Temporal and spatial shifts in the foliar fungal endophyte community of Norway spruce (*Picea abies*) over 150 years in Finland

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Abstract

Herbarium specimens are invaluable for studying plant. Yet their potential to reveal historical plant-associated microbiomes remains largely unexplored. To address this gap, we reconstructed the temporal and spatial dynamics of foliar fungal endophytes in Norway spruce (*Picea abies*) by sequencing the fungal internal transcribed spacer (ITS) region from herbarium needle specimens collected in Finland between 1861 and 2023. We analyzed community shifts across 50-year and 20-year intervals and between southern and northern regions, assessing the influence of temperature and precipitation. The results showed that the community was dominated by Ascomycota, with *Lophodermium*, *Ceratocystis, Yarrowia*, *Saccharomyces*, and basidiomycota *Heterobasidion* as the most abundant genera. Among the 50-year intervals, *H. parviporum* was detected, and the abundance of *Lophodermium picea* increased in 20-year interval (2001–2023, $P < 0.05$). The fungal community composition differed significantly between the interval 1951–2000 and both 1851–1900 and 1901–1950 ($P < 0.05$). The southern region exhibited distinct fungal community and lower alpha diversity indices compared to the north ($P < 0.05$). Functional prediction revealed a significant increase in Saprotroph, Symbiotroph and Pathotroph-Saprotroph-Symbiotroph guilds in the 1901–1950 interval ($P < 0.05$). While temporal and spatial factors significantly structured the communities, no direct correlation with historical temperature or precipitation was found. Our findings demonstrate that herbarium specimens are a powerful resource for uncovering long-term microbial dynamics and highlight the primary roles of time and geography in shaping the foliar fungal microbiome.

Introduction

For centuries, botanists have collected over 480 million plant specimens and stored them in over 3000 herbaria (Heberling & Isaac, 2017). These collections are cornerstone resources for taxonomy, systematics, and the documentation of biodiversity(Besnard et al., 2018). Beyond the plants themselves, herbaria provide critical long-term data for ecological and evolutionary research, including studies on species distribution shifts, conservation status, and responses to environmental change such as pollution and climate warming (Holmes et al., 2016; Willis et al., 2017; Lang et al., 2019; James et al., 2018). For instance, herbarium records have been used to track plant extinctions (Lienert et al., 2002; Aedo et al., 2015) and document species migrating to higher altitudes and latitudes in response to climate change (Feeley, 2012; Wolf et al., 2016).

The unrepeatable trait of every specimen from one location, species and time provides great value to reconstruct historical situations (Guerin, 2014; Nualart et al., 2017). A few studies have investigated plant specimens to explore the plant distribution and the extinction of certain local species(Lienert et al., 2002; Stehlik et al., 2007; Aedo et al., 2015). Under the context of global change, researchers could leverage the long-term data from herbarium collections to learn about the ecological and evolutionary changes during this intensely dynamic period (Lang et al., 2019). For example, plants were found to move to higher altitudes or latitudes in South America and California because of climate change (Feeley, 2012; Wolf et al., 2016). Interestingly, some investigations into local herbarium samples also linked the invasion pattern of alien species with some historical events for example increased mechanization and the Second World War (Fuentes et al., 2008; Vilović et al., 2020).

The advent of modern genomic techniques has expanded the utility of these collections into the realm of microbial ecology. The DNA preserved within herbarium specimens offers a unique opportunity to sequence and identify historical plant-associated microorganisms, including pathogens and symbionts (Heberling & Isaac, 2017; Lang et al., 2019). The plant microbiome is now recognized as a key factor influencing host physiology, health, and resilience to biotic and abiotic stress (Müller et al., 2016; Finkel et al., 2017). Fungal endophytes, which reside asymptomatically within plant tissues, are a ubiquitous and functionally diverse component of this microbiome, engaging in mutualistic, commensal, or pathogenic relationships with their hosts (Rodriguez et al., 2009; Lugtenberg et al., 2016). Their composition is known to be shaped by host identity and environmental factors (Long et al., 2010; Bowman & Arnold, 2018). The symbiotic relationships between endophytes and their hosts vary in different forms, as well as the ecological functions exhibited by the endophytes (Lugtenberg et al., 2016).

Despite this potential, studies reconstructing historical plant microbiomes from herbarium specimens remain scarce, leaving a critical gap in our understanding of microbial community dynamics over time. Preliminary studies have demonstrated feasibility, successfully recovered nodule bacteria from historical legumes (Petipas et al., 2024) and sequenced endophyte DNA from samples over a century old while exploring the temporary change in associated microbial assemblages (Daru et al., 2018; Bieker et al., 2020). These works suggest that herbaria act as repositories of microbial biodiversity, enabling investigations into long-term ecological patterns that are impossible to study otherwise. One study which sequenced ancient microbial community from rhizosphere soil and roots concluded that typical biodiversity features and assembly rules were shared between them and contemporary microbial communities, indicating that the preservation may not change these characteristics (Grasso et al., 2025). Hence, by incorporating endophyte microbes in global herbaria into contemporary plant microbial research, it is highly possible to understand the shifting patterns of microbial community in geography and temporal scales, as well as factors influencing the microbial distribution (Daru et al., 2018).

In this study, we sequenced the foliar endophytic fungal community from herbarium specimens of Norway spruce (*Picea abies* (L.) Karst.) in Finland. Our aims are to (i) characterize temporal and spatial shifts in the fungal community, (ii) assess the influence of historical temperature and precipitation on microbial community structure and diversity, and (iii) predict the ecological function of the detected fungi. We hypothesized that (i) fungal community would shift across time periods, (ii) spatial origins (northern vs. southern Finland) influence the composition of the fungal community, and (iii) historical climate variables are significant drivers of fungal diversity and community assembly.

Materials and methods

Sample collection and pre-processing

The needle specimens of Norway spruce in this study were obtained from three herbaria: the Finnish Museum of Natural History (LUOMUS), the Botanical Museum of University of Oulu and the vascular plant collections of University of Turku. We attained the sampling permission from all three herbaria before the study. For the specimens in the Finnish Museum of Natural History, we used sterile forceps to pick small branches from the original collection into 50 ml falcon tubes. For the rest of the needle specimens, we received them in envelops from University of Oulu and University of Turku. After collecting all the samples, we sterilized the needle surface to remove the adhering microbes following the modified procedure of Park et al.(2013) by washing samples with distilled water, immersing in 70% ethanol solution for 1 minute and 1% sodium hypochlorite solution for 5 minutes subsequently, and rinsing them for 2–3 times with distilled water to remove traces of disinfectants.

DNA extraction, PCR amplification, sequencing and grouping information

The genomic DNA was isolated from each herbarium sample using the plant DNA kit NucleoSpin Plant II (MACHEREY-NAGEL, Düren, Germany) following the official protocol. The extracted DNA product was checked by a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The fungal internal transcribed spacer 2 region (ITS2) was amplified using forward primer ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3')(Martin & Rygiewicz, 2005). All PCR reactions were carried out with 15 μL of Phusion High- Fidelity PCR Master Mix; 0.2 μM of forward and reverse primers, and about 10 ng template DNA. During amplicon library construction and sequencing, each PCR amplification included a negative control using ddH_2O instead of template DNA. Subsequent experiments were performed only when no band appeared in the negative control. Thermal cycling consisted of initial denaturation at 98℃ for 1 min, followed by 30 cycles of denaturation at 98℃ for 10 s, annealing at 50℃ for 30 s, and elongation at 72℃for 30 s and 72℃ for 5 min. Suitable PCR products were selected through 2% agarose gel electrophoresis. Equal amounts of PCR products from each sample were mixed, subjected to end repair, A-tailing, and ligated with Illumina adapters. A total amount of 83 samples passed the quality check, and the library was sequenced on the Illumina platform (Novaseq 6000 SP flowcell, PE250) at Novogene company limited (Cambridge, UK). Raw sequences are available from the Sequence Read Archive (SRA) on the National Centre for Biotechnology Information (NCBI) website under project number **PRJNA1285108**.

Based on temperature sum, Finland has six Norway spruce breeding zones (1–6), with lower zone numbers corresponding to larger temperature sums (Fig. 1) (Jansson et al., 2013). The 83 samples' serial numbers and their collection locations in the map were marked in Fig. 1, and their corresponding information was listed in Table 1. Geographically, 58 samples from breeding zones 1–3 were classified as 'South' group while 25 samples from breeding zone 4–6 were identified as 'North' group. Temporarily, samples were assigned to four 50-year intervals and eight 20-year intervals, starting from 1851–1900 and 1861–1880 respectively and ending with 2001–2023 for both.

