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# Do microclimatic conditions in two forest types on serpentine bedrock affect culturable microfungi in pine litter needles?

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Microfungi colonising coniferous needles in litter were intensively studied in previous decades, but forest stands on serpentine soils have been overlooked. Also, the effects of microclimatic conditions on fungal communities in coniferous litter are unknown. In our study, we aimed to characterise communities of culturable microfungi colonising pine litter needles collected from two types of Scots pine forest growing on serpentine bedrock, i.e. dense forest with relatively stable microclimatic conditions and open-canopy forest on exposed rock with highly variable conditions. The composition of their fungal communities was analysed in respect to microclimatic conditions at the collection sites.

Using a combination of phenotypic and molecular data (sequences of ITS rDNA), 35 taxa were distinguished in 1078 fungal colonies recorded, out of which 25 were identified to the species level. Fungal communities were most affected by needle type (litter vs. fermentation layer) followed by maximum temperature during the previous five months. Interestingly, a higher number and abundance of species were recorded at the warmer site, in the open-canopy forest. Dominant fungi recorded in this study (*Desmazierella acicola*, *Phacidium lacerum* and *Scleroconidioma sphagnicola*) were mostly identical to those recorded in previous studies and the occurrence of less abundant taxa previously not recorded in pine litter suggests that the uppermost litter layer represents an important reservoir of fungal diversity.

**Key words:** fungal diversity, ITS rDNA, temperature and humidity, *Pinus sylvestris*.

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Koukol O., Magdalinou E., Pánková H., Borovička J., Münzbergová Z. (2022): Mají mikroklimatické podmínky ve dvou typech lesa na hadcovém podloží vliv na kultivovatelné mikroskopické houby v jehlicích borovic v opadu? – Czech Mycol. 74(2): 181–194.

Mikroskopické houby kolonizující jehlice v opadu byly intenzivně studovány v předchozí dekádě, avšak stanoviště na hadcových půdách byla opomíjena. Také vliv mikroklimatických podmínek na houbová společenstva v jehličnatém opadu není znám. V naší studii jsme se zaměřili na popis společenstev kultivovatelných mikroskopických hub kolonizujících jehlice borovic v opadu ve dvou typech lesa tvořeného borovicí lesní, rostoucích na hadcovém podloží – hustý les s relativně stabilními mikroklimatickými podmínkami a otevřený les na exponované skále s dynamicky se měnícími podmínkami. Složení společenstev bylo analyzováno ve vztahu k mikroklimatickým podmínkám na daném stanovišti.

Za použití kombinace fenotypových a molekulárních dat (sekvence oblasti ITS rDNA) bylo rozlišeno 35 taxonů mezi 1078 získanými izoláty, z nichž 25 bylo určeno do druhu. Společenstva hub byla nejvíce ovlivněna typem jehlic (z hrabanky vs. z fermentační vrstvy), následně maximální teplotou během předchozích pěti měsíců. Zajímavé je, že větší množství druhů a větší abundance jednotlivých druhů byla zaznamenána na teplejší lokalitě, v otevřeném typu lesa. Dominantní druhy nalezené v této studii (*Desmazierella acicola*, *Phacidium lacerum* a *Scleroconidioma sphagnicola*) byly většinou shodné jako v předchozích studiích; výskyt několika méně hojných druhů, které dosud nebyly zaznamenány v jehličnatém opadu, naznačuje, že hrabanka představuje důležitý zdroj diverzity hub.

## INTRODUCTION

Coniferous needles in litter harbour rich fungal communities. A high diversity of microfungi in pine needles has been documented since the first systematic surveys aimed at culturable fungi (Mitchell et al. 1978, Tokumasu et al. 1994). Although recent studies using a metagenomic approach have mostly ignored fungal diversity in the uppermost soil horizon (Buée et al. 2009, Buscardo et al. 2014), Santalahti et al. (2016) observed the highest fungal richness and also the highest number of saprotrophic species in the uppermost soil layer as compared to deeper soil horizons. Fungal communities in the litter are influenced by numerous factors including chemical composition of the needles and local climatic conditions. Seasonal patterns were already observed in the past, with some fungi changing frequency and others disappearing for a particular season (Tokumasu et al. 1994, Hirose et Osono 2006), but recent surveys indicate a stable community in the uppermost soil horizon across different seasons (Santalahti et al. 2016). These large-scale surveys may however not reveal subtle changes on a fine scale, as different fungal communities may exist at sites with contrasting microclimatic conditions due to e.g. different openness or exposure of the stands.

