Patterns of Fungal Diversity in Needles, Rootlets and Soil of Endemic *Pinus Peuce*

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Research Article

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Abstract

Pinus peuce Griseb is five-needle pine native in high-altitude montane habitats of the Balkans. The aim was to assess the diversity and composition of fungal communities associated with the soil, rootlets and living needles of *P. peuce* at three high-altitude forest sites with different edaphic conditions and stand characteristics in southeastern Montenegro. In total, 90 needle, 90 rootlet and 90 soil samples were sampled. DNA amplification using ITS2 rDNA as a marker and high-throughput sequencing resulted in 17,620 high-quality reads, representing 825 fungal taxa. There were 52.5% Basidiomycota, 43.9% Ascomycota and 3.6% Mucoromycotina. There were 118 unique fungal taxa in the rootlets, 230 in the soil and 113 in the needles, with 8 taxa were shared. The most common fungi in rootlets were *Rhizopogon mohelensis* (11.0%), *Suillus sibiricus* (8.4%), *R. fallax* (6.9%), in the soil – *Phlebiopsis gigantea* (5.1%), *Tylospora asterophora* (2.9%), *Sollicocossima terricola* (2.7%), and in the needles – *Dothideomycetes* sp. 3360_7 (17.6%), *Dothideomycetes* sp. 3360_10 (10.7%), *Leotiomycetes* sp. 3360_16 (6.5%). The results showed that the functional tissues and the rhizosphere soil of *P. peuce* were inhabited by a high diversity of fungi, which were largely specific to each particular substrate, while. the site conditions had only limited effect on associated fungal communities. Associated fungal communities were largely determined by the host tree species, substrate properties and functional capabilities of associated fungi.

Introduction

Pinus peuce is one of the two European pine species from the subgenus *Strobus*. It is tertiary relict and endemic to the Balkans, where it is rare and has limited distribution between the latitudes 41° and 43° (Fig. 1), i.e. in the areas that include parts of Montenegro, Serbia, Albania, North Macedonia, Greece and Bulgaria (Jovanović 2007; Alexandrov and Andonovski 2011). *Pinus peuce* is adapted to high altitudes and grows between 1200 m and 2300 m i.e. in areas characterised by cold and high humidity mountain climate. It is predominantly found on silicate soils with a varying soil depth and fertility. On carbonate soils, it is usually present on deeper and more humid soils of northern expositions (Janković et al. 1987). *Pinus peuce* characteristically forms dense and pure forest stands, but at the upper tree line it often grows in small and scattered stands (Jovanović 2007) (Fig. 2). It can also grow in admixture with *Picea abies*, *Abies alba*, and on lower altitudes with *Fagus sylvatica.* At the upper tree line, it can form mixed forest stands with *Pinus heldreichii*. In these areas, *P. heldreichii* inhabits more xerotherm and dryer sites, while *P. peuce* – more humid and fertile sites (Janković et al. 1987). Historical data suggests that in the past both *P. peuce* and *P. heldreichii* formed a well-developed and continuous forest belt in the Balkans (Janković et al. 1987; Stevanović et al. 1995), but as a consequence of extensive exploitation, nowadays the total area occupied by *P. peuce* is only about 20,500 ha (Alexandrov and Andonovski 2011).

Pinus peuce is one of the most valuable conifer tree species in the Balkan (Jovanović 2007; Stevanović et al. 1995; Alexandrov and Andonovski 2011) due to its high ecological adaptability, the provision of ecosystem services such as protection of soil and water as well as biodiversity conservation. In Montenegro, at the optimum growth conditions *P. peuce* reaches up to 30 m in height and up to 1 m in diameter. Commonly, stems have branches down to the ground, while root systems have a characteristic central root with large lateral roots, which penetrate deep into the soil. In preserved forests, trees can reach the age of ca. 200 years, and individual specimens over 300 years. Even at high altitudes, *P. peuce* produces high yields of wood, which is very durable. In the past, wood was used in constructions, furniture production, woodcarving and cooperage (Jovanović 2007; Alexandrov and Andonovski 2011), but nowadays it is not harvested. The resin of *P. peuce* provides high quality derivatives and in the past was used in the chemical industry, optics and pharmacy. In traditional medicine, the resin was used to cure wounds, pectoral, skin and stomach diseases, varicose veins and other illnesses (Alexandrov and Andonovski 2011).

In the last 50 years, only limited natural regeneration was reported from *P. peuce* forests, though recent observations show more extensive regeneration in the abandoned mountain areas (Fig. 2). *Pinus peuce* is classified as nearly threatened by the International Union for Conservation of Nature Red List of Threatened Species (IUCN) and it is protected in the Balkan countries. As a protected and highly value trees species, *P. peuce* requires special attention, i.e. the development and application of conservation measures (Janković et al. 1987; Stevanović et al. 1995). It is expected to adapt well to the climate change and can be a promising tree species for forestry and nature conservation in high altitude mountain areas (Jovanović 2007; Alexandrov and Andonovski 2011).

Fungi play key roles in montane forest ecosystems and represent an essential part of biodiversity (Baldrian 2017; Lazarević and Menkis 2020). They directly influence several physiological processes in trees and contribute to carbon, nutrient and water cycling (Stewart et al. 2018). Despite their importance and possible impact on tree health, information on fungi associated with *P. peuce* is scarce. Moreover, the knowledge about fungal communities associated with pines from subgenus *Strobus* is also limited. Among different fungi associated with trees, ectomycorrhizal (ECM) fungi directly influencing the uptake of nutrients and water in pines forests, affect soil characteristics and overall plant vitality. They can be essential for the successful regeneration, i.e. establishment and growth of tree seedlings, in particular on marginal habitats under harsh environmental conditions (Menkis et al. 2012; Lazarević and Menkis 2018). Saprotrophic fungi that are decomposers of dead organic matter drive nutrient cycling in forest ecosystems (Baldrian 2017; Terhonen et al. 2019). Fungal pathogens can affect health and growth of forest trees, while fungal endophytes and epiphytes can support ecological adaptation of host trees (Stewart et al. 2018).

The aim of this study was to assess the diversity and composition of fungal communities associated with the soil, rootlets and living needles of *P. peuce* from three high-altitude forest sites with different edaphic conditions and stand characteristics in south-eastern Montenegro.

Material And Methods

Study sites

The study sites were at Bogićevica, Visitor and Zeletin (detailed description of each site is below) in south-eastern Montenegro (Fig. 1, Table 1). These sites represented characteristic *P. peuce* forest stands growing at ca. 1600–2100 m altitudes and included mixed coniferous or coniferous and deciduous forest stands. The geographical distance between the Bogićevica site and two other sites (Visitor and Zeletin) was about 12–15 km. The distance between Visitor and Zeletin sites was about 5 km. All three sites differed from each other in terms of forest stand composition and age, soil characteristics and altitude (Table 1). Climatic conditions were similar for all three sites.

The climate at the study sites, according to the Köppen climate classification, is a humid cold temperate boreal climate with cool summer and cold winter (Dfc, Dfwcx) (Burić et al. 2014). The winter minimum is below − 30°C and the summer maximum is above 10°C. The arid period is absent. However, the primary precipitation maximum is in the late autumn or winter. The secondary precipitation maximum occurs in the late spring or early summer. The winters are usually cold and snowy, while summers are usually cool. In summer, at the altitudes above 1500–1600 m the average air temperature can be above 10 °C for up to four months.

Table 1
Study sites where the soil, rootlets and needles of *P. peuce* were sampled.

