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Research Article

Climate-driven shifts in plant and fungal communities can lead to topsoil carbon loss in alpine ecosystems

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Abstract

Alpine tundra ecosystems suffer from ongoing warming-induced tree encroachment and vegetation shifts. While the effects of tree line expansion on the alpine ecosystem receive a lot of attention, there is also an urgent need for understanding the effect of climate change on shifts within alpine vegetation itself, and how these shifts will consequently affect soil microorganisms and related ecosystem characteristics such as carbon storage. For this purpose, we explored relationships between climate, soil chemistry, vegetation, and fungal communities across seven mountain ranges at 16 alpine tundra locations in Europe. Among environmental factors, our data highlighted that plant community composition had the most important influence on variation in fungal community composition when considered in combination with other factors, while climatic factors had the most important influence solely. According to our results, we suggest that rising temperature, associated with a replacement of ericoid-dominated alpine vegetation by non-mycorrhizal or arbuscular mycorrhizal herbs and grasses, will induce profound changes in fungal communities toward higher dominance of saprotrophic and arbuscular mycorrhizal fungi at the expense of fungal root endophytes. Consequently, topsoil fungal biomass and carbon content will decrease.

Keywords: alpine ecosystems, climate change, fungal community

Introduction

Linkages of vegetation and soil microbes largely determine ecosystem responses to climate change (Classen et al. 2015, Sayer et al. 2017) as well as the effects of ecosystem processes on climate (Jansson and Hofmockel 2020). Despite the obvious importance of these linkages, they are still poorly understood (Arraiano-Castilho et al. 2021). Disentangling the direct and indirect effects of climate on microorganisms and soil properties will allow better predictions of the effects of climate change on ecosystems. In return, this will allow us to determine the effects of above- and belowground changes on biogeochemical cycles and climate.

Ongoing climate change will have particularly profound consequences on vegetation, soil fungi as well as biogeochemical processes in mountains (Hagedorn et al. 2019), because the warming will strike harder in high-latitudes and high-altitudes compared to temperate or lowland sites (Pepin et al. 2015). Mountain vegetation will experience dramatic changes represented by upwards shifts of the tree line, but also by changes in composition within alpine vegetation itself. The total extent of the alpine biome is estimated at ~3% of the total land surface of the Earth (Körner et al. 2011), and soils store 90% of the total C stock in this ecosystem (Körner 2021). Therefore, even a small shift in soil C storage will have a large effect on the overall soil C balance of high-altitudinal ecosystems (Parker et al. 2015). Although aboveground responses of mountain vegetation to global change have been widely studied, parallel changes are occurring in the soil, where plant roots and fungi interact.

Alpine areas are characterized by low, compact vegetation, and severe abiotic conditions. Alpine plants endure low temperatures and air pressure, nutrient-poor soils, short growing seasons, and high solar radiation (Körner 2021). Interestingly, various mycorrhizal types can be found among alpine plants. While woody shrubs such as dwarf willows, dwarf birch, or dwarf pine form ectomycorrhizal (EcM) symbioses, dwarf shrubs from the Ericaceae family associate with a relatively narrow group of soil fungi to form ericoid mycorrhiza (ErM) (Smith and Read 2008, Kohout 2017). Alpine vegetation can be also dominated by graminoids such as grasses and sedges or small perennial herbs (Körner 2021). Vegetation of this type is usually arbuscular mycorrhizal, or non-mycorrhizal (NM; Smith and Read 2008).

Besides largely determining the composition of soil fungal communities (Bahram et al. 2020), the dominant plant mycorrhizal type may also strongly affect soil C content. Some studies have shown that soil C storage is greater in ecosystems dominated by EcM plants than in those dominated by AM plants (Averill et al. 2014). Interestingly, in a global-scale study, based on data derived from global soil databases, Soudzilovskaia et al. (2019) indeed showed a positive relationship between the biomass share of EcM plants and soil C content, while the relationship between AM plants and soil C was weaker and mostly negative except in tundra

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habitats where the relationship was positive. This indicates potentially more complex relationships between dominant mycorrhizal types in vegetation and soil C storage in alpine ecosystems. Besides that, much less is known about the relationship between the abundance of ErM plants and the soil C content, although several studies have documented a positive relationship at small spatial scales (Ward et al. 2021).

Although part of the relationship between vegetation composition and soil C pool can be explained based on plant traits (De Deyn et al. 2008, Rossi et al. 2020), it is becoming clear that symbiotic fungi and their interaction with free-living fungi also play an important role in this relationship. Fungi with the ability to form ErM are known for their substantial saprotrophic capabilities (Martino et al. 2018), some species can even decompose highly recalcitrant substrates, such as lignin (Vohník et al. 2012). The mycelia of some EcM and ErM fungi have melanized hyphae resistant to decomposition, which has been proposed to play a central role in soil C sequestration (Fernandez and Koide 2014, Clemmensen et al. 2015). Highly melanized mycelia are also typical for root-associated dark septate endophytes (DSEs), which are especially common in cold regions such as alpine vegetation (Olsrud et al. 2007, Newsham et al. 2009) and present in roots of almost all alpine plants (Hambleton and Currah 1997, Jumpponen and Trappe 1998, Knapp et al. 2019). Compared to other functional groups, the role AM fungi play during C sequestration in soils is less clear. The almost negligible saprotrophic capabilities of AM fungi (Tisserant et al. 2013) means that they have no direct effect on soil C loss, but indirectly some AM fungi can stimulate decomposition rate by interacting with soil saprotroph (Ward et al. 2022) and bacteria (Verbruggen et al. 2016). Therefore, not only the dominant mycorrhizal type in vegetation but also the composition of soil fungal communities *per se* can have profound effects on soil C pool in alpine ecosystems.

The composition of fungal communities can be influenced by various environmental conditions such as climate (Tedersoo et al. 2014, Větrovský et al. 2019) or pH (Wubet et al. 2012). Similarly, vegetation features are recognized as an important driver in the structuring of fungal communities directly, or indirectly by changing other environmental parameters (e.g. (Bahram et al. 2016, Odriozola et al. 2021). Dominant plant species have been shown to be an important driver of community composition of plant-associated (Gao et al. 2013, Urbanova et al. 2015, Nguyen et al. 2016) as well as saprotrophic fungi (Hannula et al. 2017, Awad et al. 2019) in various ecosystems. Besides that, large-scale studies have identified climate as a primary driver of fungal species distribution (Tedersoo et al. 2014, Větrovský et al. 2019).

