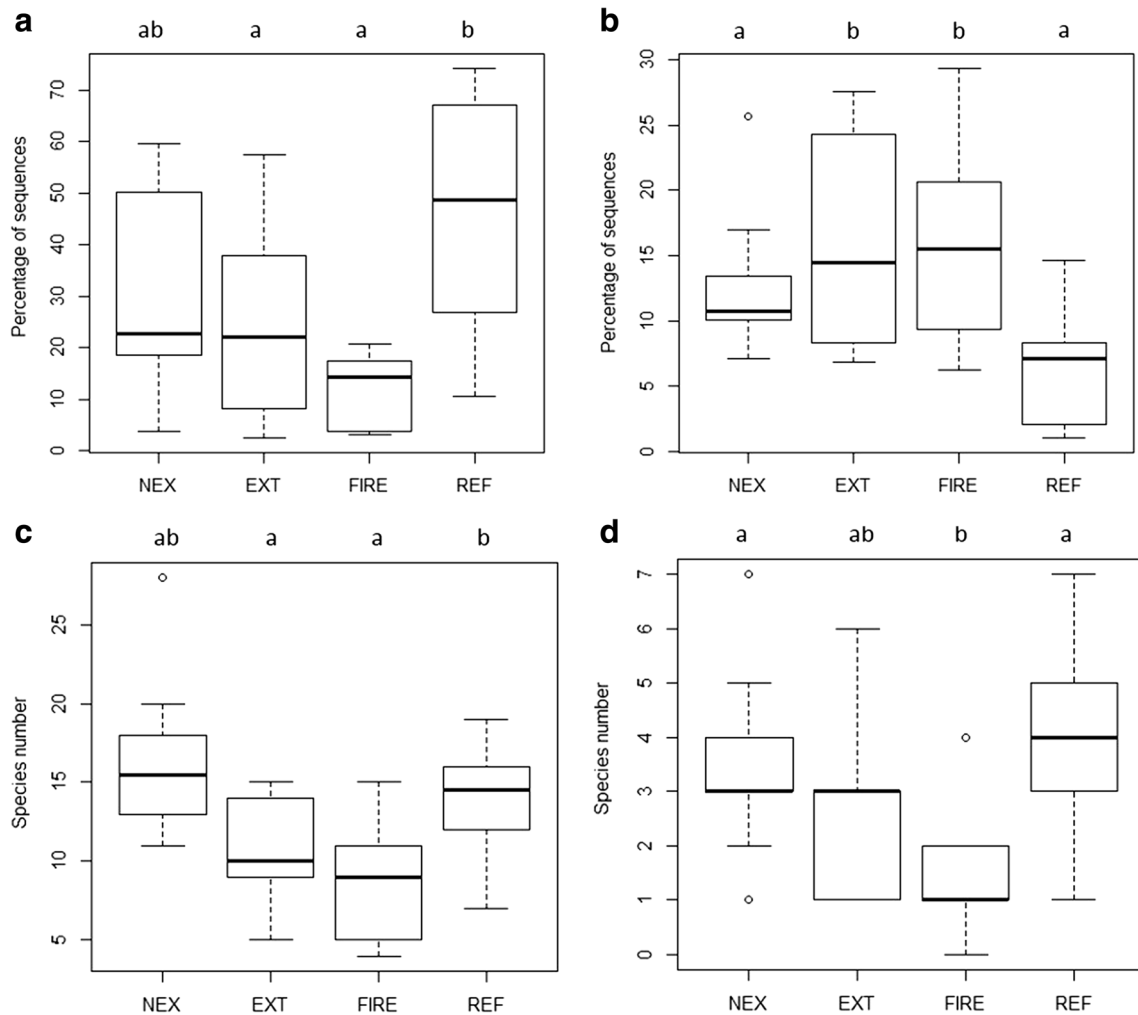


**Fig. 2** Differences in **a** percentage of ECM sequences among treatments, **b** percentage of sequences assigned to fungi forming ericoid mycorrhizae among treatments, **c** number of ECM species among treatments, **d** number of subordinate ECM species among treatments (dataset I—3367 fungal sequences)