Site	Position	Altitude (m)	Soil type *	Forest stand
Bogićevica (Mt. Prokletije)	N 42.5650422, E 20.0332079	1970–2100	Rendzina to brown acid soil on quartz sandstone	Pure *Pinus peuce* stand (90–200 year-old)
Visitor (Mt. Visitor)	N 42.6146217, E 19.8821091	1880–2000	Brown soil on basic igneous rock	Pure *Pinus peuce* stand (10–60 year-old)
Zeletin (Mt. Visitor)	N 42.6330433, E 19.8359449	1600–1800	Brown soil on cherts	Mixed *Pinus peuce Abies alba* and *Fagus sylvatica* stand (100–120 year-old)
*based on (Fuštić and Đuretić 2000)				

At the Bogićevica site, at the altitude is of ca. 1700 m, *P. peuce* is growing in admixture with *P. abies* and *A. alba* (at lower altitudes *Abieti –Picenion* forest type is situated), while at the altitude of ca. 1800 m, it is growing in admixture with *P. abies*. Above this altitude, *P. peuce* forms pure forest stands, which extends up to the uppermost mountain ridges. The sampling site was located in pure, old growth *P. peuce* forest stand with the majority of trees being between 100 and 200 year-old. Trees were 25–30 m in height and 50–60 cm in diameter. The sampling site was situated on the slope and between altitudes 1970 m and 2100 m, i.e. from the mountain plateau with a glacial lake Ridsko up to the top of the mountain. The slope has north and northeast-facing exposition. The forest site is characterised as a *Pinetum peucis montenegrinum* Bleč. & Tat. type. The site at Bogićevica has the silicate geological structure: quaternary conglomerates composed of quartz and calcite. Brown acid soil type on quartz sandstone (Fuštić and Đuretić 2000), is variable in depth, but being positioned on the mountain slopes and ridges, is very shallow and skeletal (more than 50% of the skeleton). Transient A(B) and (B)C soil horizon occurs, but horizon (B) is rarely present. The humus layer is thin, dark in colour and densely colonised by the roots of grasses, shrubs and forest trees. A or transient A (B) horizon is brown or dark brown in colour and is 8–12 cm deep.

At the Visitor site, *P. peuce* is growing from ca. 1600 m altitude, where it is mixed with *P. abies* and *A. alba* (at lower altitudes *Abieti –Picenion* forest type is situated). Pure *P. peuce* forest stand, which is dominated by 100–200 year-old trees, is growing above the altitude of ca. 1800 m and extends up to the uppermost mountain ridges (alt. 2210 m). The sampling site was on the exposed mountain slope (alt. 1900–2000 m), on an abandoned mountain pasture, where forest of *P. peuce* has regenerated. The site has north and northwest-facing exposition and was dominated by 10–60 year-old trees of *P. peuce*. On the south-facing slope, an old forest stand of *P. heldreichii* is situated, which has also regenerated and is mixed with *P. peuce* on the sampling site. The Visitor site is dominated by silicate geological soil structure, where the keratophyres, quartz keratophyres, andensitis, dacities and diabases are the most common eruptive rocks, but the highest mountain peaks are composed of Triassic limestone. On the sampling site, the soil is dystric cambisol on igneous rock (Fuštić and Đuretić 2000). As basic eruptive rocks are compact and resistant to weathering, the soil stays in initial stage for a very long time and its development is very slow. It is mainly shallow and skeletal (with large fragments of stones) and acid. The characteristic soil profile is A (B) C. A and B horizons are poorly differentiated in terms of colour and mechanical composition. The topsoil layer is dark. Mineralization of humus is very slow, resulting in its accumulation. The B horizon has fragments of brown, yellow or orange colour, which is due to the release of iron.

At the Zeletin site, the forest vegetation is differing compared to vegetation on near neighbouring areas. At 1200–1600 m altitude, *Fagus sylvatica* forest or mixed *F. sylvatica* and *A. alba* forest is situated (forest types *Fagetum moesiacae* Bleč. et. Lakš, and Ass. *Abieti –Fagetum)*. The sampling was carried out at the altitude of 1600–1800 m, where *P. peuce* is mixed with *F. sylvatica* and *A. alba*, in a dense, old-growth forest stand (100–150 year.) on the mountain slope with east and south-east-facing exposition and was characterised by the well-developed soil. Pure *P. peuce* forest stands occur at ca. 2060 m altitude and up to the top of the mountain, where these stands alternate with mountain pastures. At the sampling site, the soil is dystric cambisol on chert (Fuštić and Đuretić 2000). In the dense, old-growth and mixed coniferous and deciduous forest, soil is well developed and it has a A (B) C profile. The A horizon is dark brown in colour, and with a high humus content, loose, and with a crumbly structure; sand-loam or loamy. The B horizon is brown or dark brown in colour, with orange-grey shades and loamy. The soil is waterproof and dry.

Experimental design and sampling

At each study site, stands of *P. peuce* were healthy-looking i.e. showed no signs of damage or decline. The sampling was carried out in autumn 2015 and at each site included needles, rootlets and the rhizosphere soil of *P. peuce*. For the sampling of needles, at each site a twig up to 15 cm long, which included the current and the previous year living needles, was randomly collected using secateurs up to 2 m from the ground from each of 30 mature trees, which were situated at a distance of ca. 50 m from each other. Collected needles were visually inspected for the presence of disease symptoms, placed in individual plastic bags, transported to the laboratory and stored at -20 °C before used for DNA extraction.

For the sampling of rootlets and surrounding fine fraction soil, at each site 30 samples were randomly collected using a spade under 5–15 year-old trees of *P. peuce* growing in the vicinity to mature *P. peuce* trees used for sampling of needles. Soil samples with rootlets were taken down to 20 cm depth and included lateral rootlets with attached fine roots. Samples were individually packed into plastic bags, transported to the laboratory and kept at 4 °C for a maximum period of one week before processed. For

each sample, rootlets were separated from the soil, soaked in cold water for 12 h and gently washed in tap water to remove any of the remaining soil. Fine roots were separated from lateral rootlets, placed in 1.5 ml centrifugation tubes and stored frozen at -20 °C before used for DNA extraction. Individual samples of fine fraction soil, which was sieved using a sieve (mesh size 2 mm × 2 mm), were placed in plastic bags and stored at -20°C before used for DNA extraction. Taken together, the sampling resulted in 90 needle, 90 rootlet and 90 soil samples of *P. peuce*.

Soil chemical analyses and texture

Before analysis of soil physical and chemical properties, at least 100 g of soil from each sample was dried at room temperature (ca. 21°C) for 24 h. Soil pH was determined using a combined glass-electrode in 1 M KCl of soil suspension 1:2.5 (w/v). The humus content was determined by wet oxidation with 0.02 M $KMnO_4$. Total nitrogen was determined by Kjeldahl method (Džamić et al. 1996). The available phosphorus and potassium (extraction with ammonium-acetate-lactate solution at pH = 3.7) was determined according to Egner-Riehm-Domingo method using the spectrophotometry and flame photometry, respectively. Total carbonates was determined by volumetric method (Džamić et al. 1996). The available Fe, Mn, Zn and Cu (extraction with 0.005 M DTPA) as well as of exchangeable Ca and Mg (extraction with 1 M CH_3COONH_4) were determined by flame atomic absorption spectrometry (AA-6800, Shimadzu). The values of the above mentioned soil parameters were expressed on air-dried basis. Particle size distribution was analysed by the sieve and the pipette method (Džamić et al. 1996).

