

Article

Ectomycorrhizal Community on Norway Spruce Seedlings Following Bark Beetle Infestation

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Abstract: Ectomycorrhizal (ECM) fungi importantly influence seedling growth, nutrition, and survival and create an extensive mycelial network interconnecting tree species and enabling resource redistribution. Due to their symbiotic relationship with trees, they are impacted by forest disturbances, which are of increasing relevance due to climate change. The effect of disturbance on seedling colonization and their morphology is still largely unknown. Seedling growth parameters and the ECM fungal assemblage on the roots of Norway spruce (*Picea abies* (L.) H. Karst.) seedlings were assessed in mature spruce forests attacked and destroyed by bark beetle and in a mature non-attacked forest as a reference. We did not detect significant differences in number of ECM species on seedling roots among forest types, but ECM species composition changed; *Tylospora fibrillosa* (Burt) Donk, *Meliniomyces variabilis* Hambl. & Sigler, and *Phialocephala fortinii* C.J.K. Wang & H.E. Wilcox were characteristic species in the forest destroyed by bark beetle, whereas *Lactarius*, *Cortinarius*, and *Russula* were in the mature forest. Forest type further significantly influenced the height, root length, and root collar thickness of seedlings and the proportion of exploration types of mycorrhizae.

Keywords: bark beetle; ECM fungi; exploration types; seedling morphology

1. Introduction

Ectomycorrhizal (ECM) symbiosis represents an important relationship between plants and basidiomycetous or ascomycetous fungi. Approximately 6000 tree species are involved in this relationship, including taxa from the plant families *Pinaceae*, *Fagaceae*, *Betulaceae*, and *Corylaceae*, which form large forests in temperate and boreal regions [1]. The ecological importance of mycorrhizal infection on seedling growth properties, productivity, and establishment has already been demonstrated [2,3]. Seedlings benefit also from connection to the mycorrhizal network representing mycelial linkage between plants of the same or even different plant species facilitating their growth, survival, establishment, or defensive chemistry [4]. This extensive mycelial network provides a competitive advantage to seedlings due to sufficient water and nutrient supply [4]. Within the mycorrhizal network, the plants seem to be supported irrespective of their size, status, or identity [5].

Natural disturbances are an integral part of the development cycle of forest ecosystems initializing forest succession leading to changes in tree composition and cover. In the temperate and boreal forests

of Europe, the dominant disturbance events are caused by bark beetle outbreaks [6], windstorms [7], and fire [8]. Microclimatic conditions change after a disturbance that results in forest breakdown. This includes increased solar radiation and soil surface heating, greater input of precipitation and changes in soil moisture, and greater air circulation [9]. Forest ecosystem breakdown also impacts nutrient cycling and hydrology [10]. For instance, Štursová et al. [11] found that organic nitrogen was replaced by its mineral form, which negatively influenced the ECM fungal community [11,12]. Further, fungal community development is supposedly influenced by management of the affected forests, which affects soil compaction and related soil processes [13], soil microbiome, or the presence of small mammals [13,14], etc. The resulting fungal species composition could influence the plant successional trajectory, which may, in turn, govern the fungal successional trajectory.

Above-ground herbivory by bark beetle affects ECM symbioses by reducing the carbon flow to the roots and fungal symbionts [15,16]. ECM colonization is usually not reduced, but the fungal composition is changed in favor of ECM morphotypes with low demands on photosynthates [17–20], which may affect the nutrient uptake and recovery of the host [21].

The Tatra Mountains (Slovakia) were affected by a serious windstorm in 2004, which destroyed about 12,600 ha of mountain spruce forest [22]. Following this historically largest calamity in the region, bark beetle started to spread in the surrounding spruce forest in 2007 and 2008 [23]. Some forest parts were almost completely destroyed with dead tree crown projection reaching 90%, while other areas were not so seriously affected with around 6% of dead tree crown projection [23]. Veselá et al. [20] reported a decrease in the proportion of ECM fungi in favor of saprotrophs in soils and pronounced changes in ECM community composition toward the most seriously infested forest type. The aim of this study was to determine whether a similar effect is occurring on seedling roots, which would indicate a direct effect of ECM community on forest regeneration. We hypothesized that ECM species diversity decreased toward the most seriously attacked forest type together with pronounced changes in species composition with predominating stress-tolerant species. We further expected more vigorous growth of spruce seedlings in not seriously attacked forests due to their connection to the mycorrhizal network supported by mature trees and constant microclimatic conditions under the protection of shelter wood.