In our study, we primarily aimed to characterise fungal communities colonising pine litter needles in Scots pine (*Pinus sylvestris*) forest on two types of sites with contrasting microclimatic conditions (temperature and humidity), i.e. sites covered by dense pine forest with relatively stable microclimatic conditions versus

sites with sparse pine growth on exposed rock and highly variable conditions. The composition of the fungal communities was analysed with respect to both microclimatic conditions and vegetation cover in the moss layer (i.e. mosses and lichens). All sampling sites were located in forests on serpentine bedrock. Serpentine soils are stressful environments for plants due to multiple limitations posed by their physical characteristics and chemical composition. The low soil water-holding capacity and low Ca/Mg ratio combined with relatively high concentrations of toxic metals such as Ni may pose a considerable challenge to local plant species and result in a high proportion of endemic species with adaptive morphologies and a distinctive structure of serpentine plant communities (Brady et al. 2005, Pánková et Münzbergová 2011). Our secondary aim was to reveal if litter needles on serpentinite harbour a distinct fungal community.

## MATERIAL AND METHODS

Localities and collection. Litter needles were sampled in two forest types in Hadce u Želivky National Nature Monument (Central Bohemia, Czech Republic) on 13 March 2018. The first one is formed by dense Boreo-continental pine forest (*Dicrano-Pinion sylvestris* alliance) with a high occurrence of self-seeded trees such as *Frangula alnus*, *Picea abies* and *Betula pendula* growing on the top plateau and gentle slopes. The soil is generally shallow, but deeper than in the other habitat, with a thicker humus layer. The vegetation cover is dense with a high abundance of grasses. The other type includes open Peri-Alpidic serpentine pine forest (*Erico carneae-Pinion* alliance) located on exposed rock platforms with shallow soil and sparse vegetation (*Asplenion cuneifolii* and *Festucion valesiacae* alliances) but rich lichen cover (mostly *Cladonia* spp.). Vegetation cover in the moss layer ($E_0$) was evaluated for all localities and summary data for the coverage of mosses and lichens is presented in Tab. 1. Microclimatic conditions at the localities were determined with a TMS datalogger (Wild et al. 2019) continuously for the period of five months (17 October 2017 to 18 March 2018) prior to sample collection. Humidity and temperature on the surface were recorded every 15 min.

**Tab. 1.** Geographic location and characteristics of microclimatic conditions and moss and lichen covers at three localities in the Boreo-continental forest and in the Peri-Alpidic forest type.
Mean humidity is expressed in relative values; T-min., T-mean and T-max. reflect minimum, mean and maximum surface temperature (in °C) in the previous five months. Lichen and moss coverages are expressed in %.

| Locality | Coordinates | Humidity | T-min. | T-mean | T-max. | Lichen coverage | Moss coverage |
|---|---|---|---|---|---|---|---|
| **Boreo-continental** | | | | | | | |
| Loc. 1 | 49°41'18.8" N, 15°05'57.1" E | 1590.5 | –14.4 | 1.8 | 19.6 | 0.0 | 88.0 |
| Loc. 2 | 49°41'19.2" N, 15°06'11.9" E | 1649.4 | –13.9 | 3.3 | 23.8 | 0.0 | 18.0 |
| Loc. 3 | 49°41'01.9" N, 15°07'53.3" E | 1801.0 | –13.3 | 2.3 | 19.0 | 0.0 | 68.0 |
| **Peri-Alpidic** | | | | | | | |
| Loc. 1 | 49°41'13.5" N, 15°06'09.3" E | 1321.9 | –11.8 | 2.7 | 17.1 | 0.0 | 38.0 |
| Loc. 2 | 49°41'25.7" N, 15°06'17.8" E | 1708.1 | –14.1 | 3.2 | 25.2 | 3.0 | 8.0 |
| Loc. 3 | 49°41'00.8" N, 15°07'59.2" E | 1922.6 | –11.3 | 3.1 | 29.2 | 3.0 | 2.0 |