Molecular analysis

DNA extractions were done from individual needle, rootlet and soil samples, which were previously freeze-dried at -60°C for 48 h (Alpha 1–4 LD, Martin Christ, Germany). For isolation of total DNA, samples were placed in separate 2 ml tubes with screw cap and homogenised in a Fastprep machine (Precellys, Montigny-le-Bretonneux, France). About 100 mg of freeze-dried soil per sample was used for DNA extraction, which was done using Zymo soil kit (Zymo Research, Irvine, USA). For isolation of genomic DNA from the rootlets and needles, 200 mg of material was taken from each respective sample and the extraction was completed using CTAB protocol (Menkis et al. 2014). The DNA isolated from the rootlets was directly used in PCR reactions, while DNA isolated from the needles was additionally purified using JetQuick DNA purification kit (Genomed GmbH, Leinfelden, Germany). The DNA concentration of each sample was determined using a NanoDrop™ One spectrophotometer (Thermo Scientific, Rodchester, NY, USA) and adjusted to 10 ng/µL. Amplification by PCR of the ITS2 rDNA region was done using barcoded fungal-specific primer gITS7 (Ihrmark et al. 2012) and barcoded universal primer ITS4 (White et al. 1990). All samples of the same substrate (needles, rootlets, or soil) and site were amplified using primers with the same barcode, resulting in 9 different barcodes (3 substrates × 3 sites). Amplification of multiple samples with the same barcode was done owing to get a broader representation of fungal communities per each substrate and site. Amplifications were performed using the Applied Biosystems 2720 thermal cycler (Foster City, CA, USA). An initial denaturation step started at 95°C for 2 min, followed by 27 amplification cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s. The thermal cycling was ended by a final extension step at 72°C for 7 min. The PCR products were analysed using gel electrophoresis on 1% agarose gels stained with Nancy-520 (Sigma-Aldrich, Sweden). PCR products were purified using a sodium acetate protocol (Menkis et al. 2015). Purified PCR products were quantified using a Qubit fluorometer 4.0 (Thermo Fisher Scientific, Waltham, MA, USA), and an equimolar mix of all PCR products was used for high-throughput sequencing using a Pacific Biosciences platform (Menlo Park, CA, USA) and one SMRT cell at the SciLifeLab (Uppsala, Sweden).

Bioinformatics

Principles of bioinformatics followed Lynikienė et al. (2020). The sequences obtained were subjected to quality control and clustering in the SCATA NGS sequencing pipeline. The initial procedure started with quality filtering of the sequences that included the removal of sequences shorter than 200 bps, sequences with low read quality, primer dimers and homopolymers, which were collapsed to 3 bps before clustering. Only sequences containing a barcode and primer were retained. Then, the primer and sample barcodes were removed from the sequence, but information on the sample and sequence association was stored as meta-data. A single-linkage clustering based on 98% similarity was used to cluster sequences into different taxa. For each cluster, the sequence of the most common genotype was used for taxonomic identification. For clusters containing only two sequences, a consensus sequence was produced. The taxa were taxonomically identified using the GenBank database and the Blastn algorithm (Altschul et al. 1997). The following criteria were used for identification: sequence coverage > 80%; 94–97% similarity

to genus level and ≥ 98% similarity to species level. Sequences deviating from these criteria were identified only to a high taxonomic rank and were given unique names. Representative sequences of fungal non-singletons are available from GenBank under accession numbers MZ441403 - MZ442202.

Statistical analyses

The statistical analysis of the soil data was performed using IBM SPSS Statistics 23.0 (New York, USA). The significant differences between the means were determined with the one-way ANOVA and Duncan's test at $p < 0.05$. Differences in richness of fungal taxa in different study sites of *P. peuce* were compared by nonparametric chi-square testing (Sokal and Rohlf 2005). As each of the datasets was subjected to multiple comparisons, confidence limits for p-values of chi-square tests were reduced the corresponding number of times as required by the Bonferroni correction. The Shannon diversity index, qualitative Sørensen similarity index and principal coordinate analysis (PCA) in Canoco 5 were used to characterise the diversity and composition of fungal communities (Shannon 1948; Magurran 1988; ter Braak and Smilauer 1998). The nonparametric Mann-Whitney test in Minitab v. 18.1 (PA, USA) was used to test if the Shannon diversity index among different substrates was statistically similar or not.

Results

Fungal communities associated with the soil, rootlets and needles of *P. peuce* were examined from three different high-altitude sites, each of which were characterised by different forest stand composition, tree age, and soil type (Table 1). Although soils on the study sites differed according to geological structure and soil type (Table 1), soil texture in different study sites was similar as it was a sandy loam. At the Bogićevica site a sand percentage (68.5 ± 2.58, mean ± SD) was significantly higher than it was at the Visitor (65.9 ± 4.72) or Zeletin (65.8 ± 5.46) sites. The clay percentage was significantly higher at the Zeletin site (10.8 ± 1.06) as compared to the Bogićevica (8.6 ± 0.54) or Visitor (8.5 ± 1.23) sites ($p < 0.05$). At all three sites, soils were of the light structure and well drained.

Soil analyses showed that *P. peuce* forests were growing on very acidic to acidic soils, which were rich in humus and nitrogen (Table 2). Furthermore, soils were poor in available P, had moderate availability of K, high content of Fe and Mn, optimal content of Zn and low to optimal of Cu. Values for electrolytic conductivity (EC) covers the range from infertile to very fertile soils. The exchangeable Ca and Mg were in a range from low to optimal level. Among the three sites, the most favourable growth conditions was found to be at the Visitor site due to higher soil pH, the highest level of humus as well as of nutrients such as N, P, K, Ca, Mg and Zn (Table 2). The soil at the Zeletin site had lowest pH value, significantly higher level of available Fe and EC value, but significantly lower content of available P and Mn. The soil at the Bogićevica site had the lowest content of humus, N and the EC value, but the highest level of Mn and Cu (Table 2).

Table 2
Soil chemical characteristics of *Pinus peuce* stands at Bogićevica, Visitor and Zeletin sites. Values show the mean ± standard deviation. Within each row, values followed by the same letter do not differ significantly at $p > 0.05$.

Soil parameter	Bogićevica	Visitor	Zeletin
pH (KCl)	4.26 ± 0.09 b	4.78 ± 0.96 a	4.04 ± 0.07 b
Humus (%)	5.50 ± 0.56 c	14.0 ± 6.80 a	8.30 ± 1.29 b
N (%)	0.20 ± 0.02 c	0.61 ± 0.20 a	0.33 ± 0.15 b
P_2O_5 (mg/100g)	2.1 ± 0.73 b	2.5 ± 0.47 a	1.6 ± 0.54 c
K_2O (mg/100g)	9.5 ± 0.26 b	16.7 ± 2.92 a	16.7 ± 4.88 a
Ca (mg/100g)	44.5 ± 4.59 b	255 ± 255 a	108 ± 30.2 b
Mg (mg/100g)	7.59 ± 0.85 b	20.8 ± 20.1 a	11.3 ± 1.89 b
Fe (mg/kg)	68.5 ± 15.2 b	68.1 ± 15.7 b	97.0 ± 39.0 a
Mn (mg/kg)	64.3 ± 5.78 a	40.7 ± 11.7 b	32.7 ± 19.5 c
Zn (mg/kg)	1.58 ± 0.34 b	3.23 ± 1.50 a	1.59 ± 0.96 b
Cu (mg/kg)	0.99 ± 0.13a	0.58 ± 0.27b	0.50 ± 0.22b
EC (µS/cm)	43.7 ± 20.7c	145 ± 88.0 b	252 ± 173 a

Amplification and PacBio sequencing of fungal ITS2 rDNA from 90 needle, 90 rootlet and 90 soil samples resulted in 72,505 sequence reads. Quality filtering showed that 23,394 (32.3%) reads were of high-quality and were retained, while 49,111 (67.3%) low-quality reads were excluded. Clustering of high-quality reads showed the presence of 978 non-singleton taxa and of 1464 singletons, which were excluded. Taxonomic classification showed that among all taxa 825 (84.4%) were fungal (Supplementary Table 1) and 153 (15.6%) were non-fungal, which were excluded. The detected fungi were 52.5% Basidiomycota, 43.9% Ascomycota and 3.6% Mucoromycotina. Identification at least to genus level was successful for 469 (56.6%) fungal taxa (Supplementary Table 1), representing 67.9% of all high-quality fungal sequences. The number of fungal taxa detected at each site and substrate (soil, rootlets or needles) is in Table 3.

Table 3
Generated high-quality ITS2 rDNA fungal sequences and detected diversity of fungal taxa at different sampling sites of *Pinus peuce*. Within the column *No. of fungal taxa*, values followed by the same letter in chi-square test (compared among samples of the same substrate) do not differ significantly at $p > 0.05$.