Table 1
The corresponding sample marked in number with their collection year, locations and grouping information

Sample Number	Collection Year	20-year Group	50-year Group	Locations	Region
1	1962	1961–1980	1951–2000	Ahlainen	South
2	1984	1981–2000	1951–2000	Enontekiö	North
3	1989	1981–2000	1951–2000	Enontekiö	North
4	1996	1981–2000	1951–2000	Enontekiö	North
5	1991	1981–2000	1951–2000	Enontekiö	North
6	1984	1981–2000	1951–2000	Ekenäs	South
7	1878	1861–1880	1851–1900	Geta	South
8	1993	1981–2000	1951–2000	Hämeenlinna	South
9	1886	1881–1900	1851–1900	Hamina	South
10	1976	1961–1980	1951–2000	Hanko	South
11	1971	1961–1980	1951–2000	Hattula	South
12	1877	1861–1880	1851–1900	Helsingfors	South
13	1951	1941–1960	1951–2000	Helsinki	South
14	1997	1981–2000	1951–2000	Helsinki	South
15	1979	1961–1980	1951–2000	Helsinki	South
16	2006	2001–2020	2001–2050	Helsinki	South
17	1880	1861–1880	1851–1900	Inari	North
18	1987	1981–2000	1951–2000	Inari	North
19	1988	1981–2000	1951–2000	Inari	North
20	1891	1881–1900	1851–1900	Ingå	South
21	2023	2021–2040	2001–2050	East lapland	North
22	1975	1961–1980	1951–2000	Jämsä	South
23	1992	1981–2000	1951–2000	Janakkala	South
24	2013	2001–2020	2001–2050	Joensuu	South
25	1973	1961–1980	1951–2000	Jyväskylä	South
26	1938	1921–1940	1901–1950	Kajaani	South
27	1986	1981–2000	1951–2000	Karis	South
28	1927	1921–1940	1901–1950	Karkkila	South
29	1893	1881–1900	1851–1900	Karkku	South
30	2013	2001–2020	2001–2050	Kauniainen	South
31	1981	1981–2000	1951–2000	Kiiminki	North
32	2009	2001–2020	2001–2050	Kimitoön	South
33	1983	1981–2000	1951–2000	Kirkkonummi	South
34	1983	1981–2000	1951–2000	Kirkkonummi	South
35	1995	1981–2000	1951–2000	Kirkkonummi	South
36	2008	2001–2020	2001–2050	Koivisto	South
37	1877	1861–1880	1851–1900	Kolari	North
38	1980	1961–1980	1951–2000	Kolari	North
39	1921	1921–1940	1901–1950	Korsnäs	South
40	1930	1921–1940	1901–1950	Korsnäs	South

Sample Number	Collection Year	20-year Group	50-year Group	Locations	Region
41	1900	1881–1900	1851–1900	Kuopio	South
42	1973	1961–1980	1951–2000	Kuusamo	North
43	1910	1901–1920	1901–1950	Kyminlinna	South
44	1916	1901–1920	1901–1950	Lemland	South
45	1933	1921–1940	1901–1950	Lemland	South
46	1999	1981–2000	1951–2000	Lieksa	South
47	1996	1981–2000	1951–2000	Lieksa	South
48	1889	1881–1900	1851–1900	Lohja	South
49	1886	1881–1900	1851–1900	Lohja	South
50	1902	1901–1920	1851–1900	Lohja	South
51	1995	1981–2000	1951–2000	Muonio	North
52	2023	2021–2040	2001–2050	Myrskylä	North
53	2023	2021–2040	2001–2050	Myrskylä	North
54	2023	2021–2040	2001–2050	Myrskylä	North
55	2023	2021–2040	2001–2050	Myrskylä	North
56	2023	2021–2040	2001–2050	Myrskylä	North
57	1913	1901–1920	1901–1950	Orimattila	South
58	1980	1961–1980	1951–2000	Orivesi	South
59	1925	1921–1940	1901–1950	Parkano	South
60	1967	1961–1980	1951–2000	Piikkiö	South
61	1892	1881–1900	1851–1900	Pojo	South
62	1885	1881–1900	1851–1900	Porvoo	South
63	1861	1861–1880	1851–1900	Porvoo	South
64	1948	1941–1960	1901–1950	Pyhäjärvi	South
65	1996	1981–2000	1951–2000	Rovaniemi	North
66	1996	1981–2000	1951–2000	Rovaniemi	North
67	1996	1981–2000	1951–2000	Rovaniemi	North
68	1948	1941–1960	1901–1950	Ruotsinpyhtää	South
69	1898	1881–1900	1851–1900	Saarijärvi	South
70	1927	1921–1940	1901–1950	Saltvik	South
71	1979	1961–1980	1951–2000	Solf	North
72	1935	1921–1940	1901–1950	Sotkamo	South
73	1968	1961–1980	1951–2000	Turku	North
74	1969	1961–1980	1951–2000	Turku	North
75	1948	1941–1960	1901–1950	Tyrväänkylä	South
76	1948	1941–1960	1901–1950	Tyrväänkylä	South
77	1883	1881–1900	1851–1900	Vaasa	South
78	1901	1901–1920	1851–1900	Vaasa	South
79	1979	1961–1980	1951–2000	Vantaa	South
80	1999	1981–2000	1951–2000	Vantaa	South
81	1899	1881–1900	1851–1900	Veckelax	South

Sample Number	Collection Year	20-year Group	50-year Group	Locations	Region
82	1964	1961–1980	1951–2000	Vehkalahti	South
83	1877	1861–1880	1851–1900	Ylitornio	North

Sequence processing and analysis

Paired end reads were assigned to each sample and removed of barcodes and primers. The software FLASH (V1.2.11, http://ccb.jhu.edu/software/FLASH) was used to generate raw tags by merging paired end reads (Magoč & Salzberg, 2011). Clean tags of high quality were obtained after the quality filtering process in the software fastp (Version 0.23.1) (Chen, 2023). Chimeric sequences were identified and eliminated using the VSEARCH package (V2.29.3, https://github.com/torognes/vsearch) based on the reference database (UNITE database v9.0, https://unite.ut.ee) which targets the nuclear ribosomal internal transcribed spacer (ITS) region (Rognes et al., 2016; Abarenkov et al., 2024). Denoising was performed with DADA2 in the QIIME2 software (version 2024.10) to generate the initial ASVs (amplicon sequence variants), followed by the annotation to species based on UNITE database (v9.0, Bolyen et al., 2019). The absolute abundance of ASVs was normalized based on the smallest sequencing numbers which is 45, 251 among all the samples for the subsequent alpha diversity analysis, including community evenness (Pielou-e), dominance and diversity (Simpson's diversity). FUNGuild (Fungi + Functional + Guilds) tool was used to classify fungal ASVs into ecological modes and guilds to provide functional annotation (Nguyen et al., 2016). The main modes contain Pathotroph which takes advantages of the host cells and causes disease, Saprotroph which lives on breaking down dead host cells and Symbiotroph which obtains nutrients by exchanging resources with host cells. Other combined ecological modes refer to the transferable roles of the assigned fungal ASVs from one to another when facing different conditions.

One-way analysis of variance (ANOVA) was adopted to examine the significant differences of the relative abundance of fungal taxa, alpha diversity indices, functional modes among different year groups (50-year groups and 20-year groups) in software SPSS (v26.0, Chicago, USA). Normality tests indicated that the alpha diversity indices were not normally distributed between the southern and northern regions; therefore, non-parametric tests (Mann-Whitney U test) were applied in SPSS to assess regional differences. groups and following non-parametric tests were conducted in SPSS to detect the difference GraphPad Prism (version 9.5.0) was used to generate the figures regarding the results of fungal taxonomy, predicted functional structure and community alpha diversity.

To validate the authenticity of the sequencing results, the top 50 fungal species from previous research targeted at the endophytes in Norway spruce needles was used as a template to identify the same species in top 50 species in each 50-year groups (Meng et al., 2025). The principal coordinate analysis (PCoA) and subsequent permutational multivariate analysis of variance (PERMANOVA) were performed on the relative abundance of ASVs or the predicted functional modes on R software (version 4.4.1) using vegan package and then visualized with ggplot2 package (Wickham, 2016; Oksanen et al., 2025). To explore the response of fungal community to environmental factors, the meteorological data of 58 samples out of 83 samples including the annual average temperature and annual precipitation from those samples' collection years and locations was obtained on the website of Finnish Meteorological Institute (https://en.ilmatieteenlaitos.fi/statistics-from-1961-onwards/) and regarded as the environmental factors. The impact of environmental factors on fungal community structure was then visualized on PRIMER and exported with the PERMANOVA results based on distance-based redundancy analysis (dbRDA).

Results

Taxonomic composition and structure of fungal community

In total, 8607 ASVs were detected and preserved as fungal ASVs from 83 samples, of which 2105 ASVs (24.5%) and 1717 ASVs (20.0%) were assigned to phylum level and genus level respectively. At the phylum level, the most dominant group Ascomycota took up a relative abundance of 11.7%, followed by Basidiomycota (2.3%) (Fig. 2a). At the genus level, *Lophodermium* was the most abundant genus (1.2%), followed by *Ceratocystis* (1.2%), *Yarrowia* (1.1%), *Saccharomyces* (1.1%) and *Heterobasidion* (0.9%) (Fig. 2b). In 1851–1900, *Ceratocystis* and *Saccharomyces* were the dominant genera in the south and north respectively, which took up to 5.0% and 27.8% (Fig. 2b). In 1901–1950, *Heterobasidion* (4.5%) and *Yarrowia* (3.9%) dominated in the southern group. In 1951–2000, *Heterobasidion* (0.5%) and *Naganishia* (3.3%) were the most abundant genus in the south and north respectively (Fig. 2b). *Pragmopora* and *Lophodermium* were the dominant genus in the southern fungal community and the northern fungal community from year 2001–2023, which took up to 1.1% and 11.3% respectively (Fig. 2b). Among the top 15 genera, some of them contained one dominant species or the only species, such as *Lophodermium piceae* (99.9% of *Lophodermium*), *Heterobasidion parviporum* (100.0% of *Heterobasidion*), *Ceratocystis ethacetica* (100.0% of *Ceratocystis*), *Yarrowia lipolytica* (99.9% of *Yarrowia*) and *Saccharomyces bayanus* (100.0% of *Saccharomyces*) (Table S1).

At species level, there were 18 constant species which showed up in all 50-year groups (Fig. 3). Among these species, the relative abundance of *Lophodermium piceae* increased in 2001–2023 significantly ($P < 0.05$), while *Aspergillus flavus* decreased significantly in 1951–2000 ($P < 0.05$). The relative abundance of *Rhizopus arrhizus* and *Kazachstania slooffiae* were significantly higher in 1851–1900 than that in 1951–2000 and 2001–2023($P < 0.05$). *Issatchenkia orientalis* was more abundant in 1851–1900 compared to that in 2001–2023($P < 0.05$).