**Table 4** Results of canonical correspondence analysis (dataset I—3367 fungal sequences)

| Explanatory variable | Variables with significant effect (forward selection) | Adjusted explained variation (%) | Pseudo-F | p |
|---|---|---|---|---|
| Management | Management | 12.0 | 2.8 | 0.001 |
| All variables | Mosses, Management | 12.9 | 2.4 | 0.001 |
| All variables | Mosses, Willow | 9.0 | 2.9 | 0.001 |
| (management as covariate) Group of variables | | | | |
| Position | N°, E° | 8.2 | 2.7 | 0.001 |
| Regeneration | Willow, spruce | 5.6 | 2.1 | 0.001 |
| Vegetation | Mosses, *Calluna vulgaris* | 8.0 | 2.7 | 0.001 |
| Wood | Burnt snags, hanging logs | 5.6 | 2.2 | 0.001 |
| Soil | pH(H$_2$O), C:N | 4.3 | 1.9 | 0.003 |

other hosts than spruce were found in the mature forest, 5 in NEX, 2 in EXT, and 7 in FIRE (Online Resource 4). Most fruit bodies in REF were formed by *Cantharellus tubaeformis*, *Lactarius aurantiacus*, and *Cortinarius collinitus*, species known to be associated also with other trees than spruce (*Paxillus involutus*-EXT, NEX, *Laccaria proxima*-NEX, FIRE, and *Cortinarius uliginosus*-FIRE) dominated in plots affected by the windthrow.

### Ectomycorrhizae on seedlings

A total of 27 ECM species formed ectomycorrhizae on spruce seedlings (NEX: 15, EXT: 15, FIRE: 12, REF: 14). However, the number of species per seedling significantly differed only between REF and FIRE (Fig. 5). These differences did not positively correlate with seedling characteristic; seedlings in FIRE were higher ($p > 0.05$; FIRE: 28.8 ± 6.1 cm, REF: 22.8 ± 5.1 cm) and slightly older (REF-NEX, FIRE-EXT, REF-EXT, $p < 0.01$; NEX: 5.8 ± 0.9, EXT: 6.4 ± 1.3, FIRE: 5.3 ± 0.7, REF: 4.6 ± 0.7) than in REF. Management explained 10.7% of the variability in ECM species composition, with the main difference being between seedlings from the FIRE

treatment versus the other treatments (Table 5, Fig. 6). Other significant factors (percentage of *Calamagrostis villosa*, seedling height) explained only a minor part of the variability (1.9%, 1.5%). *Tylospora fibrillosa* and *Piloderma* sp. 1 occurred on most seedlings in NEX, *Piloderma sphaerosporum* in EXT, *Thelephora terrestris* and *Tylospora asterophora* in FIRE, and *T. fibrillosa* and *P. sphaerosporum* in REF. Fungi of the medium-sized exploration type of ectomycorrhizae were found mainly on seedlings in the FIRE treatment, whereas fungi of the contact exploration type occurred on seedlings in treatments not affected by management (REF, NEX) (Fig. 7).