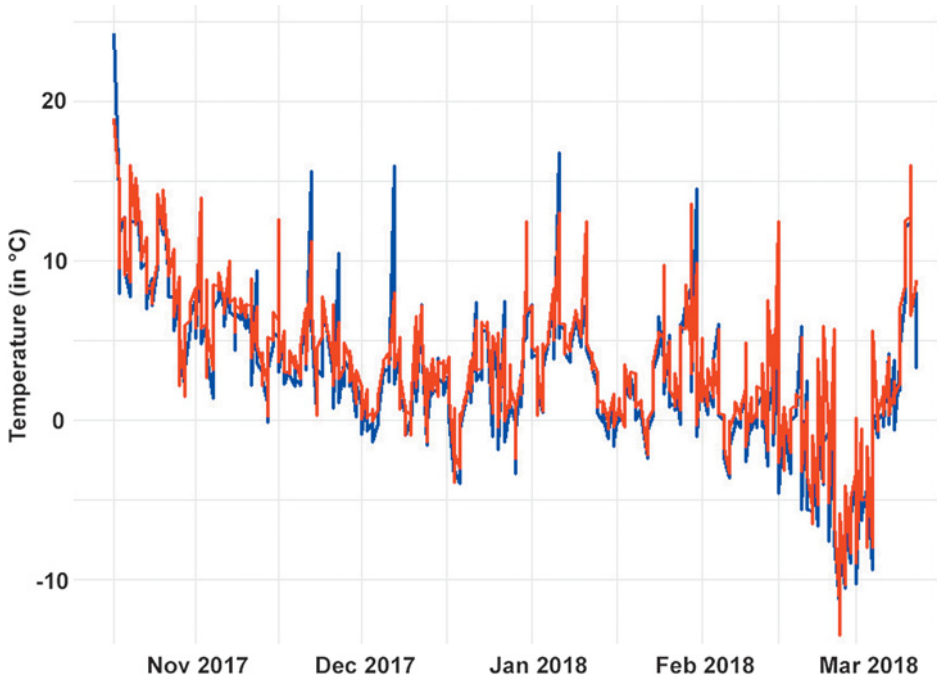


**Fig. 1.** Temperature fluctuations during the five-month period prior to the collection of litter needles. The red line indicates records from the Boreo-Continental sites, the blue line those from the Peri-Alpidic sites (means of three measurements).

For each forest type, three localities were selected, each with three sampling sites, where pine needles were collected from the organic soil horizon. Two soil horizons (layers) were distinguished: the uppermost layer consisting of brown to yellow-brown needles still retaining their integrity (litter needles in strict sense, Oi) and the layer beneath composed of black, partially fragmented and softened needles (fermentation layer, Oe). Needles were sampled into sterile plastic bags and processed in the laboratory within 48 h. In the laboratory, 25 randomly selected needle pairs were cut with scissors, and 2 cm long fragments from the middle part of each needle were surface-sterilised in 30% hydrogen peroxide for 90 s on a horizontal shaker (Koukol 2011). Fragments were placed in a Petri dish (10 fragments per dish) with malt agar prepared from brewers wort with a final sucrose content adjusted to 2% w/v and with 18 $g \cdot l^{-1}$ agar (2MA) (Fassatiová 1986). Petri dishes were cultivated at room temperature (23–25 °C). Fifty fragments were cultivated for each sampling site and horizon, rendering altogether 1800 fragments cultivated.

When possible, outgrowing fungi were directly identified based on their morphology. If sterile colonies were formed, they were grouped into morphotypes according to phenotypic characteristics (e.g. growth rate, colour, colony profile and margin). A representative strain from each morphotype was transferred onto a new plate with 2MA. Sterile cultures were kept for 6 months at lowered temperature (18 °C) combined with near-ultraviolet light (UVA) and on nutrient-poor medium potato carrot agar (Fassatiová 1986) to promote sporulation.