As compared to the template top 50 species list from one previous research, there were 5, 8, 7 and 21 fungal species observed from the top 50 species list in 1851–1900, 1901–1950, 1951–2000 and 2001–2023 which were also found in the template (Table 2).

Table 2
The list of top 50 fungal species in each 50-year groups. The same species with the template were marked with bold font.

1851–1900(5)	1901–1950(8)	1951–2000(7)	2001–2023(21)	Template
Ceratocystis ethacetica	**Heterobasidion parviporum**	*Naganishia diffluens*	**Lophodermium piceae**	*Hanseniaspora uvarum*
Saccharomyces bayanus	*Yarrowia lipolytica*	*Simplicillium lamellicola*	**Lachnum virgineum**	*Camptophora hylomeconis*
Yarrowia lipolytica	**Hypogymnia tubulosa**	**Heterobasidion parviporum**	**Mollisia scopiformis**	*Pucciniastrum areolatum*
Simplicillium lamellicola	**Aureobasidium pullulans**	*Chrysomyxa ledi*	**Tryblidiopsis magnesii**	*Cyphellophora sessilis*
Hyaloscypha gryndleri	*Cladosporium tenuissimum*	*Humicola fuscoatra*	*Lachnum pygmaeum*	*Ceramothyrium carniolicum*
Malassezia restricta	*Hyphopichia burtonii*	**Lophodermium piceae**	*Lachnellula abietis*	*Hypogymnia physodes*
Malassezia arunalokei	*Malassezia restricta*	*Glaciozyma martinii*	*Ceratocystis ethacetica*	*Neohortaea acidophila*
Glaciozyma martinii	*Phaeotheca fissurella*	*Dekkera bruxellensis*	**Tristratiperidium microsporum**	*Hormonema macrosporum*
Aureobasidium pullulans	*Ceratocystis ethacetica*	*Haploporus odorus*	*Brunnipila fuscescens*	*Geotrichum silvicola*
Lophodermium piceae	*Preussia persica*	*Yarrowia lipolytica*	*Mollisia cinerea*	*Scoliciosporum umbrinum*
Perenniporia narymica	*Heydenia alpina*	*Hyphopichia burtonii*	*Micraspis acicola*	*Perusta inaequalis*
Rhizopus arrhizus	*Hannaella zeae*	*Chaetomium longiciliatum*	*Simplicillium lamellicola*	*Capnobotryella renispora*
Kazachstania slooffiae	*Simplicillium lamellicola*	*Meira nashicola*	**Heterobasidion parviporum**	*Epibryon interlamellare*
Issatchenkia orientalis	*Fusarium oxysporum*	*Filobasidium magnum*	*Penicillium toxicarium*	*Lophodermium piceae*
Peniophora taiwanensis	*Coniochaeta decumbens*	**Hormonema macrosporum**	*Aspergillus welwitschiae*	*Fellomyces horovitziae*
Candida parapsilosis	*Wickerhamomyces anomalus*	*Diatrype stigma*	*Mollisia albogrisea*	*Exobasidium arescens*
Oidiodendron pilicola	*Rhizopus arrhizus*	*Fusarium graminearum*	**Epibryon interlamellare**	*Candida tropicalis*
Trichomerium lapideum	*Mucor moelleri*	*Ceratocystis ethacetica*	*Penicillium thomii*	*Tristratiperidium microsporum*
Oidiodendron periconioides	*Trichoderma pubescens*	*Vishniacozyma victoriae*	*Lachnum brevipilosum*	*Fuscidea pusilla*
Solicoccozyma aeria	*Hannaella luteola*	*Volutella ciliata*	*Candida parapsilosis*	*Dwayaangam colodena*
Candida albicans	*Peziza perdicina*	*Saccharomycopsis fibuligera*	*Pyrenopeziza revincta*	*Acrodontium crateriforme*
Aspergillus welwitschiae	*Lapidomyces aloidendricola*	*Pseudeurotium hygrophilum*	**Kodamaea ohmeri**	*Peltaster gemmifer*
Candida zeylanoides	*Preussia terricola*	*Rhizopus arrhizus*	*Cladophialophora humicola*	*Mollisia scopiformis*
Hyaloscypha finlandica	*Trichoderma harzianum*	*Wickerhamomyces anomalus*	**Cyphellophora sessilis**	*Scleromitrula calthicola*
Malassezia globosa	*Dioszegia hungarica*	*Amylocystis lapponica*	**Scoliciosporum umbrinum**	*Leimonis erratica*
Fluminicola thailandensis	*Issatchenkia orientalis*	*Mucor moelleri*	**Hypogymnia physodes**	*Taphrina carpini*
Starmera stellimalicola	**Perusta inaequalis**	*Peziza ostracoderma*	*Lophium arboricola*	*Neocatenulostroma microsporum*
Candida tropicalis	*Phialophora cyclaminis*	*Malassezia restricta*	*Neonectria tsugae*	*Lachnum virgineum*
Heterobasidion parviporum	*Neoascochyta paspali*	*Issatchenkia orientalis*	**Lophium mytilinum**	*Peltaster fructicola*
Nakazawaea populi	*Oidiodendron chlamydosporicum*	*Kazachstania slooffiae*	**Capturomyces funiculosus**	*Heterobasidion parviporum*
Nakazawaea wyomingensis	*Desmazierella acicola*	*Candida albicans*	**Acrodontium crateriforme**	*Exobasidium maculosum*
Chaetomium longiciliatum	*Pseudocosmospora eutypae*	*Neophaeotheca triangularis*	*Platismatia glauca*	*Sirococcus conigenus*

1851–1900(5)	1901–1950(8)	1951–2000(7)	2001–2023(21)	Template
Mortierella basiparvispora	*Cortinarius epipurrus*	**Perusta inaequalis**	*Phialocephala glacialis*	*Zasmidium fructigenum*
Trichocladium pyriforme	*Kazachstania slooffiae*	*Aspergillus welwitschiae*	*Dioszegia fristingensis*	*Capturomyces funiculosus*
Angustimassarina acerina	*Fluminicola thailandensis*	*Skeletocutis diluta*	**Neocatenulostroma microsporum**	*Hypogymnia tubulosa*
Humicola fuscoatra	*Dactylonectria macrodidyma*	*Fluminicola thailandensis*	*Orbilia sphaerospora*	*Aureobasidium pullulans*
Cephalotrichum stemonitis	*Candida parapsilosis*	*Cladosporium austrohemisphaericum*	*Pseudographis pinicola*	*Pseudopezicula betulae*
Rhodoveronaea everniae	*Alternaria photistica*	*Venturia inaequalis*	**Absconditella rubra**	*Sirotrema translucens*
Aspergillus penicillioides	*Aspergillus flavus*	**Candida tropicalis**	*Lachnum bicolor*	*Lophium mytilinum*
Umbelopsis dimorpha	*Pseudothielavia arxii*	*Candida parapsilosis*	*Phialocephala amethystea*	*Penicillium bialowiezense*
Aspergillus ruber	*Aspergillus penicillioides*	**Hanseniaspora uvarum**	*Septobasidium carestianum*	*Pachyramichloridium pini*
Monascus purpureus	**Lophodermium piceae**	*Phenoliferia psychrophila*	*Prosthemium intermedium*	*Fellhanera bouteillei*
Aspergillus flavus	*Clavaria atrofusca*	*Tortispora caseinolytica*	*Phaeosclera dematioides*	*Tryblidiopsis magnesii*
Cladosporium austrohemisphaericum	**Hanseniaspora uvarum**	*Clavispora lusitaniae*	*Cyclaneusma minus*	*Kodamaea ohmeri*
Taphrina caerulescens	**Geotrichum silvicola**	**Aureobasidium pullulans**	*Coryneum lanciforme*	*Exobasidium woronichinii*
Symmetrospora gracilis	*Diatrype stigma*	*Lophiostoma cynaroidis*	**Fellomyces horovitziae**	*Absconditella rubra*
Aspergillus fumigatus	*Ustilago hordei*	*Aspergillus ruber*	**Camptophora hylomeconis**	*Retiarius bovicornutus*
Papiliotrema flavescens	*Trichocladium pyriforme*	*Malassezia globosa*	**Dwayaangam colodena**	*Symmetrospora gracilis*
Clavispora lusitaniae	**Candida tropicalis**	*Malassezia sympodialis*	**Fellhanera bouteillei**	*Carlosrosaea foliicola*
Neoascochyta adenii	*Chaetomium interruptum*	*Montagnea arenaria*	**Pucciniastrum areolatum**	*Venturia peltigericola*

The principal coordinate analysis (PCoA) showed that PC1 axis and PC2 axis explained the total variance by 53.5% and 10.5% respectively. The subsequent permutational multivariate analysis of variance (PERMANOVA) confirmed that the southern group and northern group formed fungal communities with different structures ($P < 0.05$) (Fig. 4a & b). At 50-year intervals, the fungal community structure of group 1951–2000 was significantly different from those of 1901–1950 and 1851–1900 ($P < 0.05$) (Fig. 4a). At 20-year ranges, significant differences in fungal community structures were found between group 1941–1960 and group 1981–2000, and between group 1921–1940 and group 2021–2023 ($P < 0.05$) (Fig. 4b).

Predicted functional structure of fungal community

Among the eight functional modes, Saprotroph (7.0%) ranks the top, followed by Pathotroph (2.1%) and Pathotroph-Saprotroph (1.5%) (Fig. 5). In the southern and northern group, Pathotroph occupied 0.6% and 5.1% respectively, while Symbiotroph took up 0.6% and 0.1% respectively (Fig. 5).