2. Materials and Methods

2.1. Study Area

The research area in the Tatra Mountains, Slovakia (Figure 1), is characterized by a cold climate with mean annual temperature 4.0–5.3 °C and mean annual precipitation 833–1000 mm. The studied forest types are positioned in altitudes of 1120–1240 m a.s.l. in originally unmanaged spruce forest with larch admixture (*Lariceto–Piceetum*) on cambisols or podzolsol with granodiorite or moraine bedrock [23,24]. Before the bark beetle attack, the forest was 130–140 years old, spruce accounted for 80 to 90%, and canopy cover reached from 45 to 60% [20,23,24].

The K1 forest type is a forest attacked by bark beetle, with 10% dead tree crown projection in 2015. The bark beetle infestation started to spread in 2009. This forest part represents a slowly decaying forest composed of spruce with larch accompanied with fir and pine. Natural regeneration is represented mostly by spruce and rowan, with several individuals of larch, fir, and pine. The K2 forest type is a forest that has been destroyed by bark beetle since 2012, with standing and downed dead trees left in the forest. In this forest type, bark beetle infestation started to spread in 2007. Regeneration is poor, with mostly spruce and rowan. The REF forest type is a mature spruce forest not attacked by bark beetle. All three forest types were dominated by *Vaccinium myrtillus* L. 1753 in the understory, accompanied by *Avenella flexuosa* (L.) Drejer in K1 and REF, or *Calamagrostis villosa* (Chaix) J.F. Gmel. in K2 and REF (Table 1, Table S1).

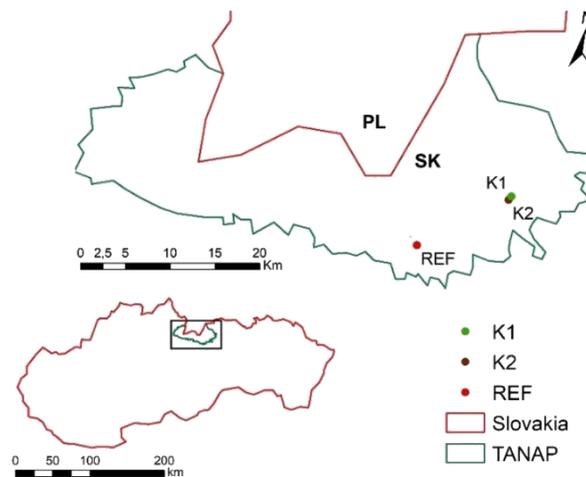


Figure 1. Map of the research area; Tatra Mountains National Park (TANAP), Slovakia (SK). Three types of forest; K1 = attacked by bark beetle, K2 = destroyed by bark beetle, REF = mature forest.

Table 1. Mean cover of trees, tree species composition, herb composition, tree regeneration, and coarse woody debris (CWD) in the K1 forest attacked by bark beetle, K2 forest destroyed by bark beetle, and REF mature forest.

	K1	K2	REF
tree cover	43 ± 10.9	0	43 ± 9.2
cover of dominant trees	<i>Picea abies</i> (L.) H. Karst. (26.5 ± 4.9), <i>Larix decidua</i> Mill. (15.0 ± 7.1), <i>Abies alba</i> Mill. (1.5 ± 1.4), <i>Sorbus aucuparia</i> L. (0.7 ± 0.5)	<i>Larix decidua</i> (1.0 ± 0.6), <i>Sorbus aucuparia</i> (3.8 ± 1.3)	<i>Picea abies</i> (36.0 ± 9.8), <i>Larix decidua</i> (7.0 ± 2.9)
cover of dominant herbs (3 dominants) *	<i>Vaccinium myrtillus</i> (37.7 ± 5.2), <i>Avenella flexuosa</i> (10.2 ± 2.1), <i>Oxalis acetosella</i> L. (4.7 ± 1.3)	<i>Vaccinium myrtillus</i> (38.1 ± 5.6), <i>Calamagrostis villosa</i> (10.4 ± 4.5), <i>Epilobium angustifolium</i> L. (8 ± 2.1)	<i>Vaccinium myrtillus</i> (32.6 ± 5), <i>Avenella flexuosa</i> (14.3 ± 3.3), <i>Calamagrostis villosa</i> (11.4 ± 2.5)
spruce seedling cover *	0.5 ± 0.3	4.5 ± 3	2.6 ± 1.3
larch seedling cover *	1 ± 1	0.2 ± 0.1	0.8 ± 0.6
rowan seedling cover *	0.3 ± 0.2	2.4 ± 0.8	0.1 ± 0.1
CWD *	2.5 ± 0.7	5.9 ± 1.1	0.5 ± 0.3

* average cover in % and standard error from 20 microsites of 4 m² per forest type.