We hypothesize that the composition of the plant community and associated fungal community would be strongly influenced by the temperature and precipitation of the driest and coldest quarters in the alpine tundra ecosystem. Further, we hypothesize that topsoil carbon content is linked to the amount of fungal biomass according to the mycorrhizal dominant type. There will be more fungal biomass in areas that are dominated by ericoid vegetation and ericoid mycorrhizal fungi. Whereas there will be less fungal biomass in sites dominated by arbuscular mycorrhizal or non-mycorrhizal vegetation and associated fungal communities. To identify these linkages between climate, vegetation, soil fungi, and topsoil C content in alpine tundra, we selected 16 sites in seven different mountain ranges across Europe. Specifically, we aimed to (i) identify the main drivers (i.e. climate, vegetation, soil chemistry, and spatial distance to test for spatial autocorrelation) of fungal community composition and soil fungal biomass in alpine tundra and (ii) disentangle the effects of abiotic and biotic variables on soil C content in alpine tundra.

Materials and methods

Study sites, sample collection, and sample processing

We sampled 16 alpine sites in nine mountain ranges across Europe (Fig. 1; Table S1, Supporting Information), covering an extensive latitudinal gradient. At each location, three plots with characteristic alpine vegetation were selected ∼100 elevation meters above the tree line. Each of the sampling plots was established in a different vegetation type if more than one type was available on the sites (e.g. grassland, dwarf-shrub dominated). Plots were established on sites with a flat surface. At each plot, five 4-cm diameter soil cores were collected up to the depth of 10 cm below the plant litter layer: one central core, and four additional cores located 2 m north, south, east, and west from the central core (Figure S1, Supporting Information). We recorded GPS coordinates and inventoried all plant species in a radius of 3 m from the central core in 48 plots. Soil cores were stored at 4°C and processed within 24 h after collection. Soil cores were separated into two samples: soil and plant roots. All remaining plant litter and stones were removed from the samples. Material from all five cores from each plot was mixed to obtain composite samples of topsoil and plant roots. The soil was sieved through a 5-mm sieve and thoroughly homogenized while roots were cut into smaller pieces (∼1 cm). All the material was freeze-dried and stored at −20°C until further analyses. Samples of the topsoil were used for fungal community analysis, while plant root samples were used for the molecular identification of plants.

Molecular analysis

DNA extraction and sequencing of PCR amplicons

Total genomic DNA was extracted from all 48 samples using a modified Miller method (Sagova-Mareckova et al. 2008). DNA was extracted in triplicates from the soil, using 250 mg of freeze-dried material. Roots were first homogenized in liquid nitrogen, and the DNA was extracted in duplicates from 250 mg of the processed material. Respective replicates were cleaned using Geneclean Turbo Kit (MP Biomedicals) and pooled prior to subsequent PCRs.

The fungal ITS2 region was amplified from soil samples, using barcoded gITS4/ITS4 primer pairs (Ihrmark et al. 2012), and the plant ITS2 region was amplified from root samples, using barcoded ITS-S2F and ITS4 primers (Chen et al. 2010). Amplifications were performed in triplicate. Each PCR reaction contained 5 μl of 5x buffer for Q5 High-Fidelity DNA polymerase (New England Biolabs, Inc.), 5 μl of 5x Q5 HighGC Enhancer (New England Biolabs, Inc.), 0.5 μl of 10 mM PCR Nucleotide mix (Bioline), 1.5 μl of 10 mg ml^{-1} BSA (GeneON), 0.25 μl of the Q5 High-Fidelity DNA polymerase (New England Biolabs, Inc.), 1 μl of each 10 μM forward and reverse primer (Sigma-Aldrich), 9.75 μl of H_2O, and 1 μl of the template DNA. The PCR conditions for the fungal ITS2 region were: initial denaturation for 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 56°C, 30 s at 72°C; followed by an extension at 72°C for 7 min. The PCR conditions for the plant ITS2 region were: initial denaturation for 5 min at 98°C; 35 cycles of 30 s at 98°C, 30 s at 56°C, 45 s at 72°C; followed by an extension at 72°C for 5 min. Amplicon triplicates were combined, purified using MinElute Kit (Qiagen), and quantified with a Qubit™ dsDNA BR Assay kit (Thermo Fisher Scientific). Sequencing libraries were prepared

Figure 1. Sampling location in Europe from the southernmost to the northernmost location: Taygetos (Greece; GRC1), Murcia (Spain; ESP2), Prokletije (Montenegro; MNE1), Pyrenees (Spain; ESP1), Vitosha (Bulgaria; BGR1), Čvrsnica (BIH; BIH2), Bjelašnica (BIH; BIH1), Alps (France; FRA1), Krn (Slovenia; SVN1), Dachstein (Austria; AUT1), Fatra (Slovakia; SVK1), Jondal (Norway; NOR1), Gisna (Norway; NOR2), Sveitarfélagið Hornafjörður (Iceland; ISL1), Vardefjell (Norway; NOR3), and Tromso (Norway; NOR4). Colors refer to mountain ranges: Taygetos Mountains (pink), Baetic System (orange), Dinaric Alps (green), Pyrenees (light blue), Rila-Rhodopes (dark blue), Alps (red), Carpathian Mountains (olive-green), Scandinavian Mountains (purple), and Iceland Mountains (turquoise).

using the TruSeq PCR-free Kit (Illumina) and sequenced in-house using Illumina MiSeq (2 × 250).

Bioinformatic data processing

Plant (ITS-S2F/ITS4) and fungal (gITS4/ITS4) datasets were analyzed separately. Sequence data were processed using the SEED 2.0 pipeline (Větrovský et al. 2018). Briefly, fastq-join (Aronesty 2013) was used for joining the pair-end reads. Quality filtering was performed with a mean quality score of 30 as a cutoff. The ITS2 region (both fungal and plant) was extracted using ITSx v1.0.8 (Nilsson et al. 2010) prior to further processing. Following the removal of chimeric sequences, the remaining sequences were clustered at a 97% similarity level using UPARSE implemented within USEARCH (Edgar 2013). The most abundant sequence for each operational taxonomic unit (OTU) was chosen (representative sequence), and BLASTn was used for identifications against relevant databases: UNITE version 8.2 (downloaded on 25th March 2021) for fungi (Nilsson et al. 2019) and PLANiTS (downloaded on 2nd March 2021; Banchi et al. 2020) combined with GenBank for plants. Sequences identified as non-fungal or non-plant were discarded from fungal or plant datasets, respectively. Samples with less than 13 000 fungal ITS sequences or less than 2000 plant ITS sequences were removed. Putative ecology categories were assigned to fungal genera using FungalTraits (Polme et al. 2021). Sequence data were deposited in SRA (BioProject PRJNA869039).