The increase of Saprotroph, Symbiotroph and Pathotroph-Saprotroph-Symbiotroph was observed in 1901–1950, followed by the decrease of Saprotroph and Pathotroph-Saprotroph-Symbiotroph in 1951–2000 ($P < 0.05$) (Fig. 6a, d & f). Saprotroph-Symbiotroph and Pathotroph-Symbiotroph increased in 2001–2023($P < 0.05$) (Fig. 6e & g). The relative abundance of Pathotroph-Saprotroph peaked in 1851–1900 and was higher than that in 1951–2000 and 2001–2023 ($P < 0.05$) (Fig. 6b).

The PC1 axis and PC2 axis explained 36.3% and 23.1% of total variance respectively (Fig. 7a & b). As to the fungal community functional structure, no significant difference was found between the southern group and the northern group or in 50-year groups (Fig. 7a & b). In 20-year groups, significant difference in the fungal community functional structure was found between 1961–1980 and 2021–2023 ($P < 0.05$) (Fig. 7b).

Impact of environmental factors on fungal community

The dbRDA1 axis and dbRDA2 axis explained 4.4% and 1.3% of total variation respectively (Fig. 8). Based on the PERMANOVA results of dbRDA, temperature ($P = 0.18$) and precipitation ($P = 0.06$) had no significant influence on the fungal community, while the interplay of region and year groups shifted the fungal community significantly ($P < 0.05$) (Fig. 8).

Fungal community alpha diversity

No significant change in fungal alpha diversity indices was observed in 50-year groups (Figure S1). In 20-year groups, lower dominance and higher Simpson's diversity was observed in 2021–2023 than that in 1901–1920 ($P < 0.05$) (Fig. 9). Significant differences in all three alpha diversity indices were observed between the southern and northern groups, with the northern group characterized by higher diversity, and evenness, and lower dominance in its fungal community ($P < 0.05$) (Table 3).

Table 3
The results of non-parametric tests showing the difference of alpha diversity indices between the southern group and the northern group. S.D. represents the standard deviation.

Indices	South	North	Non-parametric tests
	Mean (S.D.)	Mean (S.D.)	Two-tailed Significance
Dominance	0.61(0.28)	0.38(0.26)	**0.002**
Pielou's evenness	0.26(0.19)	0.37(0.16)	**0.006**
Simpson's Index of Diversity	0.40(0.28)	0.62(0.26)	**0.002**

Discussion

Despite a few examples in using herbarium collections in microbial study, the combination research which takes advantage of the total DNA obtained from specimens to learn the shift of microbial community is not largely done (Daru et al., 2018; Heberling & Burke, 2019; Bieker et al., 2020; Grasso et al., 2025). Our research, which investigates how endophytic fungal communities colonizing Norway spruce (*Picea abies*) needle specimens respond to temporal, spatial, and climatic factors, represents the first piece of the puzzle in the largely unexplored field of ancient tree microbiomes.

However, in this study, the proportion of sequences assigned to phylum level was around 24.45% using DADA2 process combined with UNITE annotation, which was much lower than the classified percentage of existing leaf-associated fungal community cases ranged from 90% to 100% (Coleman-Derr et al., 2016; Yao et al., 2019; Meng et al., 2025). In our results, the failure of large proportion of fungal ASVs to be annotated with any taxonomic information is probably due to the largely vacant incorporation of ITS2-derived high throughput sequences into UNITE database (Nilsson et al., 2019). The unassigned fungal ASVs were therefore referred to as "dark taxa" as they lack a taxonomic identity in the form of a name (Ryberg & Nilsson, 2018). It is rational that we found certain amounts of identical species in each 50-year intervals with the template top species, which supports the authenticity of the sequencing results. Despite the unusual annotation results, Ascomycota dominated the endophytic fungi in the overall herbarium needle samples, which is in accordance with the previous results which used culture-based isolation or culture-independent method to examine the foliar endophytic fungi in several fresh plant leaf materials (Guo et al., 2000; Arnold et al., 2007; Osono, 2007; Zambell & White, 2015; Bálint et al., 2015; U'Ren & Arnold, 2016; Coleman-Derr et al., 2016). Another research which investigated the leaf specimens of *Andromeda polifolia* and *Ledum palustre* also claimed the dominance of Ascomycota among endophytic fungi (Daru et al., 2018). The high proportion of Ascomycota in herbarium samples was consistent with majority of research focused on leaf endophyte, regardless of whether it is fresh leaves or herbarium leaves.

At the genus level, three genera *Lophodermium*, *Ceratocystis* and *Heterobasidion*, which contains important plant pathogens on conifer trees, were found to be among the most abundant genera (Ortiz-García et al., 2003; Asiegbu et al., 2005; de Beer et al., 2014). The top species *L. piceae* was frequently detected in Norway spruce needles, typically in over 90% of the recovered endophytic isolates throughout the entire distribution range (Sieber, 1988; Müller & Hallaksela, 1998; Müller et al., 2001, 2019). However, the ecological role of *L. piceae* in Norway spruce needles remains not fully understood, as it was not inferred as an important pioneer needle decomposer nor as an antagonistic endophyte which could assist the host against insect herbivores (Müller et al., 2001; Müller & Hamberg, 2021; Müller & Hamberg, 2023). It is most probable that increase in *L. piceae* abundance in the most recent group might have resulted from differences in the sampled needle ages, because the infection rate of *L. piceae* tend to enlarge as needle becomes older (Barklund, 1987; Sieber, 1988; Müller & Hamberg, 2021). The only species *C. ethacetica* in genus *Ceratocystis* was reported to cause rot disease in various economy plants including sugarcane, palm and pepper (Borges et al., 2019; Vo et al., 2022; Rocha et al., 2023). However, no evidence showed that the soilborne *C. ethacetica* could infect the conifer needles, it may live in needles as asymptomatic endophytes as fungi could switch their ecological roles under different prevailing conditions (Carroll, 1988; Rai & Agarkar, 2016; Ayika et al., 2024). *H. parviporum* belongs to *H. annosum* species complex and preferably infects Norway spruce by colonizing its heartwood (Garbelotto & Gonthier, 2013). The pathogen establishes primarily on the surface of tree stump or open wounds by airborne spores, then spreads to nearby tress by mycelium growth through root contacts (Garbelotto & Gonthier, 2013). The occurrence of *H. parviporum* in the needles from Norway spruce trees has previously been reported (Kovalchuk et al., 2018; Meng et al., 2025), however the frequency (0.01% and 0.07%) remained much lower. It is reasonable to presume that some of the sampled Norway spruce trees have been exposed to the root and stem rot disease by the time of collection. *Yarrowia lipolytica* was the dominant species within the top genus *Yarrowia*, which is a yeast fungi capable of degrading proteins and lipids (Nicaud, 2012). The natural habitats of this species usually include oil-polluted environments, marine and hypersaline environments (Bankar et al., 2009; Thevenieau et al., 2009; Beopoulos et al., 2010). *Yarrowia lipolytica* was also isolated from salt-excreting leaves of *Atriplex halimus* plants and the spines of *Euphorbia milli* L., and was found to improve the growth of maize with or without salt stress by facilitating its metabolism and hormone secretions (Zvyagilskaya et al., 2001; Gul Jan et al., 2019). It could therefore be inferred that this species may reside inside the needles as beneficial endophytic microbes. Another top genus *Saccharomyces* consists of yeast fungi *Saccharomyces bayanus*. Except for the existence of *S. bayanus* in fermentation process, one variety of it was found in the sap, exudate and bark of broadleaved trees (Borovkova et al., 2022), but no leaf residence has been reported. The presence of *S. bayanus* in herbarium needles was probably due to the contaminant during collection and preserving process.

We expected the dynamics of 18 constant species could indicate the basic shifting pattern of different functioning across the collecting year groups. *Aspergillus flavus* had complex ecological roles as soil saprotrophic fungus that infects seed crops, commonly parasitic species or beneficial endophyte (Raymond et al., 2000, Klich, 2007; Amaike & Keller, 2011, Ismail et al., 2019). It remains unclear what kind of ecological role *A. flavus* play in Norway spruce needles, which needs further work to clarify. *Rhizopus arrhizus*, *Kazachstania slooffiae* and *Issatchenkia orientalis* were all higher in 1851–1900 than that in 2001–2023. *R. arrhizus* is a soil-associated saprotrophic fungal species complex which could also be found in dung or decaying vegetation, and was reported to be leaf rot pathogens to *Lonicera japonica* in China (Ghosh & Rani Ray, 2011; Dai et al., 2023). The high incidence of *R. arrhizus* in the earliest collection years likely reflects either a period of intensified infection or post-collection contamination within herbarium specimens. It is strange to detect the existence of *K. slooffiae* in plant tissue, as it is usually regarded as a commensal in the swine gut microbiome which belongs to the Saccharomycetaceae family (Feehan et al., 2022). *Issatchenkia orientalis* is an ecnomic yeast applicable in the production of ethanol, organic acid by metabolic engineering, and could be isolated from fruit wines,fermented food and rotting bagasse (Kwon et al., 2011; Xiao et al., 2014; Miao et al., 2018). It is possible that *R. arrhizus, K. slooffiae* and *I. orientalis* presence into the needles all happened after the herbaria storage, and the latest collected samples had the least amount of them due to the least accumulation time of contamination species. Another consistently occurring yeast-like fungus, *Aureobasidium pullulans*, is a well-adapted saprotroph commonly inhabiting both the phyllosphere and the carposphere, and it functions as an effective biocontrol agent against post-harvest diseases (Mounir et al., 2007). In view of the massive colonization by yeasts as foliar epiphytic fungi, it is also possible that the constant presence of yeast fungi including *Candida tropicalis*, *Kluyveromyces marxianus*, *I. orientalis* and *K. slooffiae* in our results may indicate the imcomplete removal of phyllosphere microbes. Except for these, the constant existance of *Malassezia globosa* and *Candida parapsilosis*, which are relevant to human skin and gastrointestinal tract, indicated that the contamination was rather frequent in herbarium collections (Dawson, 2007; Trofa et al., 2008). It is possible to introduce skin-associated microbes during in specimen process in herbaria (Warinner et al., 2017). As regard to the identical species identified with the template list in 2023, the most amount was present in 2001–2023, which is reasonable because they shared the same collection year.