2.2. Seedlings and Ectomycorrhizae

Twenty seedlings per forest type were collected in an area of 75 × 75 m in September 2015. The seedlings were at least 10 m apart from each other with the distance between microsites inferred from the observed variability of particular patches in mountain spruce forest.

The following morphometric parameters were measured: height of the aboveground part, root length, root collar perimeter, and age (according to the number of nodes). The surrounding vegetation cover of each selected seedling in a 5 m diameter circle was visually estimated together with coarse woody debris cover and litter cover.

In the laboratory, the root system of each seedling was carefully washed under tap water to remove soil particles and the upper, middle, and lower root fragments (with approximately 100 root tips per fragment) were documented under stereomicroscope (Canon EOS 1000D; Canon, Óta, Japan) to evaluate mycorrhizal root tips. Ectomycorrhizae were preliminarily divided into morphotypes and each morphotype was excised and stored in 70% ethanol for molecular identification.

2.3. DNA Extraction, PCR, and Sequencing

Genomic DNA was extracted from ectomycorrhizal root tips using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was then used as a template for PCR amplification with primer pairs ITS1F/ITS4 [25,26] or ITS1F/ITS4B [27]. Multiple PCR products obtained from single root tip were separated from an agarose gel using the Gel Extraction Kit (Qiagen, Hilden, Germany). The PCR reaction was performed in a 25 µL reaction mixture containing 1× reaction buffer (Bioline, London, UK), 500 nM of each primer, 0.75 U of MyTaq DNA polymerase (Bioline, London, UK), and 20 ng of template DNA filled with dH₂O up to 25 µL. The amplification conditions were 94 °C for 2.5 min, followed by 37 cycles of 94 °C for 30 s, 55 °C for 40 s, 72 °C for 30 s, and a final extension of 72 °C for 4.5 min. Sequencing of one strand using ITS4/ITS4B primer was performed by SEQme (Seqme, Dobříš, Czech Republic).

2.4. Data Processing and Analysis

The sequences were manually corrected (end trimming and replacing of ambiguous bases using IUPAC ambiguity code) in Chromas Lite v.2.6.5 (Technelysium Pty Ltd., Brisbane, Australia) and aligned against the UNITE [28] and GenBank [29] databases. A 97% similarity threshold was the criterion for species approval. The sequences were deposited in GenBank (accession numbers: MN046989, MN047080). We made a species assignment for the whole photoset based on molecular identification of the morphotypes. Exploration types were adopted from Agerer et al. [30], online database www.deemy.de, or from our observations.

2.5. Statistical Analysis

Morphometric characteristics (height of aboveground part, length of main root, root collar perimeter, and age—derived from the number of nodes) and ECM species number (ECM species presence/absence data from a particular seedling) from twenty seedlings per forest type (K1, K2, REF) were tested by analysis of variance with Tukey's post-hoc contrasts in R 3.5.3 [31]. ECM species presence/absence data from a particular seedling were used to prepare a plot of ECM species occurrence from the twenty seedlings per forest type. Semi-quantitative data (ECM species from particular seedlings based on number of occupied studied root fragments) were analyzed by canonical correspondence analysis (CCA) and the proportion of exploration types based on ECM species presence/absence data assigned to a particular seedling were analyzed by redundancy analysis (RDA) in Canoco 5 [32]. CCA and RDA analyses were then conducted for each dataset using forward selection to determine the significant environmental variables (Monte Carlo permutation test, 999 permutations). The explanatory variables tested were: forest type, morphometric characteristics of the seedlings, and the cover of seedlings, litter, coarse woody debris, ferns, mosses, and herbs, including dwarf shrubs dominating in the vegetation surrounding the seedlings (*Avenella flexuosa*, *Calamagrostis villosa*, *Calluna vulgaris* (L.) Hull., *Epilobium angustifolium*, *Homogyne alpina* (L.) Cass., *Juncus* sp. L., *Luzula luzuloides* (Lam.) Dandy et Wilmott, *L. sylvatica* (Huds.) Gaudin, *Maianthemum bifolium* (L.) F.W. Schmidt, *Oxalis acetosella*, *Rubus idaeus* L., *Sambucus* sp. L., *Vaccinium myrtillus*, and *V. vitis-idaea* L.).