Climatic data

We selected three climate variables that are relevant to the Alpine ecosystem—Mean annual temperature (MAT), precipitation of the warmest quarter (a proxy for drought), and precipitation of the coldest quarter (a proxy for snow at these specific locations). The corresponding bioclimatic predictors, respectively Bio1, Bio18, and Bio19 were retrieved from the CHELSA database (Karger et al. 2017).

Soil chemistry

Topsoil pH was measured in distilled water (1:10 w/w) as described previously (Stursova and Baldrian 2011). Total topsoil C was measured in an external laboratory of the Institute of Botany of the Czech Academy of Sciences (Průhonice, Czechia) using sulphochromic oxidation.

Fungal biomass analysis

Three replicates from each soil sample were generated for neutral lipid fatty acids (NLFA) and phospholipid fatty acids (PLFA) analyses in order to assess fungal biomass, following the method described by Fernández et al. (2022). Briefly, the samples for analysis were extracted with a mixture of chloroform–methanol–phosphate buffer (1:2:0.8) according to Frouz et al. (2016). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck), and the samples were subjected to mild alkaline methanolysis (Šnajdr et al. 2008). The free methyl esters of NLFA and PLFA were analyzed by gas chromatography–mass spectrometry (Varian 3400; ITS-40, Finnigan). Biomass of AMF was estimated using 16:1ω5 concentration in the NLFA fraction (Baath 2003, Frouz et al. 2016), while the biomass of the other fungal groups in the PLFA fraction was quantified based on 18:2ω6.9 concentration (Stella et al. 2015).

Statistical analysis

To assess the taxonomic proportion of fungal and plant communities, we used the number of sequences normalized by the number of reads per sample—i.e. relative abundance (Fernandez et al. 2017). Structures of plant and fungal community composition were both analyzed by nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distance using the "metaMDS" function on the Hellinger-transformed OTU matrices. We fitted the environmental variables to the ordinations with the "envfit" function. The db-PCA axes of fungi and plant composition calculated from the Hellinger-transformed fungi and plant OTU matrices were used as community composition index (see Table S1, Supporting Information, for PC axis scores), then were fitted to their respective NMDS to interpret them (see Figs 2 and 4).

To identify the main drivers (climate—MAT, precipitation of coldest quarter, precipitation of driest quarter, vegetation—plant db-PCA axis score, soil chemistry—C, and pH -, spatial distance—Principal Coordinates of Neighbor Matrices (PCNM) vectors scores) of fungal community composition, we used variation partitioning based on distance-based redundancy analysis (dbRDA). For this analysis, we used the first two axes of plant db-PCA, which explained the greatest amount of variability in the dataset. Principal Coordinates of Neighbor Matrices (PCNM) vectors, which are calculated based on the latitude and longitude of each sample were calculated to describe the spatial distance and distinguish the net effect of the variables of interest from spatial autocorrelation (Borcard and Legendre 2002). Then, vectors with significant effects on the composition of fungal communities were selected by forward selection (Table S1, Supporting Information). Finally, the partial effect of each group of variables (i.e. the effect of a group once the effects of all other groups have been considered) was tested using "capscale" function, and only the significant groups were included in the final variation partitioning.

To test the effects of abiotic and biotic conditions on topsoil C content in alpine tundra we used path analyses. We tested the effect of the three climate variables (MAT, Bio18, and Bio19) on topsoil carbon content through the plant and fungal communities and the interrelationships between the variables. Fungal and plant communities are expressed through the first db-PCA axes calculated from the Hellinger-transformed fungi and plant out matrices. Fungal biomass (PLFA), AMF biomass (NLFA), and C content were log-transformed to follow a normal distribution. We built initial models based on the hypothesis that climatic factors would globally affect plant and fungal communities, fungal biomass, and carbon content. Then, plant and fungal communities would covary and affect fungal biomass and carbon content. Finally, fungal biomass would affect directly carbon content. Because it was not possible to include too many parameters in a model with 46 observations, we designed and tested different models including only one climatic factor and one biomass factor. We simplified the initial models by removing the less significant regression (highest *P*-value) and recalculating the model until we got good statistical support (i.e. Model test Chi-square *P*-value > .5; CFI > 0.95; TLI > 0.95; RMSEA < 0.08; *P*-value RMSEA > 0.05; SRMR < 0.08) and significant regressions ($P < .05$). When the paths from climatic variables to plant and fungal communities were not significant, we tested both alternatives of keeping them (H1) or not (H2) in the models (Figure S2, Supporting Information).

All statistical analyses were performed in R v. 4.0.5 (R Core Team 2022). The "vegan" package (Oksanen 2007) was used for community composition analyses (i.e. rarefaction, OTU count data transformation, ordinations, environmental correlations with ordination analyses, and variation partitioning). The "eulerr" package (Larsson et al. 2021) was used to build Venn diagrams to display the result of variation partitioning. Finally, "lavaan" (Rosseel 2012) and "semPlot" (Epskamp et al. 2022) packages were used for path analysis and model visualization, respectively.

Results

Sequence data

The samples BIH2c and NOR2a were removed due to a small number of fungal sequences. The fungal data set consisted of 848 765 sequences after singleton, chimera, and non-fungal sequence removal. These sequences were clustered into 7804 fungal OTUs and their relative abundance revealed that topsoil fungal communities were dominated by Ascomycota (4531 OTUs; 58% sequences), followed by Basidiomycota (1954 OTUs; 33% sequences), and Mucoromycota (443 OTUs; 5% sequences). The majority of fungi belonged to saprotrophs, followed by EcM, plant pathogens, and root endophytes (Table 1). The plant data set consisted of 103 037 sequences after the removal of singletons for a total of 117 remaining plant OTUs. Most sequences belonged to herbal roots (52%) followed by roots of dwarf shrubs (20%). Most OTUs belonged to herbs (60%), followed by graminoids (23%) (Table 1).