## Discussion

### Quantitative differences among treatments

Our data show that the percentage of ECM sequences decreased with increasing management and disturbance intensity in favor of other functional groups of fungi (e.g., saprotrophic species, ericoid mycorrhizae); however, the differences between EXT and NEX were not significant (Fig. 2a). The
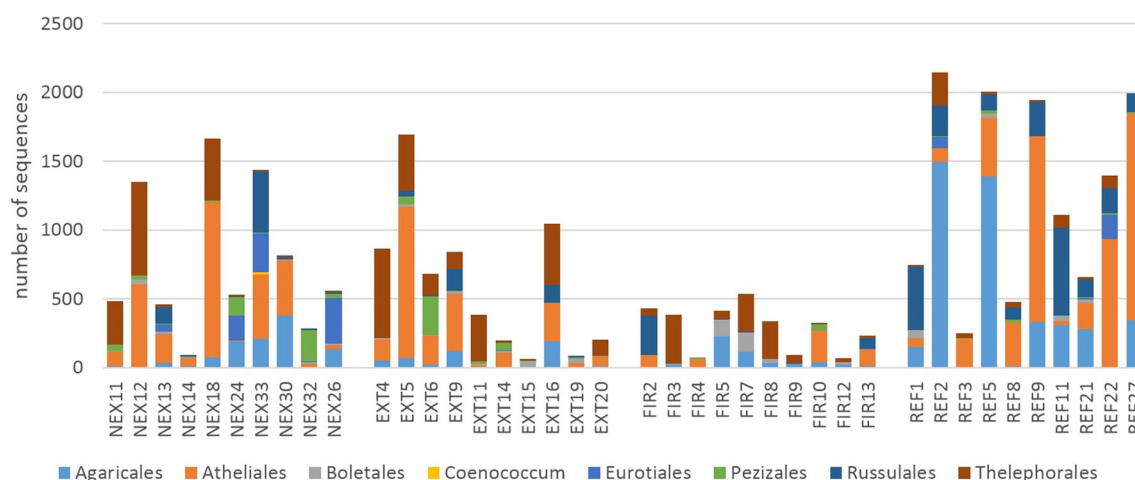