3. Results

3.1. Morphometric Characteristics of Norway Spruce Seedlings

Seedlings in the forest destroyed by bark beetle (K2) had statistically taller aboveground parts ($p < 0.05$), a longer main root ($p < 0.05$), and thicker root collar than seedlings from the forest attacked by bark beetle (K1) and the mature forest (REF) ($p < 0.05$, Figure 2a–c, Table S2). On the other hand, seedlings from REF were older, had significantly more nodes (a proxy for age, six nodes on average) than seedlings from the K1 and K2 forest types (five nodes on average; $p < 0.05$, Figure 2d).

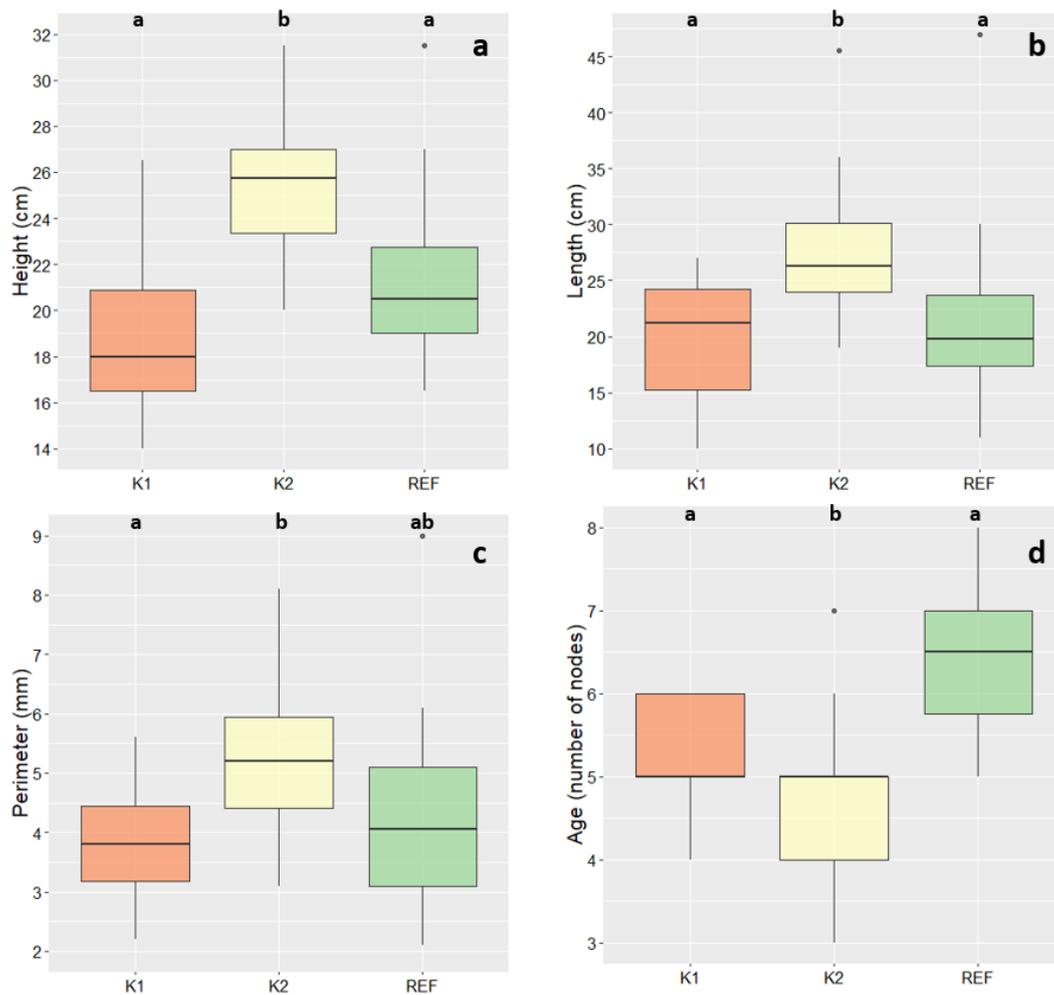


Figure 2. Morphometric characteristics of Norway spruce seedlings: (a) height of aboveground part, (b) main root length, (c) root collar perimeter, and (d) age in K1—seedlings in forest attacked by bark beetle; K2—seedlings in forest destroyed by bark beetle; REF—seedlings in mature forest. The diagrams represent median, quartiles, and range ($1.5 \times \text{IQR}$) from 20 seedlings.