Plant communities in alpine topsoil

The plant community matrix was used for principal component analysis (PCA). The first two axes of the PCA of the plant community explained 24.6% and 10.8% of the variation in the community composition, respectively. The first axis (PC1_plant) describes a gradient of vegetation composition leading from a community dominated by ericaceous dwarf shrubs to a community dominated by AM and NM herbs, while the second axis (PC2_plant) corresponds to a gradient from graminoid to herbaceous and *Salix cinerea*-dominated vegetations (Fig. 2; Figure S3, Supporting Information). All analyses were performed on OTU relative abundance matrices, as they had more informative values than occurrences. We obtained similar results when using presence–absence data for the same analysis.

Fungal communities in alpine topsoil

Variation partitioning (db-RDA) assessed the contribution of spatial distance (represented by eight significant PCNM vectors chosen by forward selection), vegetation (PC1_plant axis, PC2_plant axis), soil properties (carbon content, soil pH), and climatic conditions (MAT, precipitation of coldest quarter, and precipitation of warmest quarter) on the composition of fungal communities in the alpine topsoil (Fig. 3). The pure effects of each variable were significant ($P < .001$). Altogether the tested variables explained 41.2% of the fungal communities' variance. Further, the total variance explained by spatial distance was 23.3%, but fungal communities were more affected by the combined environmental variables than by the spatial components alone. Vegetation, soil properties, and climate, together explained 17.9% of the total variance while the net effect of spatial distance explained 14.9%. Among the environmental factors, vegetation was the most important with 15.2% of total fungal community variance explained versus 11.4% and 7.9% for soil properties and climate, respectively. Nonetheless, the net effect of climate was the most important with 7.9% of total variance explained versus 3.4% and 3.3% for the soil properties and vegetation, respectively.

To better understand the effects of studied environmental characteristics on the composition of fungal communities, the NMDS

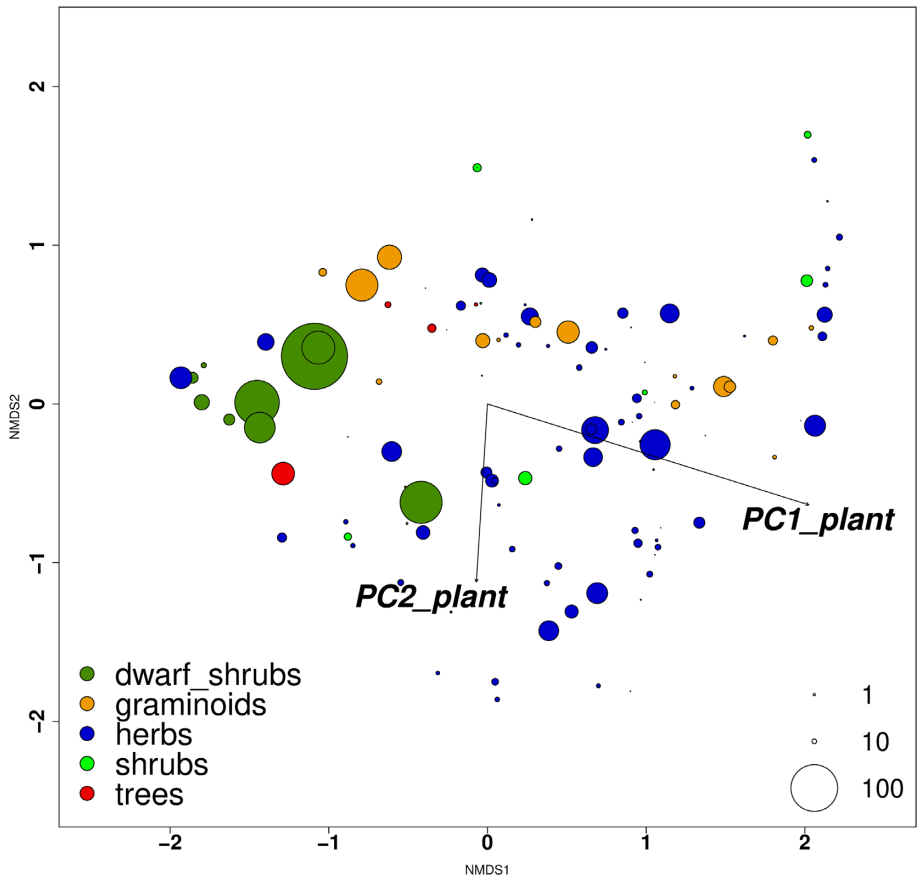

Figure 2. NMDS ordination of plant species across the European alpine ecosystem displaying plant OTUs as dots whose area is a proportion of the relative abundance. Dots are colored according to plant functional type with dwarf shrubs in dark green, graminoids in orange, herbs in blue, shrubs in light green, and trees in red. Vectors fitted vegetation composition (two firsts axis of db-PCA calculated from the Hellinger-transformed OTU matrix). Stress value = 0.1737787