**Fig. 3** Taxonomic assignment of ECM sequences (dataset I—3367 fungal sequences)
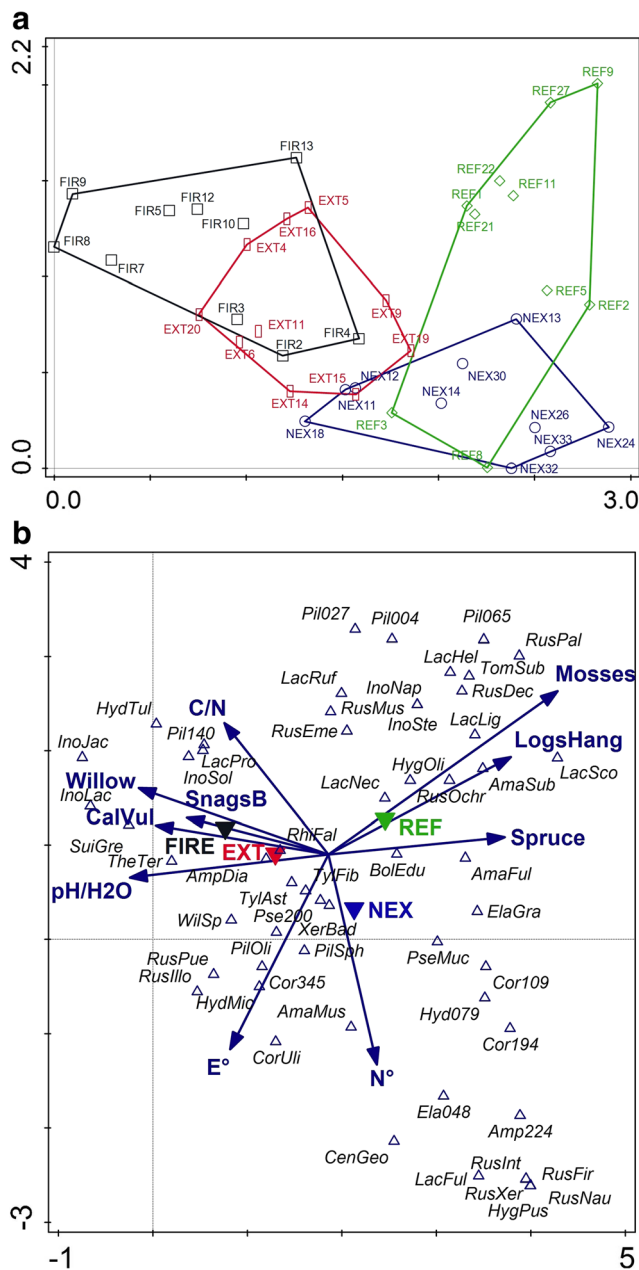
Fig. 4 **a** Ordination diagram from a DCA analysis of ECM species abundance (number of sequences) in the sampling points (dataset I—3367 fungal sequences). **b** Ordination diagram from a DCA analysis of ECM species abundance (number of sequences) in the sampling points (dataset I—3367 fungal sequences). Only environmental factors with significant effect in the CCA analysis are shown. LogsHang–hanging logs, SnagsB–burnt snags, CalVul–*Calluna vulgaris*. E°, N°–geographic position. Species: *AmaFul-Amanita fulva, AmaMus-Amanita muscaria, AmaSub-Amanita submembranacea, AmpDia-Amphinema diadema, Amp224-Amphinema* sp. *224, BolEdu-Boletus edulis, CenGeo-Cenococcum geophilum, Cor109-Cortinarius* sp. *109, Cor194-Cortinarius* sp. *194, Cor345-Cortinarius* sp. *345, CorUli-Cortinarius uliginosus, ElaGra-Elaphomyces granulatus, Ela048-Elaphomyces* sp. *48, Hyd079-Hydnotrya* sp. *079, HydMic–Hydnotrya mechaelis, HydTul–Hydnotrya tulasnei, HygOli-Hygrophorus olivaceoalbus, HygPus-Hygrophorus pustulatus, InoJac-Inocybe jacobi, InoLac-Inocybe lacera, InoNap-Inocybe napipes, InoSol-Inocybe soluta, InoSte-Inocybe stellatospora, LacPro-Laccaria proxima, LacSco–Laccaria scotica, LacFul-Lactarius fuliginosus, LacHel-Lactarius helvus, LacLig-Lactarius lignyotus, LacNec-Lactarius necator, LacRuf-Lactarius rufus, PilOli-Piloderma olivaceum, Pil004–Piloderma* sp. *004, Pil027-Piloderma* sp. *027, Pil65-Piloderma* sp. *065, Pil140-Piloderma* sp. *140, PilSph-Piloderma sphaerosporum, PseMuc-Pseudotomentella mucidula, Pse200-Pseudotomentella* sp. *200, RhiFal–Rhizopogon fallax, RusDec-Russula decolorans, RusEme-Russula emetica, RusFir-Russula firmula, RusIll-Russula illota, RusInt-Russula integra, RusMus-Russula mustelina, RusNau Russula nauseosa, RusOch-Russula ochroleuca, RusPal-Russula paludosa, RusPue-Russula puellaris, RusXer-Russula xerampelina, SuiGre-Suillus grevillei, TomSub-Tomentella sublilacina, TylAst-Tylospora asterophora, TylFib-Tylospora fibrillosa, TheTer-Thelephora terrestris, WilSp-Wilcoxina* sp., *XerBad-Xerocomus badius*

slowly compared to a forest left for natural development, because the ECM fungi lost a higher proportion of their previous niche. The total number of ECM species and the number of subordinate ECM species (Fig. 2c, d) detected in soil also decreased with increased management and disturbance intensity. Because both NGS datasets (3367 and 500 reads) showed