The southern group and northern group had different microbial communities and varied alpha diversities, which agreed with our hypothesis. Geographic locations have been regarded as the influencing factor to the diversity and composition of endophytic fungal community (Hoffman & Arnold, 2008; U'Ren et al., 2012). The geographic heterogeneity of fungal communities has been attributed to factors such as dispersal limitation, constraints on host range, local adaptive evolution, and environmental filtering (Lumbsch et al., 2008; Peay et al., 2010). Earlier findings concluded that foliar endophytic fungal diversity associated with host assemblages or individual hosts was greater at lower latitudes, however, our results showed that the northern group harboured higher community diversity (Arnold & Lutzoni, 2007). This contrary finding may result from the challenging growing condition of Norway spruce trees in southern Finland facing various turbulence risks including drought stress and disease infection under the context of global warming (Venäläinen et al., 2020). In the southern boreal zone, Norway spruce forests face an elevated likelihood and severity of wind damage, as well as heightened susceptibility to bark beetle outbreaks and *Heterobasidion* spp. diseases, both of which were more prevalent in southern than in northern Finland (Ikonen et al., 2017; Müller et al., 2018; Blomqvist et al., 2018). As a result, poorer health status of host plants is likely to alter the community composition and decrease the diversity indices of plant-associated fungi (Li et al., 2024; Meng et al., 2025). The variation in endophytic fungal community structure and diversities between the southern and northern specimens reflect both the complicated influence of geographic location and the underlying divergent growing conditions exhibited by these two breeding regions under the progress of global warming.

At the functional level of fungal community, there was no difference between the southern group and northern group. The guild concept applicable in functional classification focused on a variety of species that utilize the same class of environmental resources similarly regardless of their taxonomic relationship (Root, 1967). It offers an alternative perspective on community composition by emphasizing trophic strategies rather than species richness or taxonomic identity (Nguyen et al., 2016). Despite the fungal community differed in structures between the southern and northern group, the consistent composition of tropic modes was not disrupted by the geographic locations or the divergent climate stress.

At the time scale, the difference of fungal community structure of group 1951–2000 from that of 1851–1900 and 1901–1950 could be associated with the magnificent historical reform in forest management during that period. Historical records indicate that, since the 1950s, the implementation of intensified forest management in Finland has substantially altered the original forest landscape, including its species composition, age structure, and patch size distribution (Saarinen et al., 2001, Kalliola et al., 2003).In addition, the forest management in Finland experienced significant change from the late part of the 20th century, during when selective thinning from below substituted clear cutting and became the prevalent logging strategy both in practice and legislation (Tasanen, 2004; Myking et al., 2016). Forest management influences structural attributes at both stand and landscape scales, including tree species composition, age-class distribution, and the availability of dead wood (Kouki et al., 2001). These structural modifications have, in turn, been associated with shifts in the abundance of numerous animal, plant, and fungal species (Rassi et al., 2001). The lowest dominance and highest diversity of fungal community in the 2021–2023 may result from the unique features of these samples, as they were collected in June 2023, and then stored at -80 degree until DNA isolation in April 2024 instead of being stored at the herbaria. The herbaria storage condition may introduce environmental bacteria involved in decomposition or storage contamination (Warinner et al., 2017; Bieker et al., 2020), which further disturb the fungal DNA integrity and decrease the overall diversity of observed community.

Saprotrophs, Pathotrophs, and Saprotroph-Pathotrophs were the dominant trophic modes, consistent with the acknowledgement that endophytes inhabit plant tissues asymptomatically, ranging from mutualistic to antagonistic interactions or exhibit potential pathogenicity (Schulz & Boyle, 2005; Giauque & Hawkes, 2016). The constrained living space of endophytes within the host plant tissue leads to the strong dependence of their nutrient resource on colonized substrate (Rodriguez et al., 2009; Giauque & Hawkes, 2016). Conversely, the internal environment of plants exerts strong selective pressures on the assembly of endophytic microbiota and retains its filtering capacity even during senescence (Xiao et al., 2025). Hence, the dynamics of different trophic modes of fungal endophytes could be explained by the shifting nutritional conditions of needles among different periods. For example,

the needle substrate collected in 1901–1950 was likely to contain more available cellulose and hemicellulose and turned out more favourable for saprotrophs compared to the past 50 years and the subsequent 50 years (U'Ren & Arnold, 2016).

We expected that there was significant influence of annual precipitation and annual average temperature on fungal community, but merely the precipitation was found nearly significant. However, temperature and precipitation were reported to associate strongly with endophyte community composition in many cases (Zimmerman & Vitousek, 2012; Giauque & Hawkes, 2013, 2016; Gomes et al., 2018; Huang, 2020). In woody plants, most fungal endophytes spread horizontally via spores or hyphal fragments from aging tissues, dispersed possibly by herbivores, wind, or rain (Rodriguez et al., 2009; Vaz et al., 2018). The climate variability not only alters fungal community composition directly by shaping fungal phenology, but also indirectly by influencing fungal interactions with other microbial species and hosts (Diez et al., 2013; Andrew et al., 2016). Precipitation emerged as a critical determinant of endophytic community structure, influencing the dispersal of fungal spores and regulating the colonization dynamics of endophytes (Vacher et al., 2016; Gomes et al., 2018). The moderate temperature could raise the viability of fungal propagules and favour the plant colonization (Collado et al., 1999). The absence of significant influence of climate factors on fungal communities may lie in the low resolution of the regional collected meteorological data with the specific collected sites. During our climate data collection process, we managed to find the climate recordings from the nearest meteorological station to the needles' locations which were mostly municipality names instead of exact geographical location (longitude and latitude). Owing to the data absence, we finally identified 58 samples with climate recordings out of 83 samples. The incompleteness and ambiguity of data from both the samples' locations and corresponding climate recordings was likely to cover the associated pattern between environmental factors with fungal endophyte communities. In addition, the interplay of regions (south and north) and year groups was again confirmed to be strongly correlated with the variation in fungal endophyte composition by redundancy analysis.

Our study investigated the needle specimens of Norway spruce and drew several points that would inspire more research into utilizing herbarium samples. Although it remained unclear the exact ecological roles of some constant species in the needles, they may adopt diverse roles in endophyte networks, for example *H. parviporum*. The role of spatial parameters in shaping endophytic fungal communities, both in terms of composition and alpha diversity, is well established. However, it remains unclear whether regional climate (temperature and rainfall) among geographic locations also exert an influence, and to what extent, as it was largely affected by geographic locations (Vajda & Venäläinen, 2003; Tietäväinen et al., 2010). Historically, the reform of Finnish forestry regulations back in 1951–2000 was inferred as a significant turning point to fungal assemblage owing to the widely induced change in forest structural attributes.

Although our study took an ambitious trial in microbial research using ancient herbarium specimens, we gained some insightful points, certain kinds of biases or difficulties were encountered. Firstly, we were unable to obtain high-quality DNA from more than 100 samples that were discarded, likely due to ancient DNA damage that fragmented the molecules, impaired normal replication, and introduced erroneous incorporations (Dabney et al., 2013). Despite the applied homogenous analysis, the inadequate sampled number would intensify the random feature of the needle collections and the individual variance within groups, which might justify or merit the need for further robust sampling. Secondly, large amount of ASVs were unable to be annotated to phylum level, probably due to the widespread existence of unknown fungal sequences and the variance of ASVs from the same species. The improvement of fungal database and annotation approach would be helpful to address this existing trouble. Thirdly, it seems highly probable that the herbaria storage would introduce some contamination species, for example some yeast species from the environment and skin-associated pathogens. Certain attention should be paid to remove the highly suspicious taxa from the datasets in the future research involved in herbarium samples.

Conclusion

Across over 150 years, the regional distribution had a great influence on fungal community composition associated with herbarium needles. Overall, higher diversity indices were observed within the northern group than the southern group. On the time scale, the period 1951–2000 witnessed a different community from the period 1851–1900 or from 1901–1950, largely due to the national reform in forest management. No direct influence of historical temperature or precipitation was found on the fungal community structure.

Declarations

Data and materials availability

The datasets generated and analysed during this study are available in the NCBI SRA database under number **PRJNA1285108**.

Author Contribution

F.A and R.K. formed the research idea. O. M. provided the access to the study materials. Z. W and A. L collected the samples and did a part of the laboratory work. W. M. did the majority of the laboratory work, analyzed the data, prepared the figures and wrote the manuscript. F. A., Z. W. and A. L. reviewed the manuscript.