Fungal identification. In order to identify sterile as well as sporulating strains of problematic genera (e.g. *Trichoderma*), genomic DNA was isolated from 7–14 d old cultures using a Zymo Research Fungal/Bacterial kit (Zymo Research, Orange, USA). Nuclear rDNA containing internal transcribed spacers (ITS1 and ITS2), 5.8S and D1/D2 domains of the 28S region were amplified with primer sets ITS1/NL4 or ITS1/ITS4 (White et al. 1990, O'Donnell 1993). PCR products were purified using a GenElute PCR Clean-Up Kit (Sigma-Aldrich, St. Louis, USA). For identification of some morphotypes in which the ITS region was not sufficient, a fragment of the genes encoding the elongation factor 1α (EF1-α) was amplified using primer sets 983F/2218R (O'Donnell et Cigelnik 1997). The same primers were used for sequencing at the Sequencing Laboratory of the OMICS Core Facility, BIOCEV (Vestec, Czech Republic). Sequences were compared for homology in GenBank using the BLAST algorithm (Altschul et al. 1997). Only 99–100% matches with reliable sources (ex-type sequences, taxonomic studies) were taken as identification evidence. Fungal species names were checked with Index Fungorum (http://www.indexfungorum.org/names/Names.asp).

Chemical analyses. For chemical characterisation of the substrates, both needles from litter (Oi) and fermentation (Oe) soil horizons were collected from each sampling site. Specimens were pooled together for each locality, air-dried, milled to fine powder followed by extraction in 1M HCl according to Borovička et al. (2019). Concentrations of extractable macronutrients (Ca, Mg, K, P) and metals (Ni, Cr) were determined in the laboratory of the Institute of Geology of the Czech Academy of Sciences using Element 2 high resolution inductively coupled plasma mass spectrometry (ICP-MS). Control soil samples collected from four pine forests on sandstone, calcareous sandstone, or schist in Central Bohemia, Czech Republic were processed for comparison.

Statistical analyses. Abundances of each morphotype were calculated as the number of needle fragments with (at least one) colony of a particular morphotype. Morphotypes recorded in a single colony were excluded from the analyses of species composition (see below). Abundances were joined for morphotypes later shown to represent identical species based on morphological or molecular data.

First, factors determining numbers of fungal species and their abundances in the samples were explored using generalised linear mixed effect models with sampling site and locality as random factors. Both variables were modelled with a Poisson distribution and log-link function, as they are both non-negative small integer numbers. The predictors were needle type (litter or fermentation layer), temperature and humidity in the soil (minimum, maximum and mean values for the previous five months) and vegetation cover on the sampling sites. Chemical composition of the soil was not used in the tests, as most variation in soil chemistry was explained by needle type and so soil chemistry would not add any new information to the tests. In addition, the sample size in this study was too low to test the effects of many predictors. Because we had too many predictors for a limited number of samples even when excluding litter chemistry, the effect of needle type, which was expected to play a dominant role, was tested first. Subsequently, forward step-wise selection of the other predictors was performed with needle type as a covariate in case it was significant. The mixed effect models were run in the package lme4 for R (Bates et al. 2015).

The effect of the same predictors (i.e. needle type, soil temperature and humidity, and vegetation cover) on the composition of the fungal communities was tested using Redundancy analysis (RDA) in the vegan package for R (Oksanen et al. 2019). Again, the effect of needle type was tested first. Subsequently, forward step-wise selection of the other predictors with needle type as a covariate was used in case it was significant. Sampling site was used to define the structure of the data. Significance of the effects was assessed using permutation tests (999 permutations) permuting only within the sampling site (for litter type) or only between the sampling sites (for the other predictors).

**Tab. 2.** Fungal taxa isolated from pine needles in litter, their putative identification and abundance (number of colonised needle fragments) at the three studied localities for each forest type.