3.2. ECM Community on Root System of Norway Spruce Seedlings

In total, we detected 30 ECM species; one taxon was identified only to the genus level and one to order (Table S1). The overall number of ECM taxa per forest type was the highest in the REF site (22 species) followed by K1 (19 species) and K2 (16 species). The average number of ECM species per seedling was the lowest in K2 with 2.7 species, followed by K1 and REF with 3.3, and 3.5, respectively (Figure 3). These differences were not statistically significant ($p = 0.19$).

The dominant ECM species in all three forest types was *Tylospora fibrillosa* (present in 16.9% (K1), 30.2% (K2), and 10.1% (REF) of seedlings) and *Cenococcum geophilum* Fr., present in 15.4% (K1), 11.3% (K2), and 23.2% (REF). These were accompanied by *Amphinema byssoides* (Pers.) J. Erikss., present in 10.8% (K1), 3.8% (K2), and 10.1% (REF), and *Phialocephala fortinii* (3.1% (K1), 17.0% (K2), and 1.5% (REF), Figure 4).

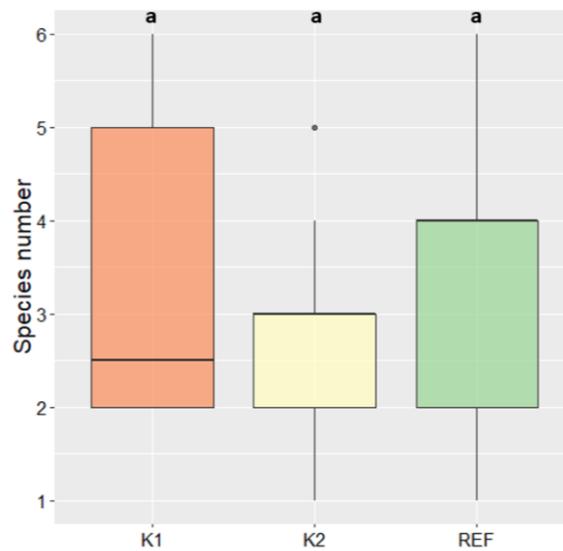


Figure 3. Ectomycorrhizal (ECM) species number from 20 Norway spruce seedlings per forest type in K1, the forest attacked by bark beetle; K2 forest destroyed by bark beetle; REF mature forest. The diagrams represent median, quartiles, and range ($1.5 \times \text{IQR}$).