Study site	Substrate	No. of fungal sequences	No. of fungal taxa	Shannon diversity index (H)
Bogićevica	Rootlets	3060	228ab	4.0
	Soil	1219	227a	4.1
	Needles	1850	119a	3.5
All Bogićevica		6129	448	
Visitor	Rootlets	3424	202a	3.1
	Soil	1960	293b	4.8
	Needles	837	79b	3.3
All Visitor		6221	441	
Zeletin	Rootlets	2331	212b	4.1
	Soil	2219	311b	4.8
	Needles	457	76c	3.4
All Zeletin		5007	435	
All sites		17357	825	

In rootlets, the richness of fungal taxa was significantly higher at the Zeletin site than at the Visitor site ($p < 0.05$), but in this respect, both of these sites did not differ significantly from the Bogićevica site ($p > 0.05$) (Table 3). In the soil, the richness of fungal taxa was similar between the Zeletin and Visitor sites ($p > 0.05$), but differed significantly from those at the Bogićevica site ($p < 0.05$) (Table 3). In the needles, the richness of fungal taxa was significantly higher at the Zeletin site vs. the Visitor site ($p < 0.05$), and both of these showed a significantly higher richness of fungal taxa than at the Bogićevica site ($p < 0.05$) (Table 3). The number of unique fungal taxa differed among different substrates (roots, soil or needles) as shown in Fig. 3. When all sites were taken together, there were 118 unique fungal taxa in the rootlets, 230 in the soil and 112 in the needles (Fig. 3). There were 340 fungal taxa shared between the rootlet and soil samples, but only 18 taxa were shared between the rootlet and needle samples, and 23 between the soil and needle samples. Overall, only 8 fungal taxa were common to all substrates (rootlets, soil and needles) (Fig. 3).

Within each substrate (soil, rootlets or needles), the composition of fungal classes was similar among different sites (Fig. 4). Consequently, at different proportions Agaricomycetes (37.4% – 50.2% and 59.8% – 77.4%) dominated fungal communities in the soil and rootlets, respectively, while Dothideomycetes (61.3% – 69.6%) dominated in the needles (Fig. 4).

Table 4
Relative abundance (%) of the 20 most common fungal taxa associated with the rootlets of *Pinus peuce* in Montenegro.

Fungal taxon	Phylum	GenBank	Sequence	Sequence	Study sites			All
		reference	length, bp	identity, %	Bogićevica	Visitor	Zelatin	
Rhizopogon mohelnensis	Basidiomycota	KX610702	341	339/341 (99)	11.8	17.3	1.8	11.3
Suillus sibiricus	Basidiomycota	KM882918	330	328/330 (99)	2.7	19.9	-	8.7
Rhizopogon fallax	Basidiomycota	KC152199	345	344/345 (99)	6.7	7.2	7.4	7.1
Suillus granulatus	Basidiomycota	KX230633	331	331/331 (100)	0.0	17.5	-	6.8
Thelephora terrestris	Basidiomycota	KT447173	313	313/313 (100)	10.3	0.5	-	3.8
Phialocephala fortinii	Ascomycota	LC131028	236	236/238 (99)	5.8	0.9	3.3	3.3
Suillus luteus	Basidiomycota	KX230614	331	331/331 (100)	1.7	5.9	-	2.9
Meliniomyces bicolor	Ascomycota	HQ157926	238	238/238 (100)	0.8	0.6	6.9	2.3
Mycena leptocephala	Basidiomycota	HQ604773	310	310/311 (99)	2.0	0.6	3.4	1.9
Tomentella bresadolae	Basidiomycota	KP753365	314	313/314 (99)	-	-	6.6	1.7
Agaricomycetes sp. 3360_15	Basidiomycota	JX042709	277	241/284 (85)	1.4	-	4.1	1.6
Suillus variegatus	Basidiomycota	MG597425	329	329/329 (100)	-	-	5.6	1.5
Archaeorhizomyces borealis	Ascomycota	NR_126144	215	215/215 (100)	0.8	-	4.5	1.5
Tylospora asterophora	Basidiomycota	AF052558	288	288/288 (100)	0.4	-	5.0	1.4
Trechispora sp. 3360_33	Basidiomycota	JX392812	320	318/320 (99)	0.5	2.9	-	1.3
Agaricomycetes sp. 3360_18	Basidiomycota	KJ595006	338	318/341 (93)	1.3	1.1	1.5	1.3
Tremellomycetes sp. 3360_44	Basidiomycota	KR265945	253	247/253 (98)	3.4	-	-	1.2
Gyoerffyella sp. 3360_31	Ascomycota	KU516480	242	242/242 (100)	0.5	1.7	0.6	1.0
Inocybe rufoalba	Basidiomycota	KX602273	274	274/274 (100)	2.6	-	-	0.9
Agaricomycetes sp. 3360_35	Basidiomycota	JF519610	305	305/305 (100)	0.0	0.3	2.8	0.9
All of 20 taxa					52.7	76.4	53.5	62.4

Table 5
Relative abundance (%) of the 20 most common fungal taxa associated with the soil of *Pinus peuce* in Montenegro.

Fungal taxon	Phylum	GenBank	Sequence	Sequence	Study sites			All
		reference	Length, bp	Identity, %	Bogićevica	Visitor	Zelatin	
Phlebiopsis gigantea	Basidiomycota	KX426965	302	302/302 (100)	22.9	-	-	5.2
Tylospora asterophora	Basidiomycota	AF052558	288	288/288 (100)	5.3	-	4.1	2.9
Solicoccozyma terricola	Basidiomycota	KY558367	329	329/329 (100)	3.1	0.6	4.5	2.8
Neohygrocybe ingrata	Basidiomycota	KF291225	269	268/270 (99)	4.1	4.5	-	2.6
Hygrocybe intermedia	Basidiomycota	EU784321	344	294/295 (99)	-	6.7	-	2.4
Russula vesca	Basidiomycota	DQ422018	321	321/321 (100)	-	-	5.5	2.3
Inocybe whitei	Basidiomycota	JF908146	286	286/287 (99)	0.1	-	4.7	2.0
Mortierella humilis	Mucoromycotina	MG052956	333	333/333 (100)	0.9	3.1	1.2	1.8
Dothideomycetes sp. 3360_46	Ascomycota	FJ554252	241	241/241 (100)	3.9	0.3	1.8	1.7
Archaeorhizomycetes sp. 3360_50	Ascomycota	KR266833	211	210/211 (99)	-	1.3	3.0	1.7
Clavulina cristata	Basidiomycota	EU862223	333	332/333 (99)	1.0	-	3.5	1.7
Mucoromycotina sp. 3360_37	Mucoromycotina	KR265910	297	290/298 (97)	2.1	2.6	0.4	1.6
Hygrocybe singeri	Basidiomycota	KC581351	318	313/319 (98)	0.1	3.9	0.1	1.5
Microbotryomycetes sp. 3360_57	Basidiomycota	KC818329	303	303/303 (100)	0.3	1.2	2.0	1.3
Pseudotricholoma metapodium	Basidiomycota	KJ417308	308	307/309 (99)	-	3.6	-	1.3
Leotiomycetes sp. 3360_63	Ascomycota	HQ021989	242	242/242 (100)	0.6	2.2	0.8	1.3
Agaricomycetes sp. 3360_49	Basidiomycota	KM402954	292	292/293 (99)	-	-	2.7	1.1
Leohumicola minima	Ascomycota	HQ691252	241	241/241 (100)	0.2	2.4	0.0	0.9
Laccaria laccata	Basidiomycota	KM067834	300	300/300 (100)	-	-	2.3	0.9
Cenococcum geophilum	Ascomycota	HM189723	242	242/242 (100)	0.1	-	2.1	0.9
All of 20 taxa					44.7	32.4	38.7	37.9

Table 6
Relative abundance (%) of the 20 most common fungal taxa associated with the needles of *Pinus peuce* in Montenegro.