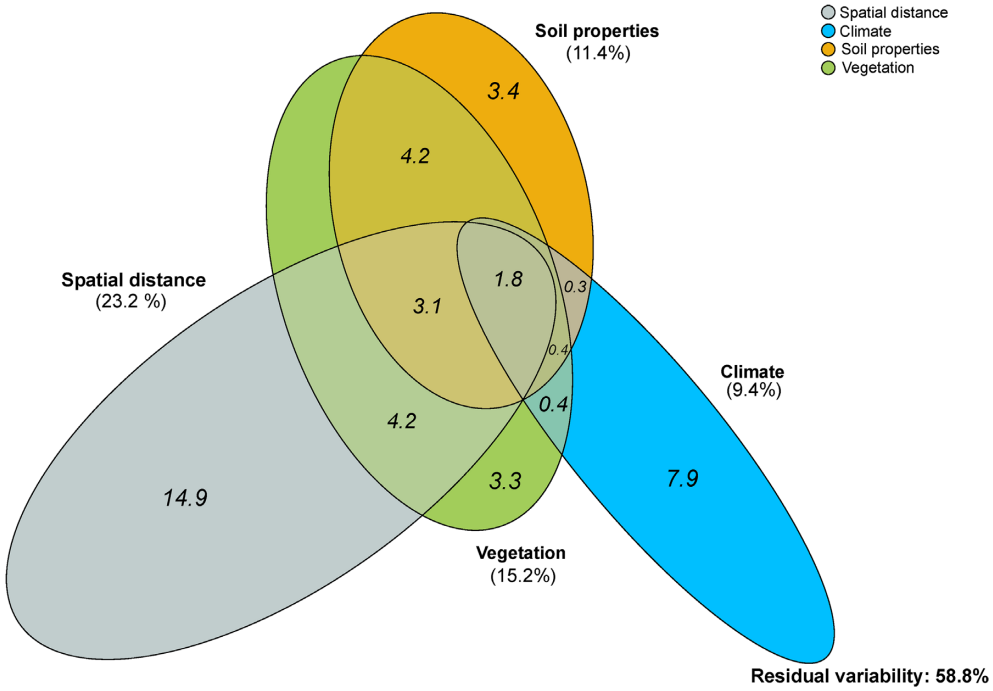

Figure 3. Variation partitioning of the fungal community VP is based on dbRDA, an analysis giving the percentage of variance explained by climate (MAT, precipitation of coldest quarter, precipitation of driest quarter), vegetation (two firsts axis of db-PCA calculated from the Hellinger-transformed OTU matrix), soil properties (carbon and pH), and spatial distance (PCNM vectors based on geographical distances between samples) on the composition of the fungal community (abundance data normalized by Hellinger transformation).

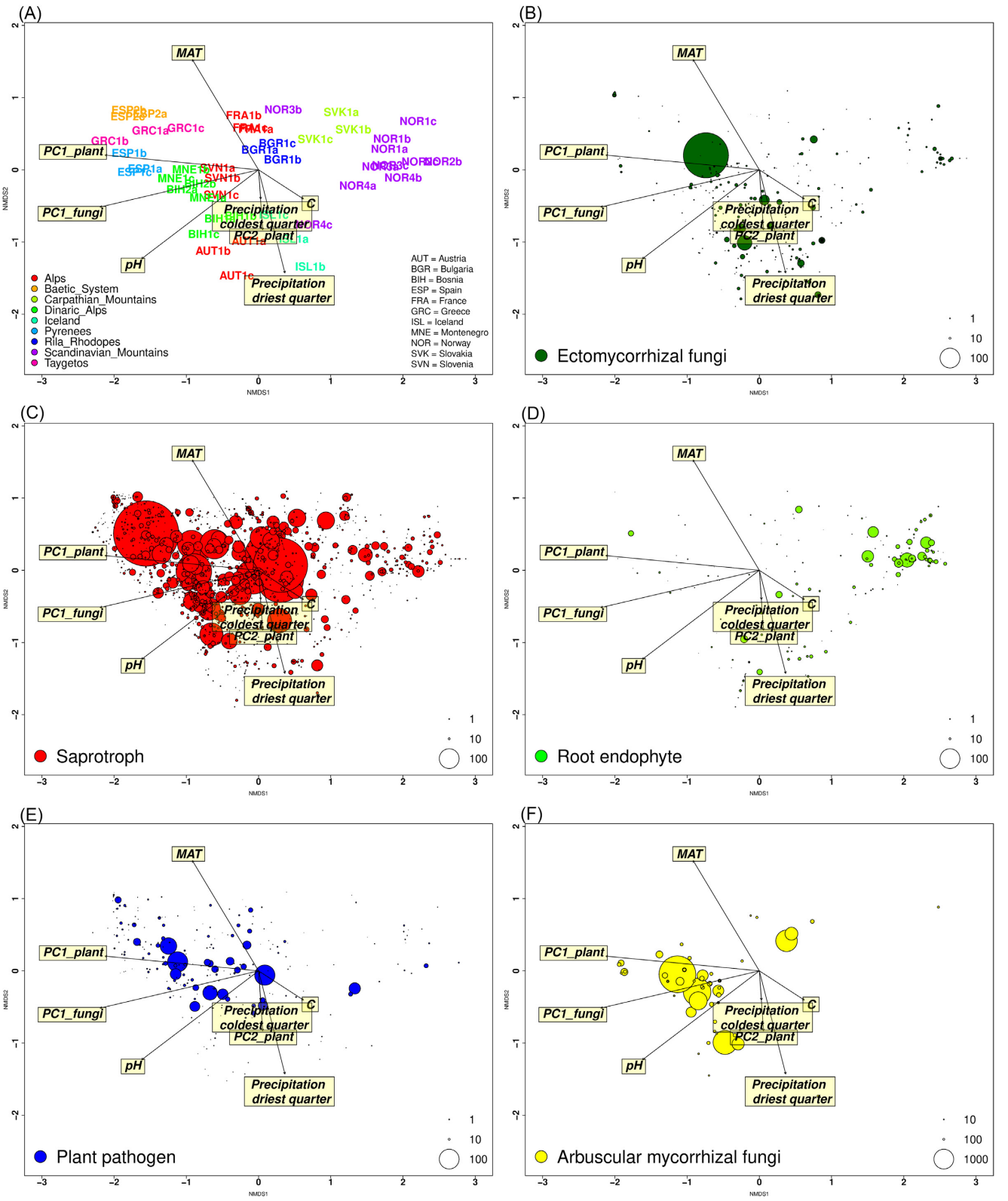

Figure 4. NMDS ordination of fungal species across European alpine ecosystem. These are variations of the same ordination displaying: (A) samples, where each name represents one sample location colored according to mountain range; (B)–(F) fungal OTUs, where each dot represents one OTU whose area is a proportion of the relative abundance and colored according to their guilds with (B) EcM fungi in dark green, (C) saprotrophic fungi in red, (D) root endophytic fungi in light green, (E) plant pathogens in blue, and (F) arbuscular mycorrhizal fungi in yellow. Vectors-fitted climate variables (MAT, precipitation of coldest quarter, precipitation of driest quarter), vegetation composition (two firsts axis of db-PCA calculated from the Hellinger-transformed OTU matrix), soil properties (carbon and pH), and fungal community composition (first axis of db-PCA calculated from the Hellinger-transformed OTU matrix). Stress value = 0.1346181.