| Taxon | Boreo-continental | | | Peri-Alpidic | | |
|---|---|---|---|---|---|---|
| | Loc. 1 | Loc. 2 | Loc. 3 | Loc. 1 | Loc. 2 | Loc. 3 |
| *Desmazierella acicola* Lib. | 36 | 41 | 16 | 50 | 55 | 29 |
| *Phacidium lacerum* Fr. | 36 | 39 | 35 | 35 | 22 | 28 |
| *Scleroconidioma sphagnicola* Tsuneda, Currah et Thormann | 4 | 21 | 4 | 25 | 66 | 8 |
| *Trichoderma polysporum* (Link) Rifai | 14 | 3 | 15 | 18 | 3 | 25 |
| *Gymnopus androsaceus* (L.) J.L. Mata et R.H. Petersen | 5 | 16 | 15 | 9 | 12 | 7 |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | 14 | 9 | 13 | 3 | 10 | 8 |
| *Trichoderma koningii* Oudem. | 7 | 15 | 1 | 0 | 8 | 7 |
| *Alternaria alternata* (Fr.) Keissl. | 5 | 4 | 14 | 2 | 6 | 7 |
| *Truncatella conorum-piceae* (Tubeuf) Steyaert | 9 | 1 | 6 | 6 | 3 | 1 |
| *Herpotrichia pinetorum* (Fuckel) G. Winter | 0 | 0 | 6 | 0 | 4 | 14 |
| *Angustimassarina* sp. | 0 | 3 | 9 | 1 | 2 | 1 |
| *Phoma herbarum* Westend. | 3 | 5 | 1 | 5 | 1 | 1 |
| *Helotiales* sp. | 0 | 2 | 0 | 2 | 7 | 0 |
| *Corticiales* sp. | 1 | 0 | 5 | 2 | 1 | 0 |
| *Epicoccum nigrum* Link | 3 | 1 | 2 | 0 | 0 | 3 |
| *Mycena* sp. | 0 | 0 | 0 | 8 | 0 | 1 |
| *Plectania melastoma* (Sowerby) Fuckel | 3 | 0 | 0 | 4 | 1 | 2 |
| *Pseudocamaropycnis pini* Crous | 0 | 1 | 1 | 0 | 0 | 7 |
| *Fusarium* sp. 1 | 2 | 0 | 0 | 0 | 4 | 1 |
| *Sistotrema efibulatum* (J. Erikss.) Hjortstam | 0 | 0 | 1 | 0 | 6 | 0 |
| *Hormonema dematioides* Lagerb. et Melin | 1 | 1 | 0 | 3 | 1 | 0 |
| *Ceratobasidium* sp. 1 | 0 | 3 | 0 | 0 | 2 | 0 |
| *Cladosporium herbarum* (Pers.) Link | 1 | 0 | 1 | 0 | 2 | 1 |
| *Lophodermium pinastri* (Schrad.) Chevall. | 1 | 0 | 0 | 1 | 1 | 1 |
| *Erythricium hypnophilum* (P. Karst.) J. Erikss. et Hjortstam | 3 | 0 | 0 | 0 | 0 | 0 |
| *Knufia tsunedae* Madrid, Guarro et Crous | 0 | 0 | 0 | 0 | 0 | 3 |
| *Pleosporales* sp. | 0 | 0 | 3 | 0 | 0 | 0 |
| *Anthostomella formosa* Kirchst. | 0 | 0 | 1 | 0 | 0 | 0 |
| *Beauveria pseudobassiana* S.A. Rehner et R.A. Humber | 0 | 0 | 0 | 0 | 0 | 1 |
| *Ceratobasidium* sp. 2 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Dothideomycetes* sp. | 0 | 0 | 1 | 0 | 0 | 0 |
| *Fusarium* sp. 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| *Mycena epipterygia* (Scop.) Gray | 0 | 1 | 0 | 0 | 0 | 0 |
| *Roridomyces roridus* (Fr.) Rexer | 0 | 0 | 1 | 0 | 0 | 0 |
| *Trametes versicolor* (L.) Lloyd | 0 | 0 | 0 | 0 | 2 | 0 |
| **Total number of species** | **18** | **18** | **21** | **16** | **23** | **21** |

## RESULTS

The isolations from pine litter needles yielded a total of 1078 colonies growing from 56% of needles (790 needle fragments remaining sterile). Colonies were assigned to 98 morphotypes, which were later reduced to 35 taxa after comparing phenotypic and molecular data. Twenty-five taxa were identified to species, while the remaining ten morphotypes did not match in their rDNA sequences with those present in GenBank and were assigned a higher taxonomic rank (Tab. 2).

The communities of culturable fungi were dominated by ascomycetes (25 species), while basidiomycetes were represented by 10 species. The three most abundant species were *Desmazierella acicola* (*Pezizales*, recorded as the *Verticicladium* stage, overall abundance 227), *Phacidium lacerum* (*Helotiales*, recorded as the *Ceuthospora* stage, overall abundance 195) and *Scleroconidioma sphagnicola* (*Dothideomycetes* inc. sed., overall abundance 128).