extraction of fallen trees from damaged forests probably leads to sudden mechanical destruction of fungal mycelia. Surviving mycelia are subsequently exposed to environmental changes, e.g. lower humidity and fluctuating temperatures on plots with little or no vegetation cover. Such places are then colonized by shrubs having symbiosis with ericoid mycorrhizal fungi, which, probably due to their saprotrophic capacities (Kohler et al. 2015), are better adapted to the new conditions, thereby replacing the original fungal communities (Fig. 2b). Ericoid fungi are also known to negatively affect ectomycorrhizae (Jones et al. 2003). However, the coverage of ericoid shrubs does not correlate with the amount of ericoid sequence, so the relationships are possibly more complex. It is likely that forests after clear-cutting will regenerate more
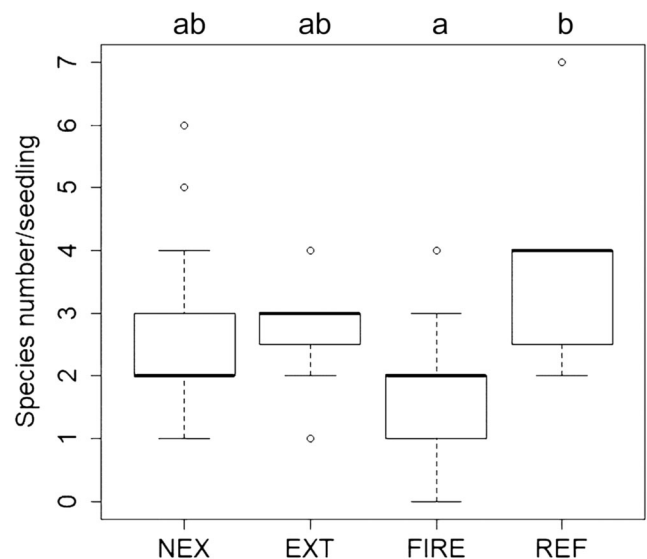


Fig. 5 Differences in the number of ECM species per seedling forming ectomycorrhizae among treatments

**Table 5** Results of canonical correspondence analysis (seedlings)

| Explanatory variable | Covariate | Adjusted explained variation (%) | Pseudo-F | p |
|---|---|---|---|---|
| ECM species occurrence on seedlings | | | | |
| Management | – | 10.7 | 3.5 | 0.001 |
| Height | – | 1.5 | 1.9 | 0.012 |
| Height | Management | 1.2 | 1.7 | 0.032 |
| *Calamagrostis villosa* | – | 1.9 | 2.2 | 0.001 |
| *Calamagrostis villosa* | Management | | – | n.s. |
| Occurence of exploration types on seedlings | | | | |
| Management | – | 16.8 | 5.2 | 0.001 |

the same pattern in the number of subordinate species (Tables 3 and 5), we considered the number of species at a certain abundance level in the NGS data to be a better parameter than total number of ECM species or diversity indices, which are possibly not suitable for the hyperdiverse NGS data. The ecological relevance of this parameter has been observed also at the treeline (Vašutová et al. 2017). The number of ECM species forming fruit bodies was reduced in plots affected by windthrow, which indicates that ECM mycelia were in suboptimal conditions (minimum C from trees, competition for water and minerals with other functional groups). Of all the treatments, only FIRE significantly impacted the number of ECM species on spruce seedlings (Fig. 6). Therefore, the amount of ECM inoculum in EXT and NEX is possibly sufficient. This finding corresponds with the analysis of dataset 2, that after quantitative differences were filtered out, there was a similar number of ECM species in the 500 sequences per subplots

(Table 2). Our finding from the fire treatment disagrees with the review by Taudière et al. (2017), who reported no consequence of fire on the richness of the ECM community. In our case, the ECM fungal community affected by fire became less complex and possibly could be less resilient (Mariotte 2014). A negative effect of management on ECM diversity of mature forests was already reported by Tedersoo et al. (2012). However, because management affects tree composition and age structure as well as soil properties, which are very difficult to disentangle, detailed studies often lead to contradictory results (Dvořák et al. 2017; Spake et al. 2016).