Table 1. Number of OTUs and proportion of sequences assigned to each fungal guilds and plant functional type. Proportion of mycorrhizal types associated to each plant life form is indicated between brackets. ErM: ericoid mycorrhizal, AM: arbuscular mycorrhizal, EcM: ectomycorrhizal, and NM: non-mycorrhizal.

	Assigned ecology	Number of OTUs (% of total OTUs)	Proportion of sequences (% of the total)
Fungal guilds	EcM fungi	544 (6.97)	8.57
	Arbuscular mycorrhizal fungi	83 (1.06)	0.12
	Saprotrophic fungi	2179 (27.92)	46.31
	Root endophytes	216 (2.77)	2.56
	Plant pathogens	358 (4.59)	2.84
	Others	398 (5.10)	3.20
	Unassigned	4026 (51.59)	36.40
Plant life forms	Dwarf shrubs (100% ErM)	8 (6.84)	20.34
	Graminoids (82.1% AM, 17.9% AM–NM)	27 (23.08)	12.15
	Herbs (73.6% AM, 19.4% AM–NM, 7% NM)	70 (59.83)	52.19
	Shrubs (71.4% EcM, 28.6% AM)	7 (5.98)	6.45
	Trees (60% EcM, 20% AM, 20% AM–EcM)	5 (4.27)	8.89

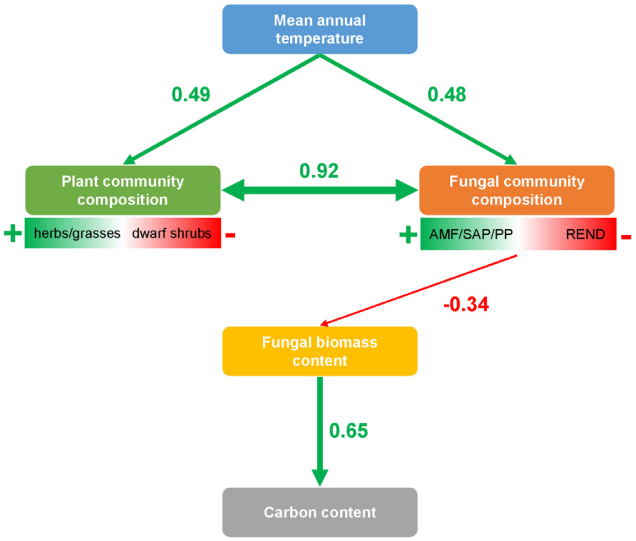

Figure 5. Path analysis modeling biotic and abiotic linkages to explain carbon content in alpine ecosystems in Europe. The final model retains MAT, plant community composition (first axis of db-PCA calculated from the Hellinger-transformed OTU matrix: from sites dominated by ericaceous dwarf shrubs to sites dominated by herbaceous and graminoid vegetation), fungal community composition (first axis of db-PCA calculated from the Hellinger-transformed OTU matrix: from sites with a higher share of root endophytes (REND) to sites with the dominance of saprotrophic fungi (SAP), arbuscular mycorrhizal fungi (AMF), and plant pathogens (PP).), and fungal biomass (PLFA). Arrows indicate significant ($P < .05$) paths between variables and their widths are proportional to the standardized path coefficients. Green and red arrows represent positive and negative pathways, respectively.

ordination was calculated. Congruently with the results of variation partitioning, soil pH, climatic variables, and the vegetation gradient mainly affected fungal community composition (Fig. 4). The first axis of fungal PCA (PC1_fungi) captured a gradient associated with the first axis of plant PCA (PC1_plant). Sites dominated by ericaceous dwarf shrubs had a higher share of root endophytes (guild which also includes ErM fungi) in the fungal communities (Fig. 4D) while sites dominated by herbaceous and graminoid vegetation harbored fungal communities dominated by saprotrophic fungi, AM fungi, and plant pathogens (Fig. 4C, E, and F). The distribution of EcM fungi was primarily explained by the second vegetation axis (PC2_Plant), with a higher share of EcM fungi on sites dominated by herbaceous plants as well as by *S. cinerea* (Fig. 2; Figure S3, Supporting Information). PC1_fungi, which explained 21.2% of the variation in fungal community structure, was used for the subsequent path analysis.

Biotic and abiotic drivers of carbon content in alpine topsoil

To test our hypotheses that soil fungi are the strongest predictor of C content in alpine topsoil and that this relationship can be influenced by the responses of plant and fungal communities to climate, we utilized path analysis modeling (Fig. 5). The final model chosen was the one including MAT, plant, and fungal communities that are both described by respective first db-PCA axis (Figs 3 and 4), fungal biomass (PLFA), and carbon (C) (Figure S2.1, Supporting Information). The model obtained good statistical support (i.e. Model test Chi-square *P*-value = .990; CFI = 1; TLI = 1.069; RMSEA = 0; *P*-value RMSEA = 0.992; SRMR = 0.015) and showed that plant and fungal communities covary strongly (path = 0.92, *P*-value < .001) and they were both correlated with MAT. Higher MAT favors AM and NM-dominated plant communities, mostly herbs, and graminoids, (path = 0.49, *P*-value < .001) associated with saprotroph and AM fungi (path = 0.48, *P*-value < .001) at the expense of ErM dwarf shrubs associated with root endophytes (including also ErM fungi). The fungal community PC axis was identified as a negative predictor of fungal biomass (path = −0.34, *P*-value = .014) meaning that root endophyte-rich fungal communities exhibit higher biomass compared to those rich in saprotrophs and AMF. Finally, fungal biomass was identified as the best predictor of C content (path = 0.65, *P*-value < .001), while plant community composition and fungal community composition direct paths were not significant predictors of C content and have been removed from the final model. Interestingly, the alternative model that includes AMF biomass (NLFA) instead of other fungal biomass (PLFA) indicates that NLFA is also a positive predictor of carbon content. However, in this case, AMF biomass is more influenced by the plant community than the fungal community. Additionally, a fungal community with a higher proportion of AMF and saprotroph fungi was found to be a negative predictor of C content (Figure S2.1.2, Supporting Information). Nevertheless, this model including NLFA instead of PLFA had weaker statistical support.