When compared to the control sites, both Oi and Oe soil horizons from the serpentine bedrock were considerably enriched with Mg and Ni, and their Ca/Mg ratios were remarkably lower than those at the control sites (Tab. 3). On average, concentrations of Mg, Ni and Cr in the Oe horizon were approx. 2×, 5× and 5× higher, respectively, than those in the Oi horizon.

**Tab. 3.** Chemical characteristics of the uppermost litter (Oi) and fermentation (Oe) soil horizons collected at serpentinite and control sites. The values represent extractable element fractions ($mg \cdot kg^{-1}$) expressed as mean ± standard deviation out of six serpentinite and four control samples.

| Element | Serpentinite | | Control | |
|---|---|---|---|---|
| | Oi | Oe | Oi | Oe |
| Ca | 7847 ± 605 | 8105 ± 868 | 7461 ± 781 | 5089 ± 584 |
| Mg | 2086 ± 563 | 3703 ± 2004 | 748 ± 61.0 | 512 ± 162 |
| Ca/Mg | 3.96 ± 0.80 | 3.18 ± 2.01 | 10.0 ± 1.25 | 10.8 ± 2.63 |
| K | 685 ± 74.1 | 867 ± 44.1 | 1697 ± 668 | 839 ± 95.7 |
| P | 277 ± 38.6 | 416 ± 65.3 | 605 ± 106 | 515 ± 48.8 |
| Ni | 13.6 ± 4.84 | 67.8 ± 41.2 | 0.99 ± 0.18 | 9.20 ± 3.37 |
| Cr | 1.25 ± 0.79 | 6.55 ± 4.93 | 1.09 ± 0.18 | 17.4 ± 9.40 |

Both the number and abundances of species were significantly affected by soil horizon ($F = 30.155$, $p < 0.001$ and $F = 44.39$, $p < 0.001$, respectively), with higher values in the uppermost litter (Oi) than in the fermentation layer (Oe). After accounting for this effect, we detected a significant effect of maximum surface temperature in the previous five months (Fig. 1), with a slightly higher number of species at sites in the Peri-Alpidic forest type (Tab. 2).

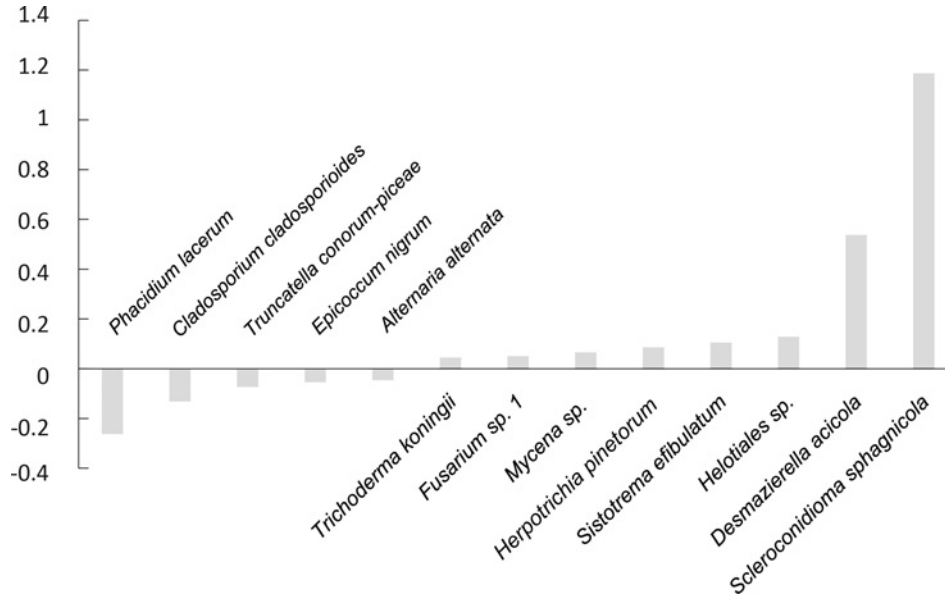


**Fig. 2.** Affinity of fungal species to warm conditions at the locality expressed as their scores along the first RDA axis. Only 13 species with the highest scores (threshold 0.04) are shown.

Similarly to species number and abundance, also species composition significantly differed between the two layers, explaining 10.5% of variation in the data. In a subsequent step-wise selection using layer as a covariate, maximum surface temperature in the previous five months was detected as the only significant predictor explaining an additional 4.1% of variation (Fig. 2).