## Differences in species composition

Management was the most important factor, which explains the variability in ECM species read abundance among the treatments; none of the other environmental factors was found
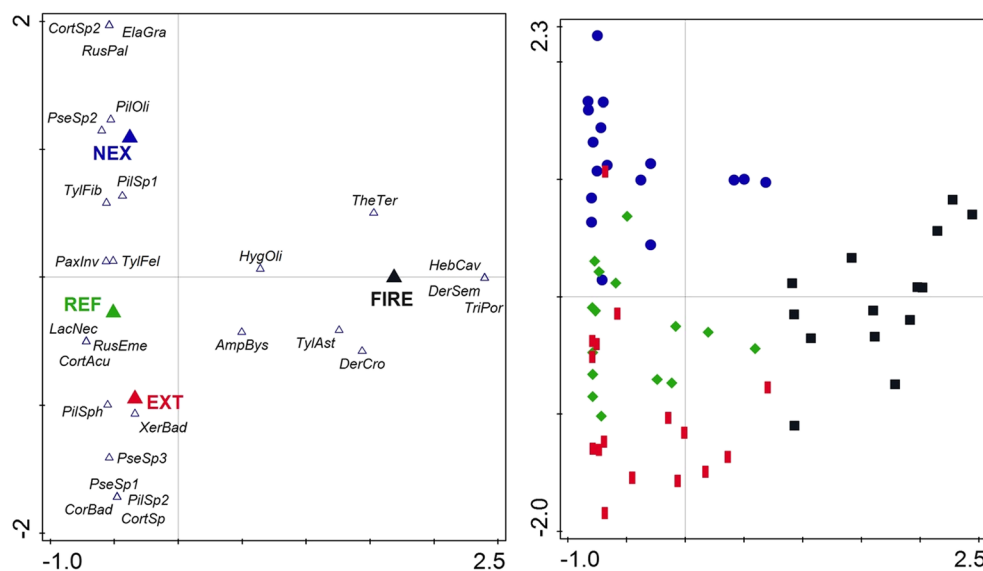


**Fig. 6** Ordination diagrams from CCA analysis of ECM species occurrence on seedlings (semiquantitative data). *AmpBys–Amphinema byssoides, CortAcu–Cortinarius acutus, CortSp1–Cortinarius* sp. 1, *CortSp2–Cortinarius* sp. 2, *CortBad–Cortinarius badius, DerCro–Cortinarius croceus, DerSem–Cortinarius semisanguineus, ElaGra–Elaphomyces granulatus, HebCav–Hebeloma* sp. 1, *HygOli–Hygrophorus olivaceoalbus, LacNec–Lactarius necator, PaxInv–Paxillus involutus, PilFal–Piloderma fallax, PilSp1-Piloderma* sp. 1, *PilSp2-Piloderma* sp. 2, *PilSph–Piloderma sphaerosporum, PseSp1–Pseudotomentella* sp. 1, *PseSp2–Pseudotomentella* sp. 2, *PseSp3–Pseudotomentella* sp. 3, *RusEme–Russula emetica, RusPal–Russula paludosa, TheTer–Thelephora terrestris, TriPor–Tricholoma portentosum, TylAst–Tylospora asterophora, TylFel–Tylopilus felleus, TylFib–Tylospora fibrillosa, XerBad–Xerocomus badius*
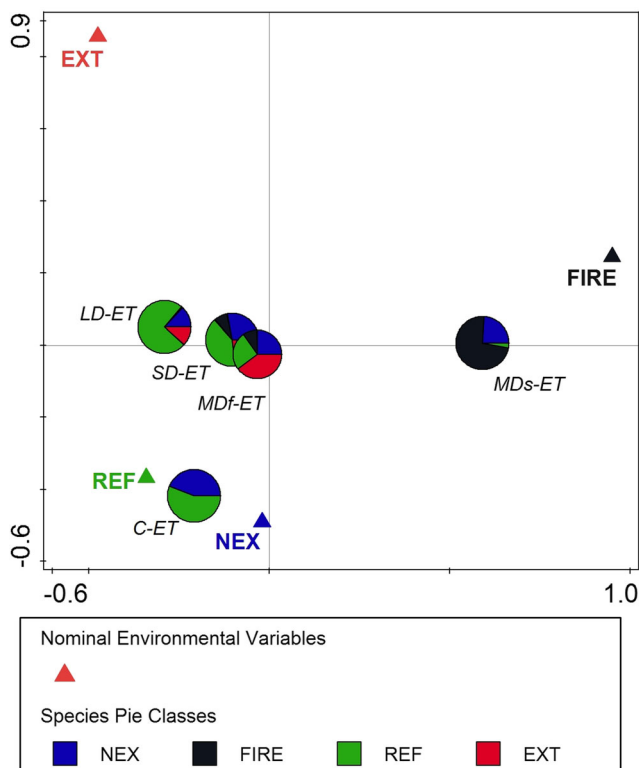
**Fig. 7** Ordination diagram from CCA analysis of exploration type occurrence on seedlings (semiquantitative data). EXT cleared area, FIRE area affected by wildfire, NEX uncleared area left for natural development, REF mature forest. Exploration types (ET): C contact, SD short-distance, MDs medium-distance smooth, MDf medium-distance fringe, LD long-distance