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Figures

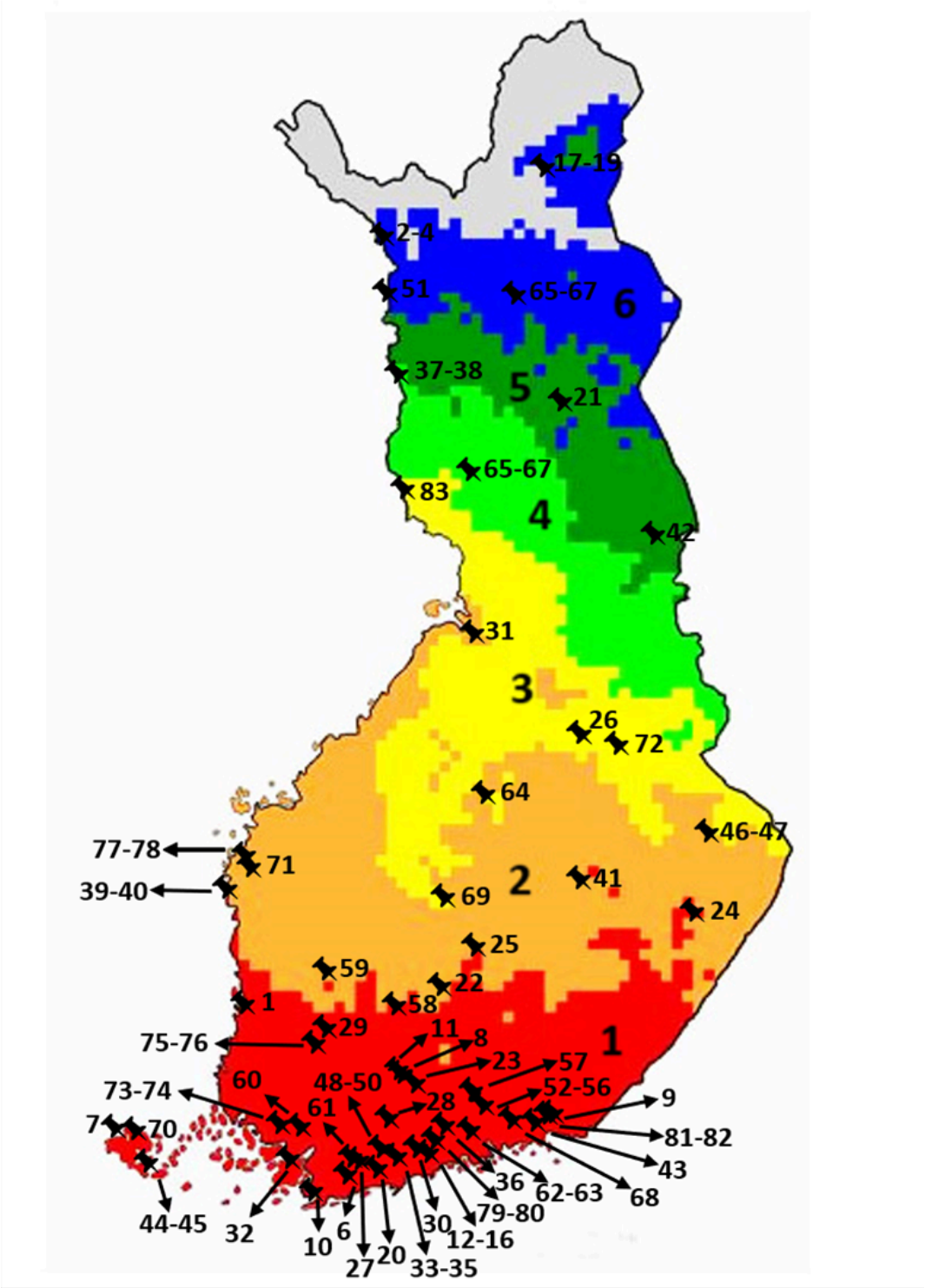

Figure 1

The location of each sample marked with serial number in the Finnish map. The different colours represent different tree breeding zones (1-6) in Finland.

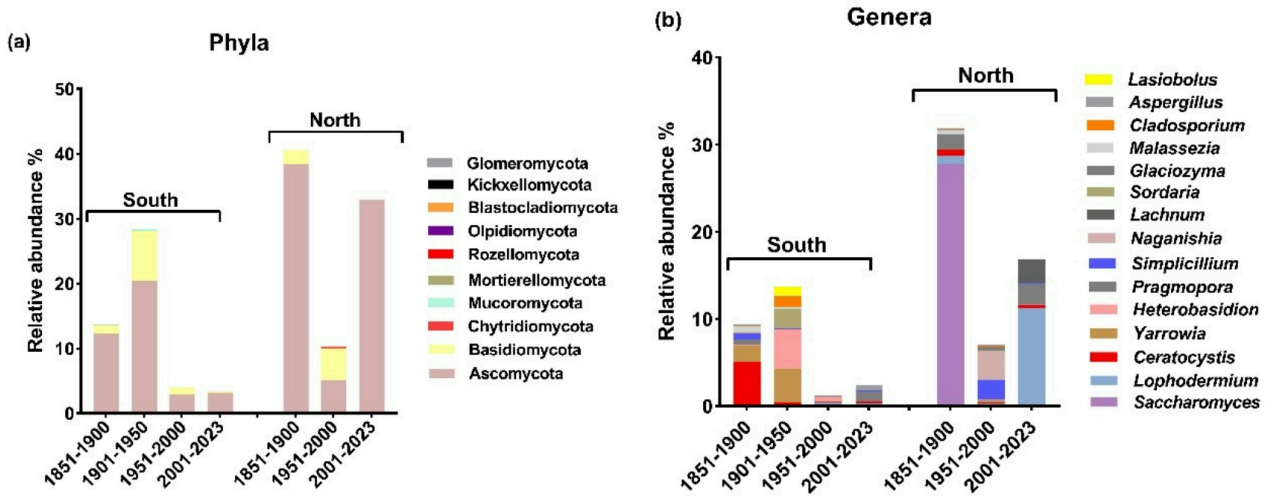

Figure 2

Fungal taxonomic composition at phylum level(a) and top 15 genera (b) from south and north in 50-year groups.

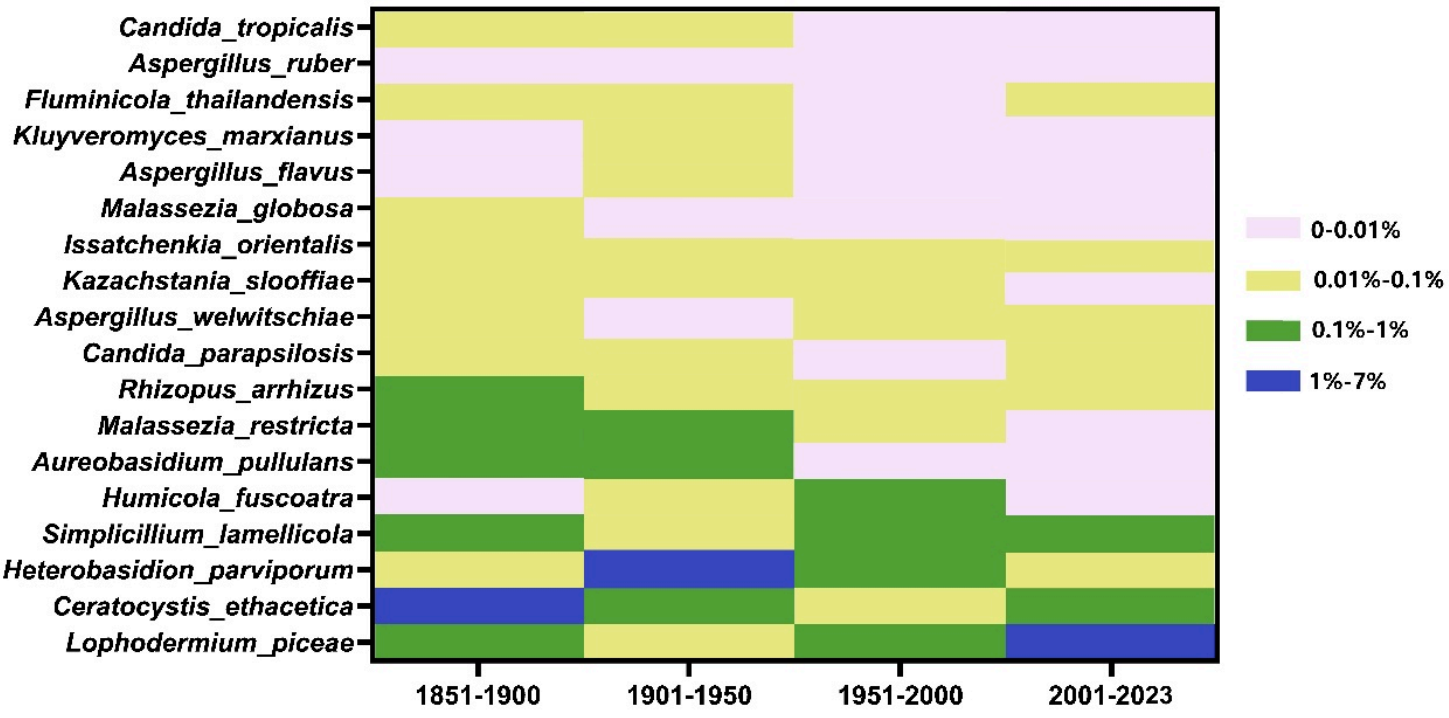

Figure 3

The relative abundance of constant species in 50-year groups. The species names from top to bottom are arranged from smallest to largest of relative abundance.