## DISCUSSION

In our study we primarily focused on how communities of culturable microfungi in pine needles in the litter depend on local microclimatic conditions. Therefore, two contrasting Scots pine forest types (open and dense pine forest) differing in vegetation cover, but situated close to each other were selected and detailed characteristics of the conditions on the surface were determined. To our knowledge, this was the first study in which fungal communities in litter were compared based on detailed microclimatic measurements rather than mean annual rainfall (Prihatini et al. 2015) or seasonal differences (Santalahti et al. 2016).

**Factors affecting fungal communities**

Out of all the factors tested, we only identified soil horizon (litter and fermentation layer) to be the most important determinant of fungal diversity and abundance observed. Although this difference could be partly attributed to the sterilisation technique used (higher penetration of hydrogen peroxide into the more fragile needles in the Oe layer), a similar pattern was also observed in pyrosequence data by Santalahti et al. (2016) and Solly et al. (2017), who recorded a distinct community in the Oi layer differing from deeper soil horizons. Also, the higher abundance of fast-growing *Trichoderma* species observed on the Oi needles (data not shown) might have prevented other fungi from growing.

After the effect of soil layer had been filtered, fungal communities were affected by the maximum temperature in the previous five months. Thus resistance to high temperatures and potentially also to desiccation rate may be expected in fungi recorded at sites in the Peri-Alpidic forest. This is particularly true for *Scleroconidioma sphagnicola*, a species with a strongly melanised mycelium able to withstand adverse abiotic conditions (Koukol et Kovářová 2007), which had the highest score along the first axis in the RDA analysis (Fig. 2). Similarly, Solly et al. (2017) observed that soil warming at the alpine treeline led to an increase in abundance of several litter-associated ascomycetes. Effects of season had already been documented, e.g. Zamora et al. (2008) observed a high divergence of fungal communities isolated in spring vs. autumn from pine needles in plantations in Northern Spain.

**Fungi in serpentine soils**

Our study pioneered the characterisation of the diversity of culturable saprotrophic microfungi from pine litter on serpentine soils. Previous studies were only aimed at plant-symbiotic fungi and soil fungi, but indicated that mycobiota may be less affected by nutrient limitation or excess of metal cations as compared to the strong impact which serpentinite has on plant communities. Muller et Hilger (2015) recorded a similar richness of fungal symbionts in the roots of the perennial herb *Onosma echioides* (*Boraginaceae*) at serpentine and non-serpentine sites, but their structure differed. The fungal community at non-serpentine sites was dominated by arbuscular mycorrhizal members of *Glomeromycota*. Their occurrence on serpentine site was lower and basidiomycetous species (potentially opportunistic symbionts) prevailed. Waseem et al. (2017) found a high diversity of ectomycorrhizal fungi associated with different endemic species of *Tristaniopsis* (*Myrtaceae*) growing on both ultramafic and sedimentary sites. Rosenstock et al. (2016) observed a higher biomass of ectomycorrhizal fungi in mesh bags placed on serpentinite compared to that on granite and amphibolite. Differences in ectomycorrhizal fungal communities associated with

these three parent materials were attributed to different pH levels. Another possible indirect effect is that vegetation cover seems to affect microbial communities in serpentine soils rather than soil properties. Bordez et al. (2016) found an unexpectedly high richness of fungal species in ultramafic soils with different successional stages of aboveground vegetation, which contrasts to the present study, where vegetation cover did not affect the species composition of the fungal communities. Cecchi et al. (2019) observed a lower fungal richness in soil microfungi isolated from serpentinite-rich soil, but the most abundant microfungi were not affected by geochemical factors of the soil. The addition of serpentinite may even promote fungal diversity. For example, Małek et al. (2021) observed that out of several treatments, soil in a serpentinite-fertilised temperate spruce forest harboured the second highest number of operational taxonomic units.