Fungal taxon	Phylum	GenBank	Sequence	Sequence	Study sites			All
		reference	length, bp	similarity, %	Bogićevica	Visitor	Zelatin	
Dothideomycetes sp. 3360_7	Ascomycota	KP897336	257	251/257 (98)	20.2	10.2	20.8	17.6
Dothideomycetes sp. 3360_10	Ascomycota	NR_145090	238	216/246 (88)	11.5	14.2	1.3	10.7
Leotiomycetes sp. 3360_16	Ascomycota	KR267039	242	242/243 (99)	4.2	12.1	5.3	6.5
Sydowia polyspora	Ascomycota	KU516591	256	256/256 (100)	5.8	6.6	5.0	5.9
Celosporium larixicola	Ascomycota	FJ997287	255	249/253 (98)	4.6	8.1	1.5	5.1
Dothideomycetes sp. 3360_34	Ascomycota	KP891395	241	239/241 (99)	3.9	6.8	3.1	4.6
Lophodermium pinastri	Ascomycota	HM060657	239	239/239 (100)	6.8	0.1	2.2	4.4
Dothideomycetes sp. 3360_36	Ascomycota	KP897893	250	230/253 (91)	2.2	1.6	11.4	3.3
Leotiomycetes sp. 3360_51	Ascomycota	FR846479	237	237/237 (100)	3.2	0.5	1.8	2.3
Helotiales sp. 3360_53	Ascomycota	HQ845750	242	242/242 (100)	2.2	3.2	0.7	2.2
Leotiomycetes sp. 3360_77	Ascomycota	JF449635	241	241/241 (100)	1.8	2.5	1.3	1.9
Dothideomycetes sp. 3360_96	Ascomycota	FR682183	242	240/242 (99)	0.4	2.5	7.0	1.9
Cryptococcus sp. 3360_109	Basidiomycota	KM216339	317	298/299 (99)	1.5	1.2	1.3	1.4
Cladosporium macrocarpum	Ascomycota	MF077231	242	242/243 (99)	1.5	0.8	2.0	1.4
Sarcinomyces crustaceus	Ascomycota	NR_121503	267	265/267 (99)	1.7	0.8	-	1.2
Phaffia sp. 3360_92	Basidiomycota	HF558647	351	309/355 (87)	1.1	1.9	0.4	1.2
Eurotiomycetes sp. 3360_126	Ascomycota	FR682181	248	248/248 (100)	1.2	0.6	0.7	1.0
Lachnellula calyciformis	Ascomycota	KC464636	239	239/239 (100)	1.6	-	-	1.0
Dothideomycetes sp. 3360_115	Ascomycota	AM901716	239	221/242 (91)	0.9	1.7	-	1.0
Epithamnolia xanthoriae	Ascomycota	KY814539	237	233/238 (98)	1.3	0.6	0.2	1.0
All of 20 taxa					77.6	76.0	65.9	75.5

The most common fungi in rootlets were *Rhizopogon mohelensis* (11.3%), *Suillus sibiricus* (8.7%), *Rhizopogon fallax* (7.1%), *Suillus graulatus* (6.8%), *Thelephora terestris* (3.8%), *Phialocephala fortinii* (3.3%), *Suillus luteus* (2.9%) and *Melinomyces bicolor* (2.3%) (Table 4). In the soil, these were *Phlebiopsis gigantea* (5.2%), *Tylospora asterophora* (2.9%), *Sollicocossima terricola* (2.8%), *Hygrocybe intermedia* (2.4%) and *Russula vesca* (2.3%) (Table 5). In the needles, these were *Dothideomycetes* sp. 3360_7 (17.6%), *Dothideomycetes* sp. 3360_10 (10.7%), *Leotiomycetes* sp. 3360_16 (6.5%), *Sydowia polyspora* (5.9%), *Coleosporium larxicola* (5.1%), *Dothideomycetes* sp. 3360_34 (4.6%) and *Lophodermium pinastri* (4.4%) (Table 6).

Principal coordinates analysis (PCA) of fungal communities explained 35.5% variation on axis 1 and 17.4% on axis 2. PCA showed that fungal communities from the same substrate (samples representing different sites) clustered together (Fig. 5). PCA also showed that fungal communities in different substrates (needles, rootlets and soil) were separated from each other (Fig. 5), showing that fungal communities in each of these substrates were largely different. In the needles, the Shannon diversity index was between 3.3 and 3.5, in rootlets – between 3.1 and 4.1 and in the soil – between 4.1 and 4.8 (Table 3). The Mann-Whitney test showed that the Shannon diversity index among different substrates did not differ significantly ($p > 0.05$). The Sørensen similarity index of fungal communities was: needles vs. rootlets – 0.06, needles vs. soil – 0.06, and rootlets vs. soil – 0.6.

Discussion

Five-needle pines, which belong to the subgenus Strobus (section Quinquefoliae), are unique in their ecology and distribution. There are 24 *Pinus* spp. in this subgenus, which are native to Europe, North and Central America and Asia (Gernandt et al. 2005). The majority of these species are characterized by a narrow distribution and high-altitude habitats i.e. growing under harsh environmental conditions. Information about fungal diversity associated with those *Pinus* spp. is still limited, while the available knowledge is mainly on the above ground sporocarps or on ECM fungi. For example, ECM communities of *P. cembra* were described from European Alps (Rainer et al. 2015; Bacher et al. 2010), of *P. albicaulus* (Mohat et al. 2008; Cripps and Antibus 2011; Jenkins et al. 2018), *P. flexilis* (Cripps and Antibus 2011) and *P. monticola* from the North America, of *P. walchiniana* from Himalaya (Sagar and Lakhanpal 2005; Tyub et al. 2018) and of *Pinus amamiana* from Japan (Murata et al. 2017). These studies emphasise the importance of ECM fungi for natural regeneration and survival of those tree species, which all have limited distribution and represent important tree species in montane ecosystems. These studies have also revealed the prevailing ECM species, which could potentially be used for seedling mycorrhization owing the conservation of these pine species. Apart from ECM fungi, data on fungi associated with needles and soil of five-needle pines is scarce, but could provide important knowledge on potential pathogens and endophytic fungi.

Survey of fungal fruitbodies in native forests in North Macedonia and Bulgaria have showed that *P. peuce* is associated with nearly 400 fungal species (Kalucka et al. 2013), while only six fungal species have been recorded in Montenegro (Perić and Perić 2004; Kasom and Karadelev 2012). *Heterobasidion annosum* and *Phaeolus schweinitzii* were reported as decay fungi of mature *P. peuce* trees (Papazov 1969; Rosnev 1985; Tomanić et al. 1998). *Cenangium ferugginosum*, *Cenangium abietis* and *Ungulina marginata* where frequently recorded on weakened trees (Papazov 1969; Tomanić et al. 1998). Previous studies on needle pathogens in native forests revealed the presence of a potentially invasive species *Dothistroma septosporum* in Montenegro (Lazarević et al. 2017) and *Cytospora pinastri* in Bulgaria (Georgieva and Marković 2018). *Lophodermium* fungi were shown to be present in forests and in forest plantations (Georgieva and Marković 2018; Tomanić et al. 1998).

The results of the present study have expanded the available knowledge on endemic *P. peuce*, demonstrating that needles, rootlets and the rhizosphere soil are inhabited by taxa-rich communities of fungi (Table 3). The detected fungal communities were largely specific to each particular substrate (Fig. 3–5), showing their adaptation and substrate preferences. In support, the qualitative Sørensen similarity index was very low when compared between the above- (needles) and belowground (rootlets or soil) substrates, repeatedly demonstrating the potential importance and functional preferences of associated fungi. Interestingly, the site conditions had only limited effect on associated fungal communities as in different sites these were similar (Fig. 4–5). The latter may suggest that associated fungal communities were largely determined by the host tree species, substrate properties and functional capabilities of associated fungi. This may also suggest that fungi detected at one particular site could be used in different habitats (e.g. for ECM inoculation, or for biocontrol of pests or diseases) i.e. within the distribution of *P. peuce*.