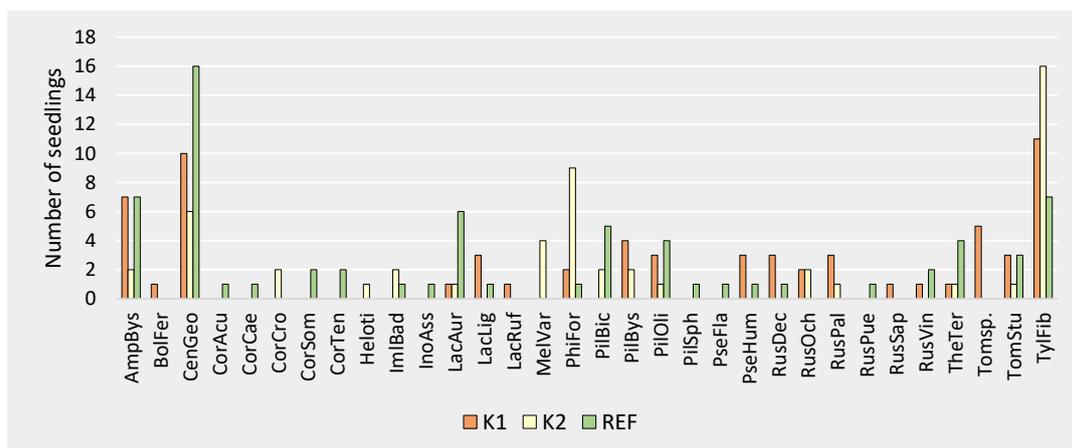


Figure 4. ECM species occurrence on Norway spruce seedlings. K1—forest attacked by bark beetle; K2—forest destroyed by bark beetle; REF—mature forest. AmpBys—*Amphinema byssoides*, BolFer—*Boletus ferrugineus* Schaeff., CenGeo—*Cenococcum geophilum*, CorAcu—*Cortinarius acutus* (Pers.) Fr., CorCae—*Cortinarius caesiobrunneus* Kytöv., Niskanen & Liimat., CorCro—*Cortinarius croceus* (Schaeff.) Gray, CorSom—*Cortinarius sommerfeltii* Høil., CorTen—*Cortinarius tenuifulvescens* Kytöv., Niskanen & Liimat., Heloti—*Helotiales* Nannf., ImlBad—*Imleria badia* (Fr.) Vizzini, InoAss—*Inocybe assimilata* (Britzelm.) Sacc., LacAur—*Lactarius aurantiacus* (Pers.) Gray, LacLig—*Lactarius lignyotus* Fr., LacRuf—*Lactarius rufus* (Scop.) Fr., MelVar—*Meliniomyces variabilis*, PhiFor—*Phialocephala fortinii*, PilBic—*Piloderma bicolor* (Peck) Jülich, PilBys—*Piloderma byssinum* (P. Karst.) Jülich, PilOli—*Piloderma olivaceum* (Parmasto) Hjortstam, PilSph—*Piloderma sphaerosporum* Jülich, PseFla—*Pseudotomentella flavovirens* (Höhn. & Litsch.) Svrček, PseHum—*Pseudotomentella humicola* M.J. Larsen, RusDec—*Russula decolorans* (Fr.) Fr. 1838, RusOchr—*Russula ochroleuca* (Pers.) Fr., RusPal—*Russula paludosa* Britzelm., RusPue—*Russula puellaris* Fr., RusSap—*Russula sapinea* Sarnari, RusVin—*Russula vinosa* Lindblad, TheTer—*Thelephora terrestris* Ehrh., Tomsp.—*Tomentella* sp., TomStu—*Tomentella stupeosa* (Link) Stalpers, TyfFib—*Tylospora fibrillosa*.

3.3. Exploration Types

Generally, short distance type (SD) was the most widespread exploration type (ET). These were represented, for example, by *T. fibrillosa* and *C. geophilum*, which were the dominant species in all forest

types (35% in K1, 68% in K2, and 38% in REF). Medium distance fringe type (MDf), represented by the genus *Cortinarius* (Pers.) Grey and several species from the genus *Piloderma* Jülich (K1 29%, K2 17%, REF 33%), and the contact type (C), represented by species from the genera *Russula* Pers. and *Lactarius* Pers. (K1 23%, K2 8%, REF 16%), were less abundant. Medium distance smooth type (MDs) accounted for 11% in K1, 4% in K2, and 12% in REF, while long distance type (LD) was only 2% in K1 and REF and 4% in K2. The species assignment to ET is listed in Table S1.

3.4. Environmental Drivers of Fungal Community Composition and Exploration Type Proportion

Canonical correspondence analysis (CCA) was used to evaluate the effect of environmental variables on ECM species composition on seedlings (Figure 5, Table 2). Forest type explained 6.7% of the variability. The first axis separated seedlings in the K2 and REF forest types, while the K1 and REF forest types were separated on the second axis (Figure 5).

The effect of environmental variables on the proportions of exploration types on seedlings were assessed by redundancy analysis (RDA). The significant drivers were found to be forest type (10.5%) and seedling microsite-cover of *Luzula luzuloides* and *Epilobium angustifolium* (14.7%) (Table 2). Exploration type SD was associated with *E. angustifolium* cover, whereas C type was related to *L. luzuloides* cover.