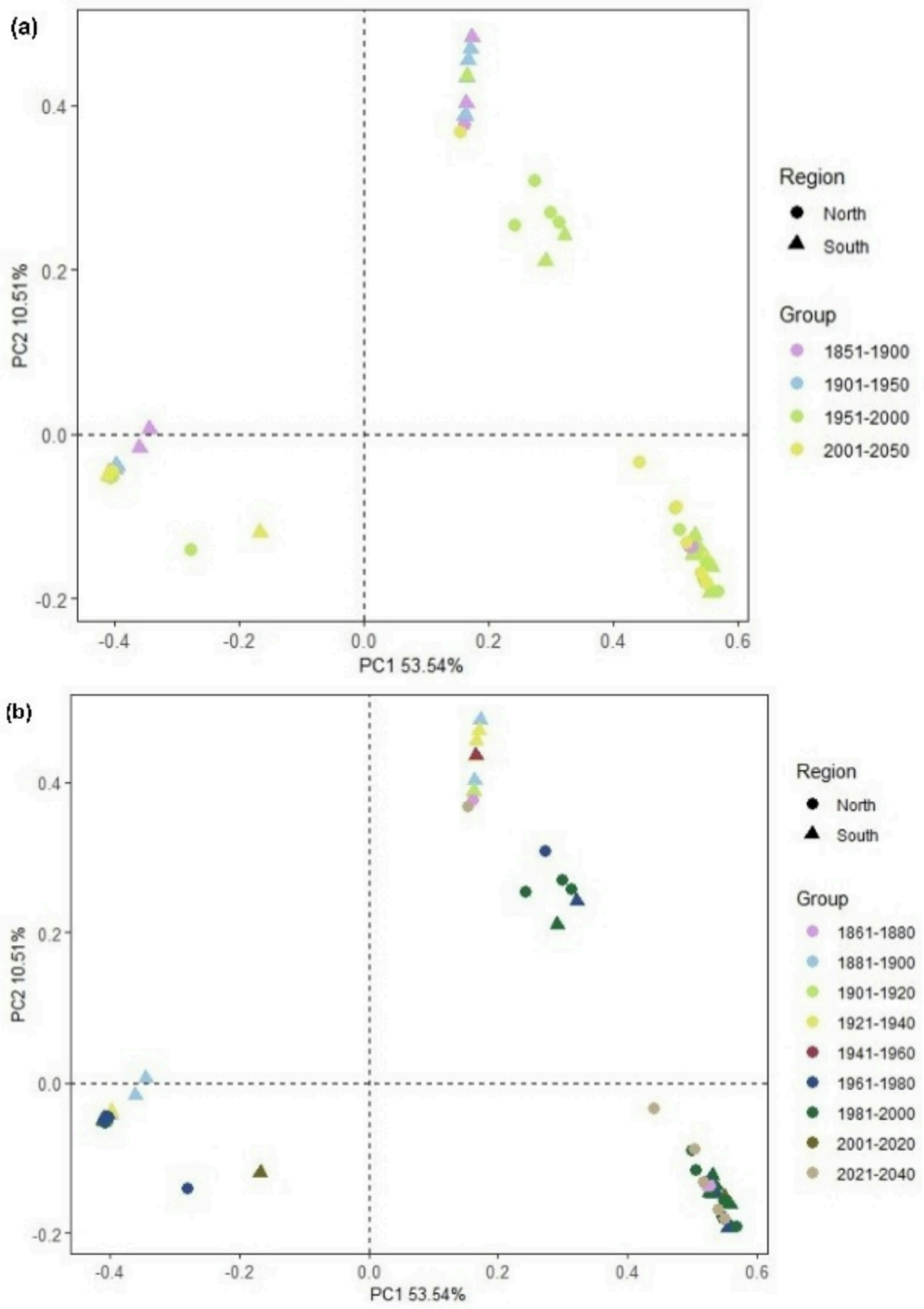

Figure 4

PCoA (principal co-ordinates analysis) illustrating the fungal community structures in 50-year groups (a) and 20-year groups(b).

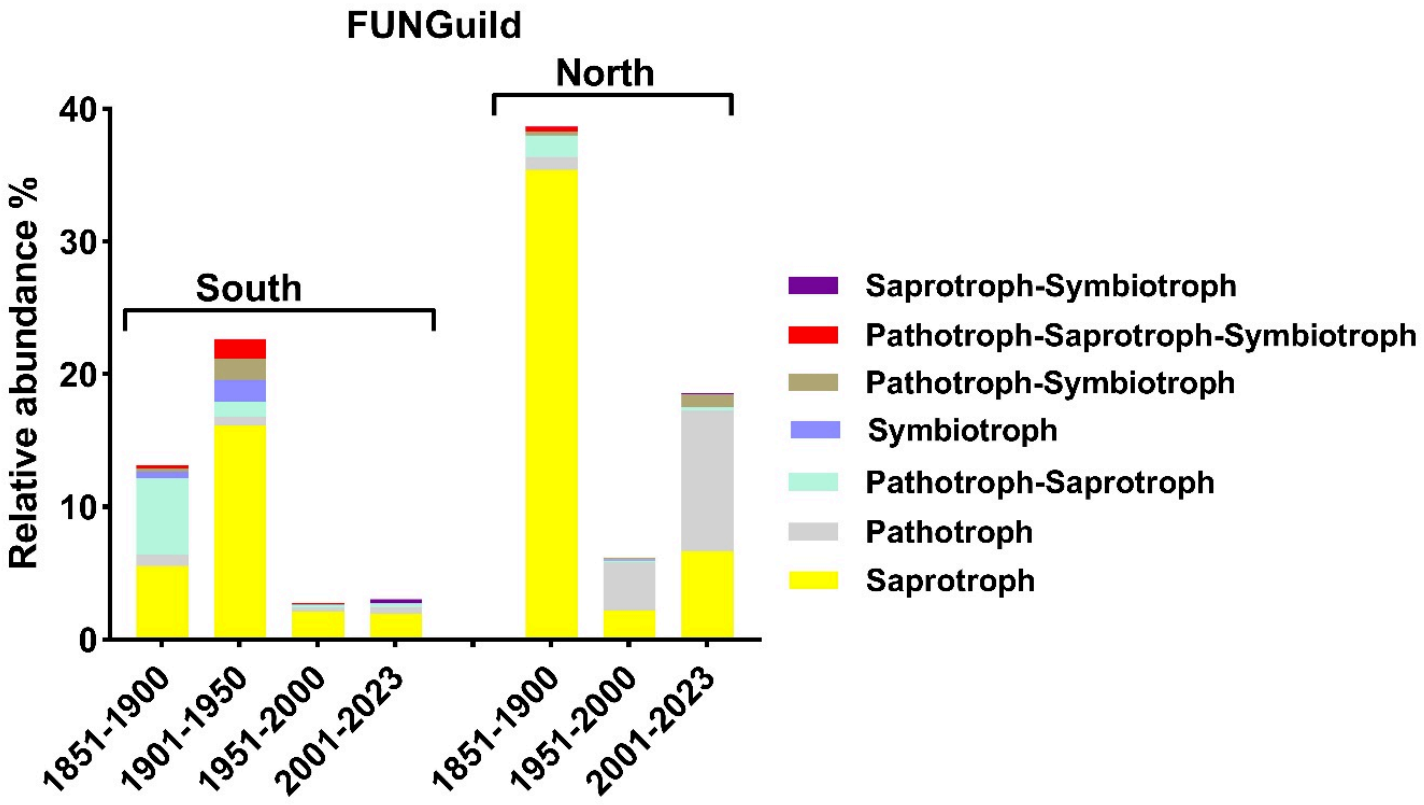

Figure 5

Fungal predicted functional composition at mode level from south and north in 50-year groups.

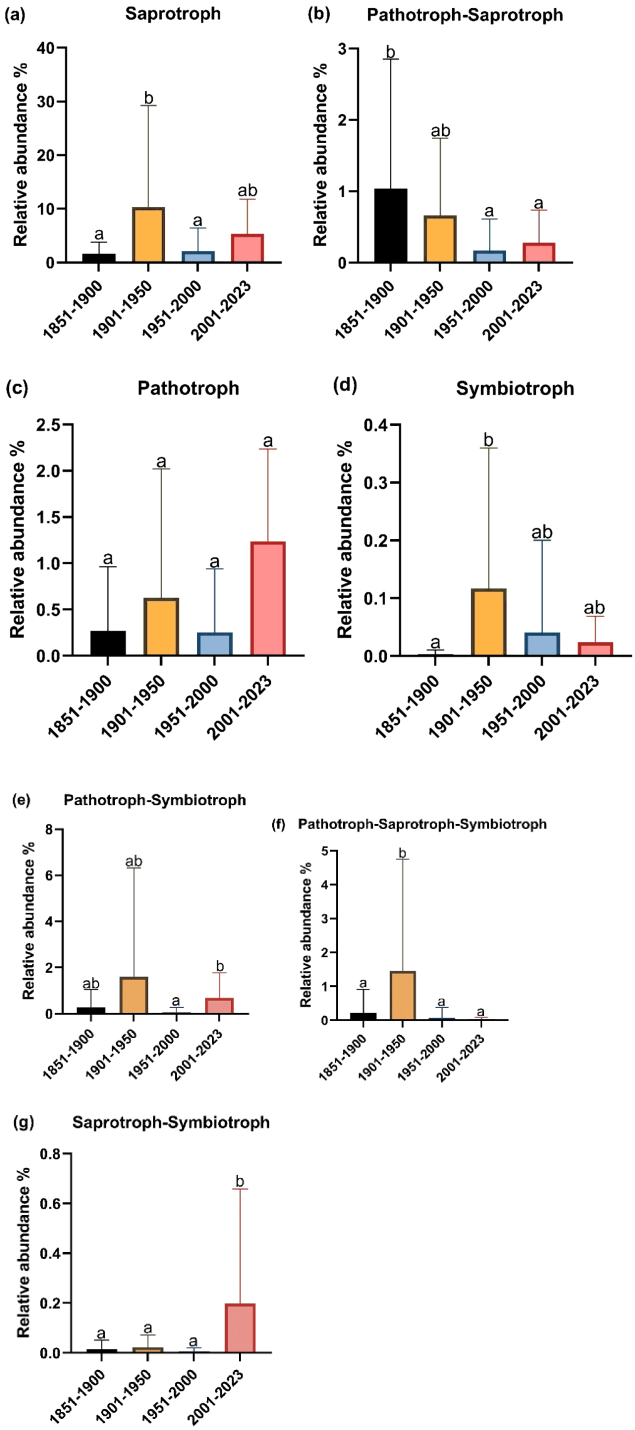

Figure 6

Fungal predicted functional mode in 50-year groups. Saprotroph (a), Pathotroph-Saprotroph (b), Pathotroph (c), Symbiotroph (d), Pathotroph-Symbiotroph(e), Pathotroph-Saprotroph-Symbiotroph (f), Saprotroph-Symbiotroph(g).