Among the principal fungi identified in the soil and rootlets, there were taxa from genera *Suillus* and *Rhizopogon*, which are closely related and almost exclusively restricted to Pinaceae (Bruns et al. 2002). A long co-evolutionary history between *Suillus* and *Pinus* species (Wu et al. 2000) represents an example of host specificity and adaptation (Jenkis 2018). Moreover, a limited number of *Suillus* fungi appear to be specific to five-needle pines (Klofac 2013), and this symbiosis can be essential for the survival under harsh environmental conditions. For example, *Suillus* fungi could be regarded as the most important and widespread symbionts of *P. cembra* in the Alps (Rainer et al. 2015) and of *P. albicaulus* in the North America (Mohat et al. 2008). *Suillus sibiricus* readily forms an ECM symbiosis with five-needle pines (Liao et al. 2016), which are found in different regions worldwide (Reiner 2015; Mohat 2008). It is a protected fungus in many European countries, including North Macedonia (Karadelev 1998), Bulgaria (Boev 2011) and Montenegro (Kasom and Karadelev 2012), where it was recorded in native forest stands of *P. peuce*. In agreement, the results of the present study provided the evidence that *S. sibiricus* is a common and an important symbiont of *P. peuce* as it was detected in both the rootlets and the soil (Table 4, Supplementary Table 1). Surveys on the aboveground sporocarp production and analyses of fine roots have shown that *Suillus granulatus* is the dominant fungus in *P. heldreichii* forests in Montenegro (Lazarević et al. 2011; Lazarević and Menkis 2018). In the present study, *S. granulatus* was only at the Visitor site, where *P. heldreichii* forest is in near proximity (see above). Liao et al (2016) have shown that *S. granulatus* can also readily form ECMs with pines from the subgenus *Strobus. Suillus luteus* and *S. variegatus* were also detected in rootlet and soil samples (Table 4, Supplementary Table 1), showing that *P. peuce* is associated with different suilloid fungi, which can colonise tree roots in high-altitude habitats. Fungi from the genus *Rhizopogon* are also primarily associated with Pinacea, but are not strictly host specific (Bruns et al 2002) and are also known to form ECM symbioses with trees in high-altitude coniferous forests (Kjøller and Bruns 2003; Mohatt et al. 2008; Lazarević and Menkis 2018). Contrary to *Suillus* spp., *Rhizopogon* fungi produce hypogeous sporocarps and their spores are mainly dispersed by animals (Grubisha et al. 2007; Mohatt et al. 2008). This makes the gene flow of *Rhizopogon* species more restricted (Grubisha et al. 2007), leading to genetic differentiation among isolated populations (Grubisha et al. 2007; Murata et al. 2017) and eventually to the evolution of different *Rhizopogon* species. *Rhizopogon mohelensis* was one of the most commonly detected fungi in this study, particularly in pure *P. peuce* forests, but less abundantly found in mixed forests. By contrast, *R. falax* was commonly detected in all study sites (Table 4). *Rhizopogon mohelensis* was reported from many countries in Europe (Holec et al. 2013). It belongs to *R. roseolus* group and sometimes can be confused with *R. rubescens*. For example, *R. salebrosus* is exclusively associated with *P. strobus* (Kohout et al. 2011), what limits such misidentification.

It appears that both *Suillus* and *Rhyzopogon* fungi possess specific ecological adaptations important for the establishment of *Pinus* spp. on marginal habitats and after forest disturbance (Kjøller and Bruns 2003; Mohatt et al. 2008). Such host specialists may often represent the dominant ECM species in high-altitude habitats characterised by extreme conditions (Bruns et al. 2002; Antibus and Cripps 2010). Certain suilloid fungi can be of special importance to five-needle pines due to host specialisation, high efficiency of nutrient and water uptake and transfer between the symbiosis partners, and an exclusion of mycoheterotrophy (Cripps and Antibus 2011). The common occurrence of suilloid fungi was particularly notable at the Visitor site containing newly regenerated *P. peuce* trees, but their abundance was lower in old-growth stands (Tables 1 and 3).

Among other fungi commonly detected in rootlets, there were *Thelephora terrestris* and *Phialocephla fortinii* (Table 4). *Thelephora terestris* is a typical ECM fungus of long-distance exploration type. This property allows efficient transportation of nutrients and water over long distances, which appears to be especially suitable for undisturbed, but very stony habitat with high soil heterogeneity (Reiner at al. 2015). *Phialocephala fortinii* belongs to a complex of dark septate endophytes, which forms non-specific associations with many plant hosts. The complex includes ECM-forming fungi as well as numerous pathogenic fungi (Tedersoo et al. 2008). Interestingly, *P. fortinii* was also commonly detected in rootlets of old growth *P. heldreichii* in high-altitude habitats, but not in its pioneer forests (Lazarević and Menkis 2017).

Tomentella brasodellea and *Tylospora asterophora* were the other two ECM fungi found in common association with *P. peuce* rootlets (Table 4). *Tomentella* fungi have been shown to be among dominant species in older coniferous forests worldwide (Lilleskov and Bruns 2005; Mrak et al. 2020). *Tylospora asterophora* is known as one of the most consistent and abundant ECM fungus associated with *P. abies* (Eberhard et al. 1999). *Melinomyces bicolor* (*Piceirhiza bicolorata*) was also commonly recorded (Table 4) and it is known to colonise roots of *Pinus*, *Picea* and *Betula* trees, but also forms ericoid mycorrhiza with shrubs from the Ericaceae family (Grelet et al. 2009). A high presence of *M. bicolor* in the present study could be influenced by the occurrence

of *Vaccinium myrtilus* in the ground vegetation. According to Horton et al. (1999), the sharing of ECM fungi between coniferous trees and plants from Ericaceae may play a major role in plant community dynamics. Soil microorganisms associated with arbutoid members of Ericaceae may enhance growth, survival, mycorrhizal root formation, and nitrogenase activity of conifer tree seedlings (Amaranthus et al. 1990). Fungal networks shared between those hosts (Pinaceae-Ericoid) remain even after disturbance events as these fungi are able to form associations with different plants (Perry et al. 1989). Ericaceous plants was shown to play an important role in the formation of ECM communities associated with *P. strobus* (Kohout et al. 2011). Besides, ericoid plants are very important in Mediterranean forests as these support ECM and ericoid fungi after fire events (Bergero et al. 2003).

Although a number of fungi were shared between the rootlet and soil samples as indicated by a high value of Sørensen similarity index, soil samples were characterised by a higher abundance of saprotrophic fungi as compared to the rootlet samples (dominated by ECM fungi) (Tables 4 and 5), which led to the differentiation of fungal communities in these two substrates (Fig. 4). Among the dominant fungi in the soil, there was *P. gigantea*, which is known as a common saprophytic fungus that causes white rot in conifer logs and stumps (Copenhaver et al. 2014), thereby playing an important role in the decomposition of conifer wood debris. Its common occurrence at the Bogićevica site was likely associated with the vast availability of dead wood, which was absent at the other two sites. *Solicoccozyma terricola* was another commonly detected saprotrophic fungus known from soils of temperate forests (Mašínová et al. 2016). *Hygrocybe intermedia* and *Neohygrocybe ingrata* were also among dominant fungi, which are known to be common in montane grasslands in Montenegro. These fungi, while being red-listed, are often associated with habitats of *P. heldreichii* (Perić and Perić 2004), which are nutrient-poor, but support high diversity of fungi (Lazarević and Menkis 2018). Interestingly, recently described ubiquitous soil fungi of the genus *Archaeorhizomyces* (Rosling et al. 2011) were also detected (Table 5). Although functional properties, reproduction structures and dispersal strategy of these fungi are largely unknown, the current observation expands available knowledge on the host tree species and geographical distribution.

A number of ECM fungi were also commonly detected in the soil, including *Tylospora asterophora*, *Russula vesca*, *Inocybe whitei*, *Laccaria laccata* and *Cenococcum geophilum* (Table 5), showing that these may be important symbionts of forest trees grown in high-altitude habitats. However, as these ECM fungi were mainly detected at the Zeletin site (Table 5), the possibility should not be excluded that their occurrence was also affected by the other three species (*A. alba* or *F. sylvatica*) present there.