In our present study, we did not include collections of litter needles for fungal isolation from non-serpentine soils as a negative control. However, we may compare the results with those obtained by Koukol (2011), who collected needles of Scots pine from a forest on sandstone rock in the same season (March) and surveyed fungal communities using the same approach. Koukol (2011) isolated fungi from 320 needles collected from the Oi layer only and obtained 35 species in 340 colonies. In the present study, 32 species were identified in 678 colonies growing from 900 needles from the Oi layer. When fungal abundances in the different forest types are compared separately, needles from the Peri-Alpidic sites yielded 0.81 colony per needle, whilst those from the Boreo-continental sites only 0.69 colony compared to 1.06 colony per needle collected on sandstone rock (Koukol 2011). The diversity and abundance of fungi at the serpentine sites were thus slightly lower, but the most abundant species were almost identical in both studies. The only exception is the anamorphic ascomycete *Sympodiella acicola* (*Venturiales*), which was absent in the present study, although it was the second most frequently isolated species on sandy soils. Whether local absence rather than the sensitivity of *S. acicola* to the specific conditions of the serpentine soil plays a role here is not clear. In concordance to previous studies, fungi should be able to balance the negative effect of serpentinite due to their phenotypic plasticity (Waseem et al. 2017), and particular strains, rather than species, may show different sensitivity and tolerance to the specific chemical composition of serpentine soils similarly to the tolerance of heavy metals at polluted sites (Colpaert et al. 2011). Although the concentrations of Ni, Cr, and the Ca/Mg ratio were even more unfavourable in the Oe layer (Tab. 3), we assume that the statistically significant lower fungal diversity and abundances in this layer were rather related to the effect of the sterilisation procedure, as already mentioned.

**Fungal identification**

We identified our isolates primarily based on morphology. Only sterile isolates were identified based on molecular data, mostly the sequence of the ITS region. Even for the sequenced strains, this marker may not be sufficient to reveal genetic divergence. In a study by Jourand et al. (2010), nickel-tolerant and nickel-sensitive phenotypes of the ectomycorrhizal species *Pisolithus albus* isolated from ultramafic and volcano-sediment soils, respectively, clustered in a single lineage based on ITS. However, amplified fragment length polymorphism indicated that they represent two distinct populations. Therefore, we may not exclude that some of the species isolated in our study represent cryptic species or ecotypes adapted to the specific chemical composition.

Our approach of identifying sterile strains based on molecular data has nevertheless produced interesting results including unusual fungal species isolated from pine litter and species with unclear species delimitation. As an example, in the Boreo-continental forest, we recorded *Pseudocamaropycnis pini*, which was described only recently from pine needles in Hong Kong by Crous et Groenewald (2016). At five out of six sites, we isolated slow-growing grey sterile colonies, the ITS sequence obtained from a representative one of which showed 100% identity with sequences originating from types of five species of the recently described genus *Angustimassarina* (Thambugala et al. 2015). They differed from three other species by only a single insertion. The sequence of EF1-α was 100% identical with *A. acerina*, but differed in just a single mutation from two other species described by Hyde et al. (2017). Giving that all *Angustimassarina* species were described as lignicolous saprotrophs from twigs of several broad-leaved trees, our record from pine needles in litter expands the niches known for this genus. It also points to the difficult identification and unclear taxonomic boundaries between species described based on single isolates, and calls for a critical revision of this genus. Neither the identity of a strain originating from both forest types and identified in our study as *Truncatella conorum-piceae* was straightforward. This saprotrophic species described from spruce cones is frequently found as a pine twig endophyte and may be also pathogenic to pine and spruce needles and cones (Blumenstein et al. 2021). The morphology of its conidia and identity of the ITS sequence to sequence MT790329 originated from a study by Blumenstein et al. (2021) supported the identification. Rather surprisingly, it was also almost 100% identical (496/498 bp) with sequence NR_154504 originating from the type of *Truncatella spartii*, a species described from a branch of *Spartium junceum* (Senanayake et al. 2015). This species was later combined into the new genus *Heterotruncatella* by Liu et al. (2019), who mentioned numerous isolates obtained from pines. Considering that *T. conorum-piceae* was not represented by any ITS sequence in GenBank before 2018, these

two species seem to be conspecific. The older epithet was overlooked by excessive emphasis placed on molecular data in description of *T. spartii* (Koukol et Delgado 2021).

## CONCLUSIONS

To conclude, we determined soil horizon to be the main factor affecting the species composition of microfungi in pine litter. However, this factor combines the effect of the sterilisation method and potentially also the chemical composition and is thus difficult to interpret. Interestingly, seemingly less favourable conditions (high temperature) at the locality may actually support a higher diversity and abundance of fungi.

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