to be more important. In agreement with our hypothesis, the ECM community composition of the mature forest was the most similar to the sites left for natural development (NEX), while it was most dissimilar from subplots in the burnt area (FIRE) struggling with reduced ECM inoculum. The higher presence of Thelephorales and Pezizales on sites affected by wind can be explained by the predominance of seedlings that often host such ECM fungi (Barker et al. 2012; Buscardo et al. 2015, Walker et al. 2016). The most common ECM species, *Thelephora terrestris*, is found to be a symbiont of young trees and regularly occurs in nurseries, although its presence may not always be advantageous for seedlings (Smith et al. 2015).

According to Jones et al. (2003), the ECM assemblage change after clearcut is a result of changes in the soil environment as well as alteration in inoculum. The importance of inoculum was noted by Glassman et al. (2016), who reported differences in the capabilities of various species to survive. It is difficult to disentangle these factors under field conditions. Management possibly directly influenced the survival of the original ECM community (dying mycorrhizae, roots of surviving trees) as well as indirectly through changing environmental factors (pH, humidity, vegetation etc.), which influence the ability of spores (either coming from the soil bank

or by wind) to establish new mycelia. The similarity of ECM species composition in neighboring sampling points (Online Resource 6) possibly indicates similar local environmental conditions. It could also be a result of vegetative spreading of mycelia, but because the size of one mycorrhizal genet is expected to be less than 30 m (Taylor 2002) and the size of one ramet, a physically connected mycelium, must be even smaller, it is not very probable. However, we could not exclude that 250 m is an effective distance for spreading inoculum by animals. Species with hypogeous fruit bodies, such as *Hydnotrya* and *Elaphomyces*, which are thought to be spread by small mammals, were common especially in the uncleared sampling points (NEX), where they can benefit from the increased amount of cover provided by dead wood.

Surprisingly, the species composition of ectomycorrhizae on seedlings from the mature forest was more similar to those growing in the extracted site (EXT). This similarity could be caused by the small number of replicates, high variability in the species composition of the species poor assemblages (1–6 ECM species per seedling), and the limited area (200 × 200 m) where seedlings were taken. However, the functional diversity of the mature forest (REF), based on the exploration type assessment, is most similar to the site left for natural development (NEX). It could also indicate that the impact of disturbance is more pronounced on the entire ECM community structure than on its small part associated with seedlings, which is well adapted for changing conditions (Nicholson and Jones 2017).

# Conclusions

The main effect of wind on the ECM fungal community was the decrease in the percentage of ECM sequences, the reduced occurrence of ECM fruit bodies, and a shift in species composition towards taxa known to be associated with seedlings (Thelephorales, Pezizales). ECM fungal communities of sites left for natural development were more similar to those of the mature forest in most quantitative parameters and species composition. These results support the idea that there is more abundant forest regeneration in sites left for natural development (Michalová et al. 2017). Nevertheless, the differences in quantitative parameters between interventionist and non-intervention management were not significant, possibly due to the high variability of the fungal communities. We clearly proved that fire significantly reduced the number of subordinate ECM species compared to sites left for natural development. If the maintenance of diversity is required, fire, as a widely used tool for forest management in boreal forests, is not suitable in the local conditions. The effect of treatment is detectable even in the symbiont assemblage on spruce seedlings. Although it has possibly no direct effect on ECM species diversity, seedlings from sites left for natural development

host ECM species of a similar exploration type as those from mature forests. Based on these data, we expected a faster succession towards the community of mature spruce forests in unmanaged stands.

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