Living needles are known to be associated with diverse trophic groups of fungi, including endophytes, epiphytes and pathogens. Many of these can be functionally important and metabolically active taxa, which respond to changes in the environment (Nguyem et al. 2016). They appear to be able to colonise different tree species, but factors driving their distribution remains largely unclear (Tehronen et al. 2019). Among the dominant fungi associated with the needles of *P. peuce*, there were *Dothideomycetes* sp. 3360_7, *Dothideomycetes* sp. 3360_10 and *Leotiomycetes* sp. 3360_16 (Table 6), which could not be identified to the species or genus level, thereby not only posing a challenge to fungal taxonomy, but also limiting the identification of their ecology and functional roles. Further, *Sydowia polyspora* was detected in *P. peuce* needles (Table 6), which is the fungus with a wide geographical distribution and common occurrence in Europe (Botella and Diez 2010). The pathogenic behaviour of *S. polyspora* to young conifers (genera *Thuja*, *Abies*, *Tsuga*, *Larix*, *Picea* and *Pinus*) was previously reported (Talgo et al. 2018). Besides, it has been recently reported as one of the most abundant needle pathogens in high-altitude *P. heldreichii* forests in Montenegro (Lazarević and Menkis 2020), showing that it is not restricted by harsh environmental conditions prevailing in these habitats. *Sydowia* symptoms include needle discoloration, necrosis and shoot dieback. However, the fungus is favoured by a warm climate, especially if the host is stressed by drought or insect attack (Munoz – Adalia et al. 2017). *Celosporium larixicola* was another commonly detected fungus (Table 6), which was recently described from needles of *Larix lyallii* in Canada (Tsuneda et al. 2010) and needles of *P. abies* in Sweden (Ngyen et al. 2016). Fungi from the genus *Celosporium* was shown to be commonly recovered from alpine habitats and may be biotrophic or necrotrophic (Brown et al. 2015). *Lophodermium pinastri* was also common in needles of *P. peuce* (Table 6). It has global distribution and is commonly associated with pines (Millberg et al. 2015; Reignoux et al. 2014). It was shown recently that *L. pinastri* colonises healthy needles latently as an endophyte, initiates active growth at the beginning of needle senescence and sporulates after the needle fall. It is a dominant coloniser of dying needles and a saprotroph contributing to the decomposition of the needles (Reignoux et al. 2014). Besides, *L. pinastri* was one of the most commonly detected fungi on *P. heldreichii* needles in Montenegro (Lazarević and Menkis 2020) and frequently reported

from *P. nigra* and *P. sylvestris* grown in forests, forest nurseries and plantations in the Balkan region (Karadžić and Miijašević 2008; Dobrova et al. 2016).

In summary, *P. peuce* in high altitude mountain habitats harbour diverse communities of fungi, composition of which appears to be largely determined by the host tree species, substrate properties and functional capabilities of these fungi.

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Figures

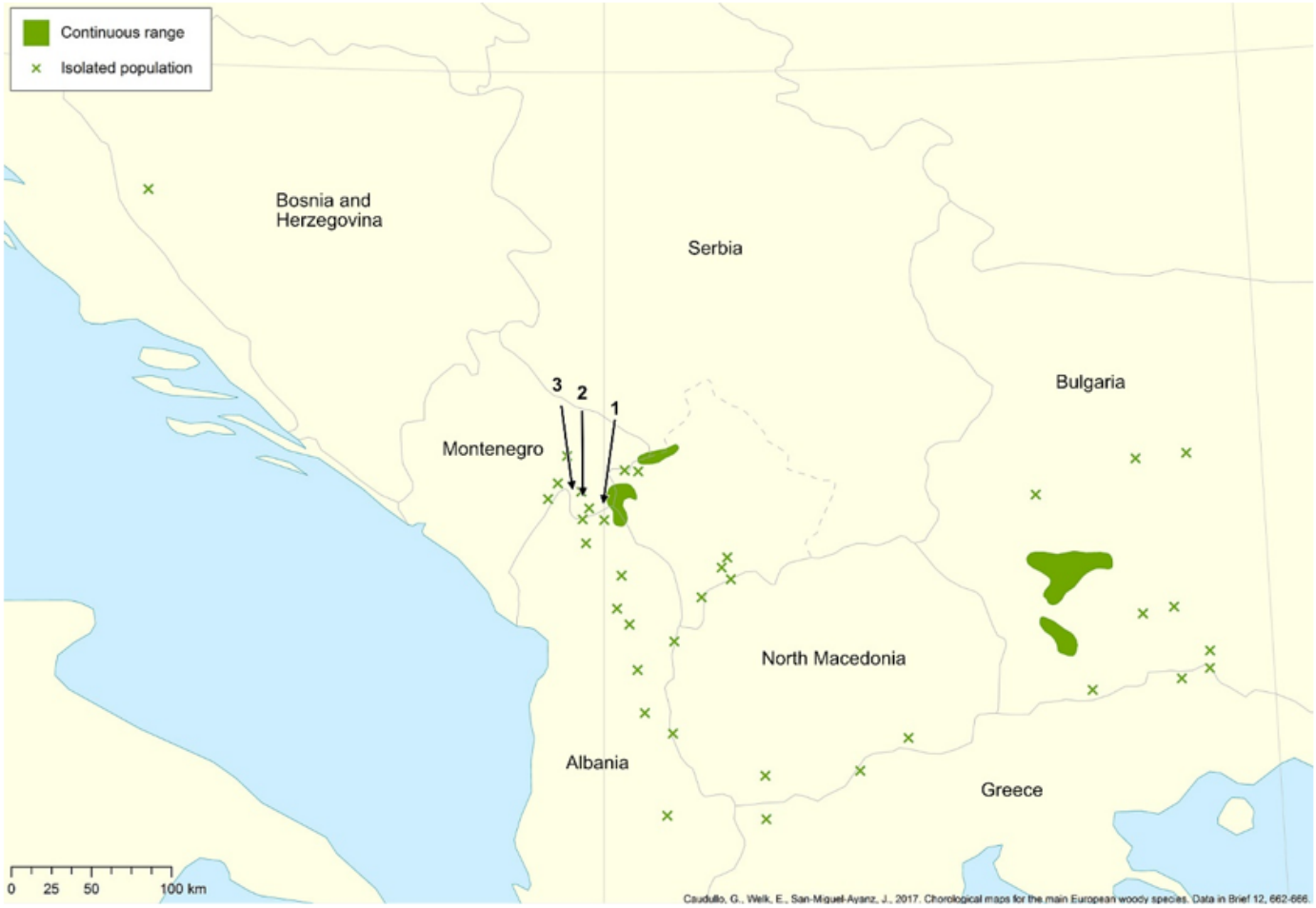

Figure 1

The map of the Balkan region showing the distribution of Pinus peuce (Caudullo et al. 2017). The sampling sites are arrowed and numbered: 1 – Bogićevica, 2 – Visitor and 3 –Zeletin.

Figure 2

Pinus peuce forest stands at the Bogićevica site in southestern Montenegro.

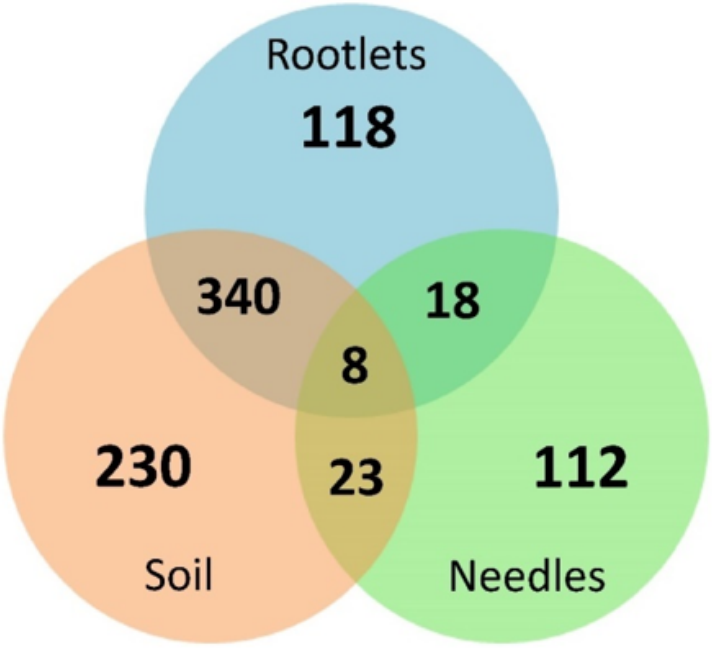

Figure 3

Venn diagram showing the diversity of fungal taxa found in rootlets, soil and needles of Pinus peuce, and the number of fungal taxa shared between different substrates. Samples from different sites are combined.