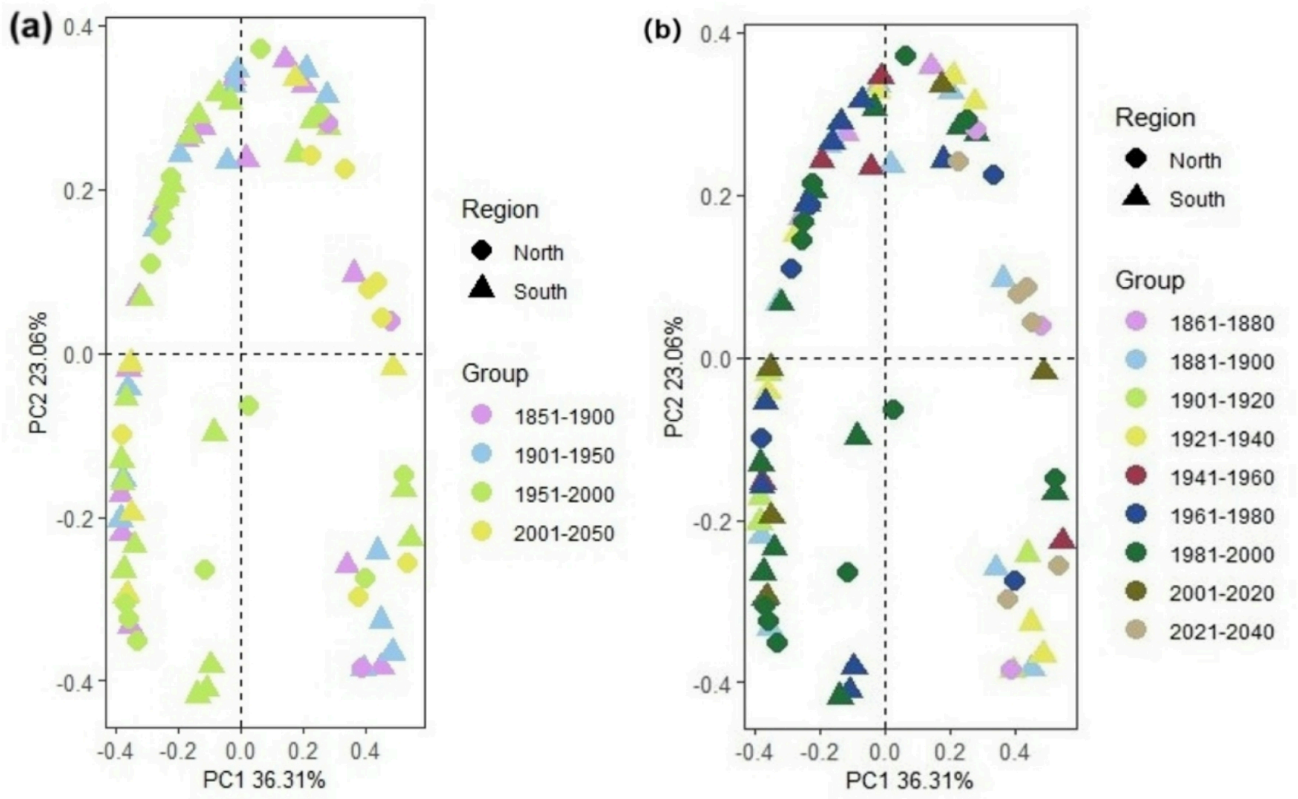

Figure 7

PCoA (principal co-ordinates analysis) illustrating the fungal community functional structures in 50-year groups (a) and 20-year groups(b).

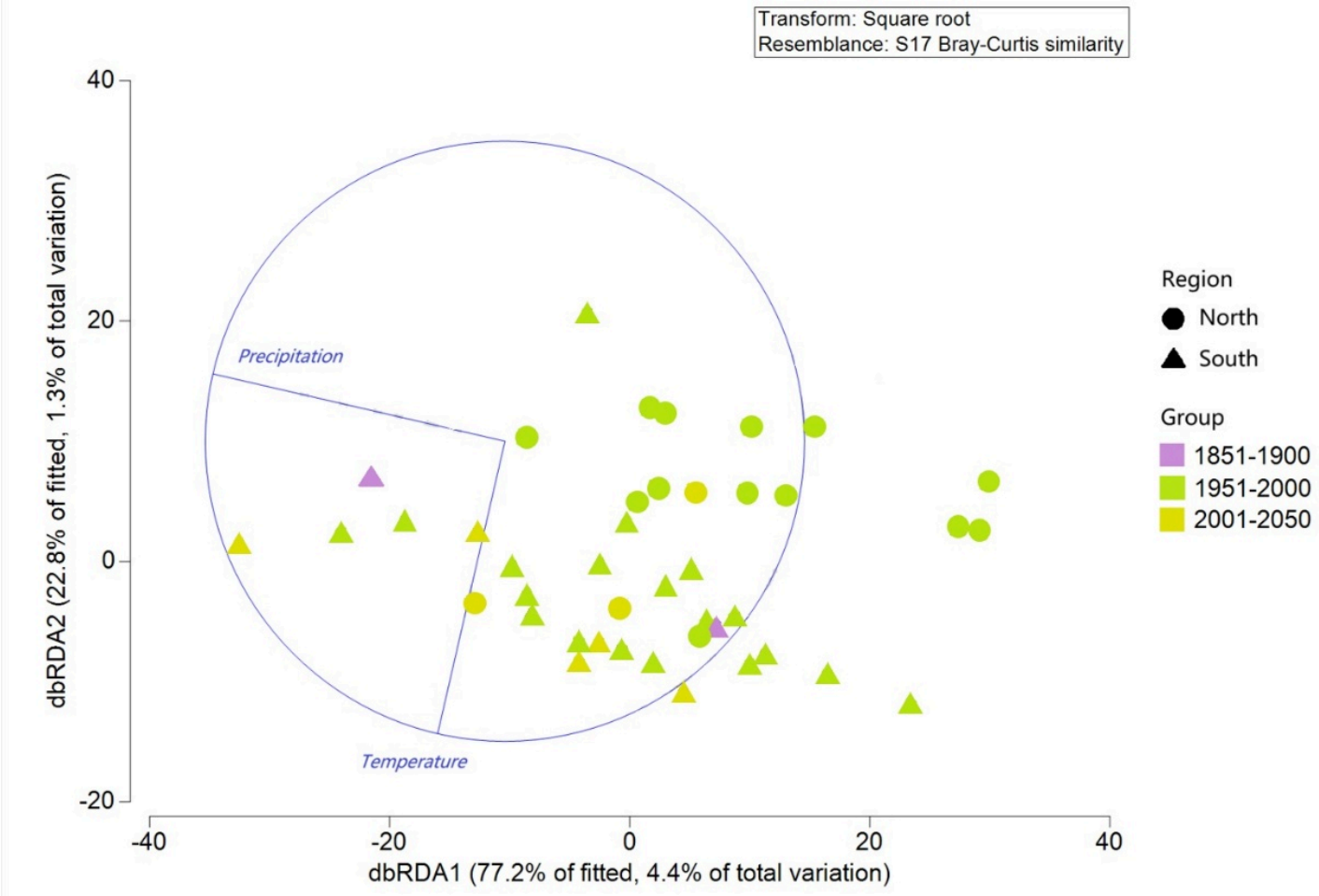

Figure 8

The distance-based redundancy analysis showing the sample ordination and the environmental factors (annual average temperature and annual precipitation).

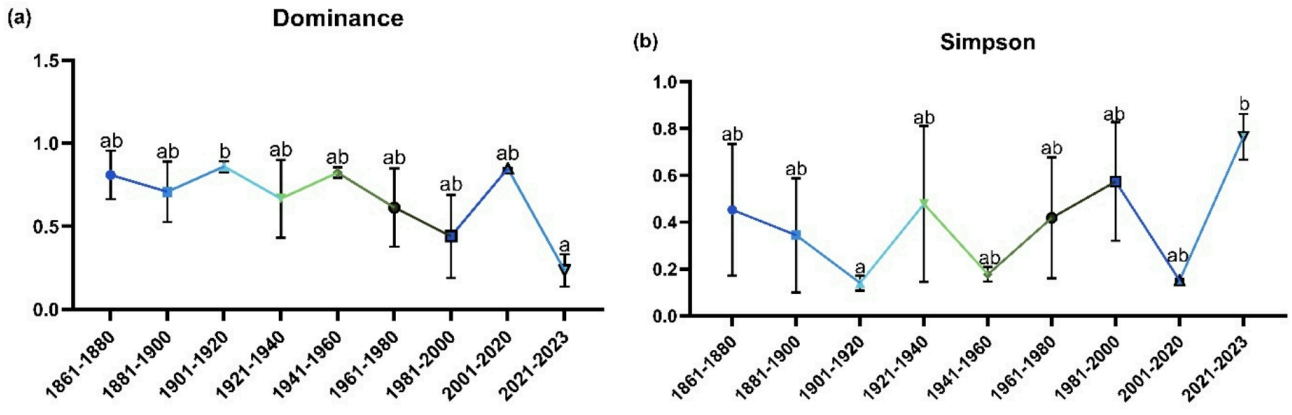

Figure 9

The alpha diversity indices of fungal communities in 20-year groups: dominance (a), Simpson's Index of Diversity (b). Letters above the column represent the significant difference ($P < 0.05$) among groups.

Supplementary Files

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- Supplemmentarymaterials.docx