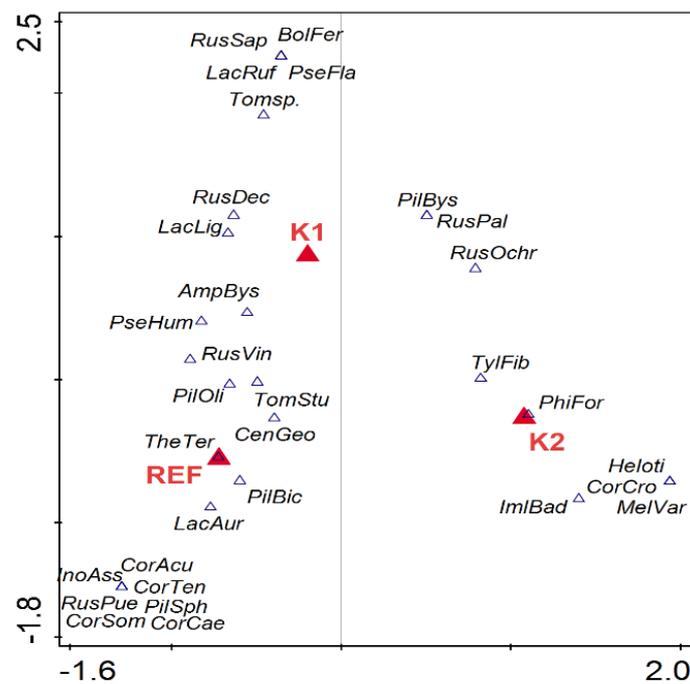


Figure 5. CCA analysis of ECM species composition of Norway spruce seedlings. Forest type was used as passive explanatory variable. K1, forest attacked by bark beetle; K2 forest destroyed by bark beetle; REF mature forest. AmpBys—*Amphinema byssoides*, BolFer—*Boletus ferrugineus*, CenGeo—*Cenococcum geophilum*, CorAcu—*Cortinarius acutus*, CorCae—*Cortinarius caesiobrunneus*, CorCro—*Cortinarius croceus*, CorSom—*Cortinarius sommerfeltii*, CorTen—*Cortinarius tenuifulvescens*, Heloti—*Helotiales*, ImlBad—*Imleria badia*, InoAss—*Inocybe assimilata*, LacAur—*Lactarius aurantiacus*, LacLig—*Lactarius lignyotus*, LacRuf—*Lactarius rufus*, MelVar—*Meliniomyces variabilis*, PhiFor—*Phialocephala fortinii*, PilBic—*Piloderma bicolor*, PilBys—*Piloderma byssinum*, PilOli—*Piloderma olivaceum*, PilSph—*Piloderma sphaerosporum*, PseFla—*Pseudotomentella flavovirens*, PseHum—*Pseudotomentella humicola*, RusDec—*Russula decolorans*, RusOchr—*Russula ochroleuca*, RusPal—*Russula paludosa*, RusPue—*Russula puellaris*, RusSap—*Russula sapinea*, RusVin—*Russula vinosa*, TheTer—*Thelephora terrestris*, Tomsp.—*Tomentella sp.*, TomStup—*Tomentella stuposa*, TylFib—*Tylospora fibrillosa*.

Table 2. Effect of environmental variables on ECM composition (canonical correspondence analysis (CCA)) and proportion of exploration types (redundancy analysis (RDA)) on Norway spruce seedlings in K1—forest attacked by bark beetle, K2—forest destroyed by bark beetle, and REF—mature forest.

Type of Analysis	Explanatory Variables	Explained Variability (%)	Pseudo-F	<i>p</i>
CCA	ECM composition			
	Site type (K1, K2, REF)	6.7	2.0	0.001
RDA	Exploration types			
	Site type (K1, K2, REF)	10.5	3.3	0.011
	Vegetation cover: <i>Epilobium angustifolium</i> , <i>Luzula luzuloides</i>	14.7	4.9	0.001

4. Discussion

Our data on ECM species composition of seedlings in forest types differently affected by bark beetle did not support the hypothesis on species diversity decrease toward the most seriously attacked forest type. Even if total number of ECM species on seedling roots per forest type decreased (REF–K1–K2), it was not significant in the case of the number of ECM species per seedling. Similarly, Vašutová et al. [14] did not find a significant difference in species number colonizing seedling roots in a windstorm destroyed area and mature forest; the only significant decrease was reported from a burnt forest stand. On the contrary, Peter et al. [33] found decreased species number with increased forest pollution intensity. Disturbances impact the ECM community in different ways depending on their intensity, duration, degree of microhabitat conditions disruption and present ECM species pool.

On the other hand, the ECM species community composition on Norway spruce seedlings changed, which was consistent with our hypothesis. When comparing this alteration with one detected in a soil fungal community after bark beetle infestation [20], where *Elaphomyces* Nees sp. and *Russula* spp. dominated in mature forests, while *Piloderma* spp., *Wilcoxina* Chin S. Yang et Korf sp., and *Thelephora terrestris* in bark beetle destroyed forest, the change in the ECM community on seedling roots was not so dramatic. Similarly, Vašutová et al. [14] detected less pronounced changes in the ECM community on seedlings than in soil. The ECM fungal community in soils is usually more complex in comparison with seedlings because of the presence of spores and mycelium of species associated with all tree age classes, while seedlings host only a specific ECM fungal spectrum related to that particular age [34,35].