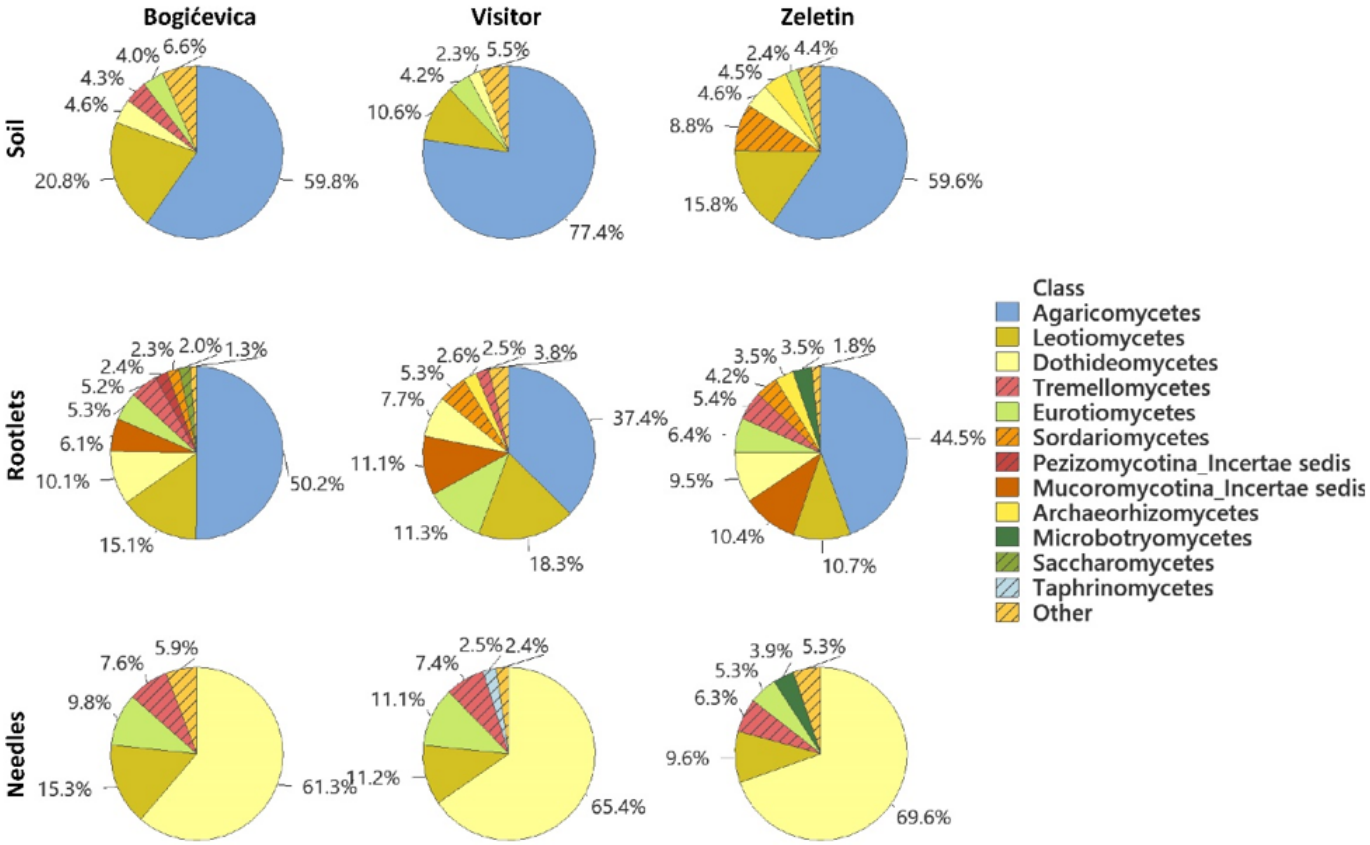

Figure 4

Relative abundance of fungal classes in the soil, rootlets and needles at the Bogićevica, Visitor and Zeletin sampling sites of Pinus peuce in Montenegro. Other represent fungal Classes with a relative abundance of <2%.

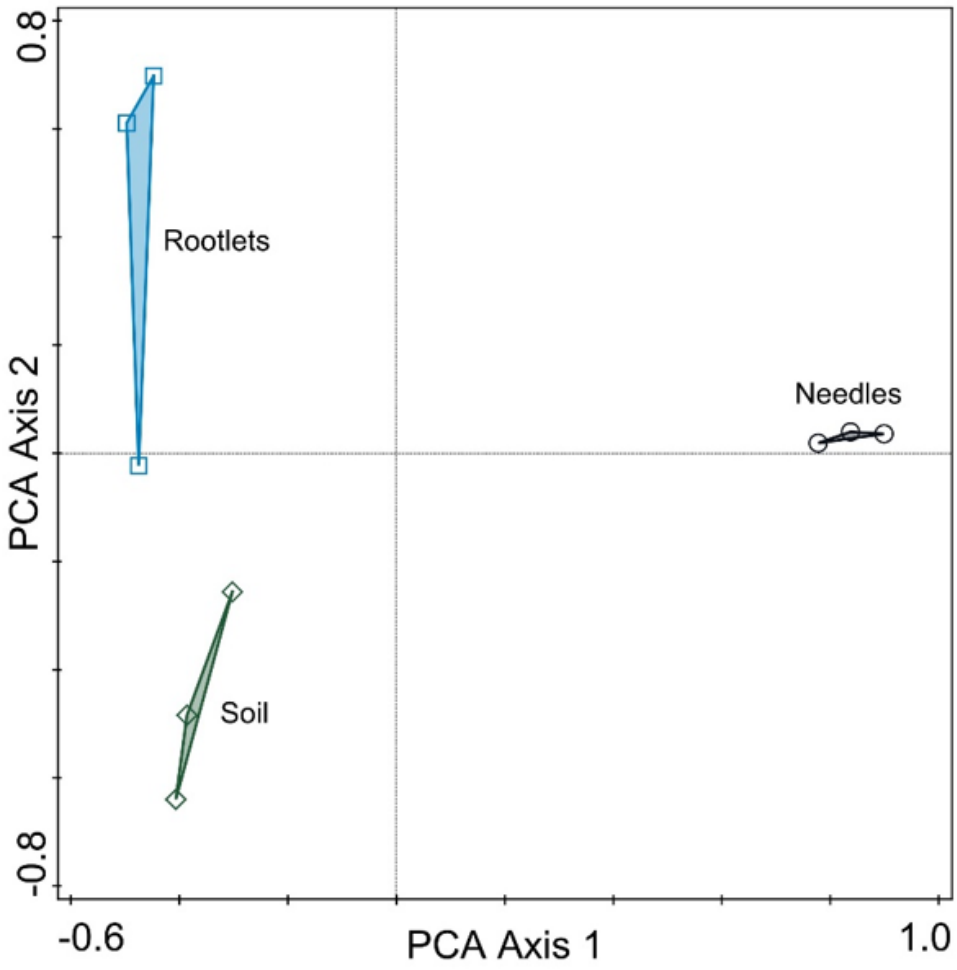

Figure 5

Ordination diagram based on principal coordinates analysis (PCA) of fungal communities from needles, rootlets and soil of Pinus peuce in Montenegro. Each point in the diagram represents a single sample. Samples of the same substrate (needles, rootlets or soil) are enveloped.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PinuspeucefungiSupplementaryTable1.xlsx