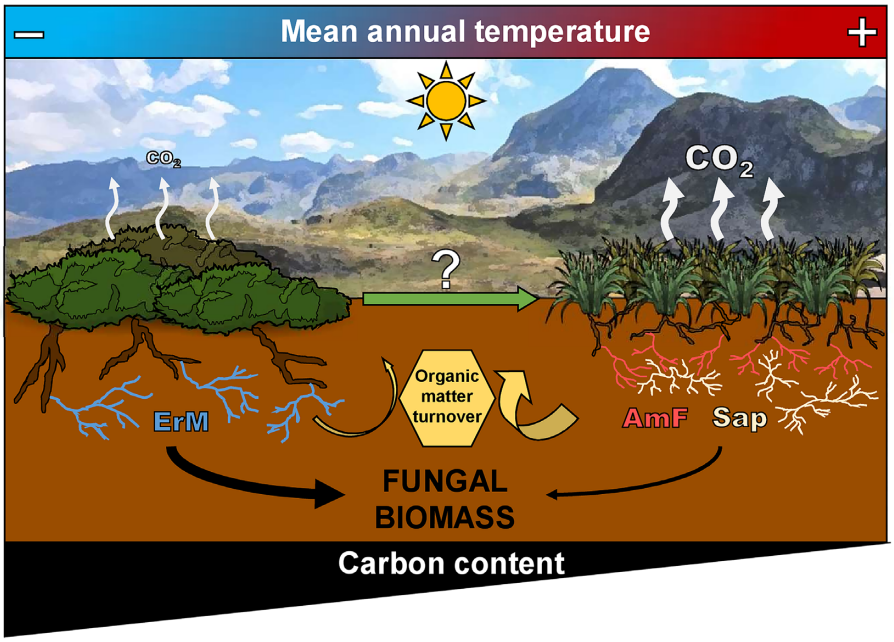

Figure 6. Conceptual figure of the hypothesis inferred from our results. Rising temperatures could lead to a change in alpine tundra vegetation (dwarf-shrub toward herbs and grass) associated with a change in fungal communities (ericoid mycorrhizal (ErM) fungi toward saprotroph (Sap) and arbuscular mycorrhizal (AmF) fungi), resulting in an increase of organic matter turnover and respiration as well as a loss of fungal biomass and carbon content in 10 cm topsoil.

Discussion

Fungal communities in alpine topsoil

Climate represents the key environmental factor that shapes soil fungal communities (Tedersoo et al. 2014) and the distribution of a wide range of fungal species (Větrovský et al. 2019). In our study, climate had the largest influence on fungal community composition variation on its own, whereas its total influence was the weakest compared to other variables. (Fig. 3). This can be partly explained by small differences in climatic conditions among the study sites compared to global-scale studies.

On the contrary, we found a strong relationship between plant and soil fungal community composition, previously also documented by numerous studies (e.g. Bahram et al. 2016, Krüger et al. 2017. The main PC axes of vegetation composition were represented by gradients from ericaceous dwarf shrubs to herbs for the first axis and from graminoids to herbs for the second axis, which corresponds not only to a shift in plant functional type but also in dominant mycorrhizal type (Fig. 2). It is becoming widely accepted that both plant functional and mycorrhizal types play an important role in structuring fungal communities (Bahram et al. 2020, Davison et al. 2020). For instance, the removal of ericoid shrubs, in forest islands, caused declines in the relative abundance of saprotrophic basidiomycetes, ericoid, and other root-associated fungal species (Fanin et al. 2022). Our study shows the importance of ericoid dwarf shrubs vegetation in structuring soil fungal communities across a wide range of alpine sites, phenomena recently also documented by local-scale studies in the Pyrenees and Alps (Grau et al. 2019, Broadbent et al. 2022).

Factors driving carbon content in alpine topsoil

Using a path analysis approach (Fig. 5; Figures S2, Supporting Information), we tested the direct and indirect effect of climate, plant, and fungal communities on C content in alpine topsoil. We found that the carbon content of alpine tundra topsoil is exclusively determined by fungal biomass. (Fig. 5). This agrees with mounting evidence of the importance of soil microorganism biomass in the accumulation of organic C in soil (Clemmensen et al. 2013). In terms of biomass, fungi dominate global soil communities, with an approximate global biomass of 12 Gt C, which is almost double that of soil bacteria (7 Gt C) (Bar-On et al. 2018). The potential importance of fungi in soil C accumulation is also supported by high fungal biomass productivity in various ecosystems (Ekblad et al. 2013, Baldrian 2017).

Besides the quantity, the quality of fungal biomass, which affects the decomposability of fungal necromass, can play a significant role in sequestration of the soil C. While chitin, a common structural polymer of all fungal cell walls, undergoes rapid decomposition by hydrolytic extracellular enzymes, the decomposition of melanin depends on nonspecific oxidative enzymes, whose production is very energy-intensive for the microorganisms and retards decomposition of fungal necromass (Malik and Haider 1982, Fernandez et al. 2013, Fernandez and Koide 2014). Compared to chitin, the production of melanin is restricted to just a subset of fungal species with the characteristic black or gray color of mycelia. Ericoid mycorrhizal (ErM) fungi and many root endophytes, particularly so-called DSEs, represent species with high melanin content in their cell walls (Sadowsky et al. 2012). Therefore, a higher abundance of these fungal species leads to the production of more recalcitrant necromass, which can lead to soil C sequestration (Clemmensen et al. 2015).

The composition of fungal communities may also have an indirect effect on soil C content due to changes in decomposition rate. It is widely assumed that mycorrhizal and saprotrophic fungi compete for nutrients (Lindahl and Tunlid 2015, Verbruggen et al. 2017). Compared to free-living saprotrophs, mycorrhizal fungi (ErM and EcM fungi) receive C from their host plants and can, therefore, invest more energy in extracting nutrients from the decomposed substrate. The selective decomposition due to mining for N by ErM or EcM fungi increases C:N ratio of soil organic

matter (SOM) leading to limited growth of saprotrophic fungi, poor degradability of SOM, and C sequestration in the soil, phenomenon well-known as the Gadgil effect (Gadgil and Gadgil 1971, Fernandez and Kennedy 2016). While the Gadgil effect can be particularly strong on sites where saprotrophs compete with EcM fungi (Kyaschenko et al. 2017), it has been recently shown by Fanin et al. (2022) that dwarf shrubs and associated ErM fungi impaired decomposition and nutrient cycling, suggesting a Gadgil effect caused by ericoid shrubs and their fungal partners. Considering the extracellular enzymatic activity of ErM fungi (Read et al. 2004, Vohník et al. 2012) as well as high gene contents for polysaccharide-degrading enzymes, lipases, and proteases (Martino et al. 2018), ErM fungi may compete with saprotrophs for N in organic soil (Fanin et al. 2022, Mielke et al. 2022) or act as sufficient saprotrophs by themselves. In this context, it remains unclear whether the observed relationship between soil C content and fungal community composition is driven by the production of more recalcitrant necromass of endophytic fungi or because fungi associated with ericoid dwarf shrubs can contribute to nutrient limitation, impairing saprotroph decomposition and leading to an accumulation of organic matter as in boreal forest ecosystem.