Tylospora fibrillosa and *Cenococcum geophilum* were dominant on seedling roots in all three forest types, but their abundance differed between forest types. *T. fibrillosa* occupied twice as many seedlings in K2 than in REF, while *C. geophilum* showed an opposite trend. REF was characterized by the presence of *Lactarius*, *Russula*, and *Cortinarius* species, associated with healthy forests [20,36,37], with some were still found in K1. A similar trend was detected by Peter et al. [33], who found *T. fibrillosa* increased its abundance on seedlings in a locality affected by air pollution with increasing damage intensity, while *Russula* and *Lactarius* species preferred sites with lower damage levels. One of the possible explanations for the increase in *T. fibrillosa* in seriously damaged forests is its ability to degrade remnants of decaying wood to acquire extra carbon due to their possessing lignin peroxidase genes [38]. Another explanation can be the prevalence of ECM fungal species with low demands on photosynthates following defoliation [17–19]. Probably *Phialocephala fortinii* and *Meliniomyces variabilis*, which increased their abundance in K2 compared to K1 and REF, are such species. *P. fortinii* is commonly treated as an endophyte but it was also found to create ectomycorrhizae with Scots pine [39], similarly as root-associated *M. variabilis*, which was found to simultaneously colonize Scots pine and *Vaccinium* [40,41]. The ericaceous shrubs may then serve as a source of fungal inoculum enabling the colonization of spruce seedlings in K2 where the mature forest was destroyed and true ECM fungi decreased their diversity and abundance [20]. ECM species from the ordo *Helotiales* were already described to increase its abundance in beetle-killed stands [21].

ECM fungal species with saprotrophic capacities may further indirectly support seedling nutrition through access of mineralized nutrients [3]; this is consistent with the biggest seedlings found in K2. However, no direct relationships between morphological parameters and ECM species composition on seedlings were observed. Thus, the more intensive growth of seedlings in K2 was probably associated with increase light level connected to greater CO₂ assimilation and photosynthetic rate [42] and decreased competition from mature trees for water and nutrients [43].

These above mentioned findings are closely related to the changes in the proportion of exploration types. The short distance exploration type almost exclusively prevailed on seedlings in the K2 forest type (68%); this type was less abundant in other forest types (ca 35%). The SD exploration type, including taxa as *T. fibrillosa* or *C. geophilum*, are thought to have lower plant C demands for their mycelial growth [44] in contrast to the more C demanding medium distance fringe type [45] or contact type, which prefer wet soils with high organic matter content [46,47]. These latter types increased in K1 with ongoing bark beetle infestation. Changes in exploration types following environmental changes are also thought to affect C and N cycling [48]. The proportion of exploration types on seedlings was partly explained by the forest type itself but a larger part of the variability was explained by cover of the herbs *Epilobium angustifolium* and *Luzula luzuloides* (Table 2). ECM fungi are impacted by chemical compounds leached into soil from litter or decaying plants or from root exudates [49,50]. Cote and Thibault [51] demonstrated that some ECM fungi are inhibited (*Paxillus involutus* (Batsch.) Fr., *Laccaria proxima* (Boud.) Pat., *L. bicolor* (Maire) P.D. Orton, *T. terrestris*), while others are stimulated (*Hebeloma cylindrosporum* Romagn. and *C. geophilum*) when exposed to leaf leachate of raspberry [52]. Similarly, stimulatory or inhibitory effects could be seen in the relationship between the above-mentioned herbs and ECM fungi on roots of Norway spruce seedlings.

5. Conclusions

Bark beetle infestation influenced the ECM fungal community on Norway spruce seedlings. We detected increased abundance of stress tolerant *Tylospora fibrillosa*, *Meliniomyces variabilis*, and *Phialocephala fortinii* and considerable decrease in species associated with healthy forests. Seedlings in forests affected by bark beetle possessed a higher percentage of short distance exploration mycorrhizal type. The bark beetle outbreak also influenced seedling morphological parameters. Seedlings were significantly bigger in the destroyed forest, which is probably associated with forest stand opening and may also be supported by the above-mentioned stress tolerant ECM species.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/9/740/s1>, Table S1: List of sequences of ECM fungi found on roots of spruce seedlings, Table S2: Seedling morphological parameters and surrounding vegetation cover.

Author Contributions: Conceptualization, P.C. and M.V.; methodology, P.C. and M.V.; investigation, P.V. and K.H.; formal analysis, P.V., K.H., and M.E.-J.; data interpretation, P.V., M.V., and K.H.; data curation, P.V. and K.H.; writing—original draft preparation, P.V. and M.V.; writing—review and editing, P.C.; funding acquisition, P.C.

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Conflicts of Interest: The authors declare no conflict of interest.

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