Congruently with the results of variation partitioning, we found a strong correlation between the composition of plant and fungal communities, as described above (Figs 3 and 5). As might be expected, while fungal root endophytes dominated communities under the ericoid dwarf shrub vegetation, AM and saprotrophic fungi made up a higher proportion of the fungal communities growing on sites dominated by herbaceous and graminoid plants, typically with AM or NM status (Fig. 4). Although we found a significant effect of fungal community composition on soil fungal biomass (i.e. sites with a higher share of root endophytes correlated with higher fungal biomass) as well as a correlation between plant and fungal communities, there was no direct relationship between plant community composition and fungal biomass in soil (Fig. 5). This shows that the potential role of vegetation in soil C sequestration in alpine habitats goes through changes in the composition of soil fungal communities. Congruently, Clemmensen et al. (2015) identified soil fungal community composition, particularly the high abundance of ErM fungi, as a key driver of soil C sequestration in boreal forests.

The composition of both plant and fungal communities was structured by MAT. Vegetation dominated by herbaceous and graminoid plants was positively correlated with MAT, while vegetation dominated by ErM dwarf shrubs was associated with the colder region. This gradient corresponds to a shift from AM or NM to ErM-dominated vegetation and one can assume that the increasing MAT, due to global climate change, will favor non-ericaceous vegetation. Although the ErM plants are indeed known to dominate colder regions (Read 1991, Bueno et al. 2017, Kohout 2017), the response of their abundance in the tundra to the ongoing climate change is less clear and potentially species-specific. Wilson and Nilsson (2009) documented the decline of *Vaccinium myrtillus* in low alpine vegetation in Norway over 20 years and its replacement with AM herbaceous species. Additionally, ErM plants and fungi are associated with an inorganic N-limited environment (Ward et al. 2022) and they could be replaced by AM plants and fungi as a result of an increased N availability associated with warming (Solly et al. 2017, Steidinger et al. 2019). Although reports of *Vaccinium* spp. expansions into the European alpine tundra can be attributed more to changes in grazing regimes than climate warming (Mayer and Erschbamer 2017, Kaufmann et al. 2021), there is robust evidence that evergreen ericoid shrubs, such as *Empetrum spp.*, are expanding in tundra regardless of grazing intensity and, therefore, most likely as a result of climate changes (Klanderud and Birks 2003, Wilson and Nilsson 2009, Vowles et al. 2017, Vuorinen et al. 2017, Vowles and Bjork 2019). Defrenne et al. (2021) suggested that warmer and drier conditions favor the abundance and growth of EcM and ErM plants in their warming experiment in peatland. Finally, compared to other biomes (Steidinger et al. 2019), shifts in relative abundances of different mycorrhizal types in tundra vegetation due to global changes are less clear, although the consequences for biogeochemistry can be striking.

Although we did not find a direct correlation between MAT and topsoil C content, increasing temperature will likely affect topsoil C content as shown in an alpine soil warming experiment, where 4°C warming induced mineralization of stable old SOM and increased soil respiration by 38% (Streit et al. 2014). Moreover, in another alpine experiment, 3 years of warming-induced a shift in fungal community composition that could accelerate the breakdown of organic matter and contribute to a decline in C storage (Solly et al. 2017). Further, once C is removed from alpine soil due to such changes, there is no way to replace it because of slow mean net addition (Körner 2021). The design of our study allowed us to disentangle the potential effects of abiotic and biotic factors on topsoil carbon content, however, we cannot infer from this study the effect on the global carbon pool in alpine tundra. It is also important to note that our study is based on observational data from various locations. The path analysis is a useful tool to evaluate causal hypotheses. However, it cannot differentiate between causality and correlation among our observed variables. Nonetheless, we built our hypotheses on previously established relationships between climatic variables, the composition of plant and fungal communities, and the accumulation of recalcitrant biomass associated with C content in topsoil. Future experiments, such as transplanting soil mesocosms at different elevations, should confirm the observed trend by controlling for changes in temperature and tracking the changes of the plant and fungal communities over time.

Conclusions

We investigated linkages between climate, vegetation, fungal community structure, and topsoil carbon content across numerous alpine sites in various European mountain ranges. Our results suggest that rising temperature may lead to a replacement of ericoid-dominated alpine vegetation by non-mycorrhizal and arbuscular mycorrhizal herbs and grasses. If this happens, such a shift will induce profound changes in fungal communities toward higher dominance of saprotrophic and arbuscular mycorrhizal fungi at the expense of fungal root endophytes, which will lead to the decrease of soil fungal biomass and subsequently to slower accumulation of carbon in topsoils (Fig. 6).

Data accessibility and benefit-sharing section

Data accessibility statement

Raw sequence reads and metadata are deposited in the SRA (BioProject PRJNA869039).

Benefit-sharing statement

Benefits generated: benefits from this research accrue from the sharing of our data and results on public databases as described above.

Authors' contributions

Andrea Moravcová (Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing), Florian Barbi (Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing), Vendula Brabcová (Investigation, Writing – review & editing), Tomáš Cajthaml (Investigation, Resources, Writing – review & editing), Tijana Martinović (Formal analysis, Investigation, Resources, Writing – review & editing), Nadia Soudzilovskaia (Writing – review & editing), Lukáš Vlk (Formal analysis, Investigation, Writing – review & editing), Petr Baldrian (Project administration, Supervision, Writing – review & editing), and Petr Kohout (Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing).

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Supplementary data

Supplementary data are available at *FEMSEC* online.

Conflict of interest. None declared.

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