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Root morphology, carbohydrate pools, and mycorrhizal associations of silver fir across contrasting canopy conditions

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Abstract: The widespread decline of Norway spruce has led to larger gaps in the canopy of Central European mountain forests, potentially affecting the regeneration of shade tolerant silver fir (*Abies alba* Mill.). We examined whether greater light availability in canopy gaps influences the morphology of fir needles and fine roots, their carbohydrate concentrations, and their fungal associations, as trees may respond to environmental changes in both their canopy and in their belowground interactions. We compared small patches of fir trees under a closed canopy and low light conditions with regenerating patches growing in gaps with greater light transmission (i.e. canopy gap). We quantified understory photosynthetically active photon flux density (PPFD%), soil water storage, shoot apical dominance, needle specific leaf area (SLA), fine root traits (AD- average diameter, SRL- specific root length, SRA- specific root area), root and leaf tissue nitrogen (N), non-structural carbohydrates (TNC), and characterized root-associated fungi via ITS2 sequencing. Canopy gaps had 1.5 times higher PPFD and reduced soil water storage. Silver fir individuals in canopy gaps also displayed greater apical dominance, thinner roots, and longer SRL and greater SRA compared with individuals under a closed canopy. Contrary to our expectations, the concentrations of soluble sugars and total non-structural carbohydrates were higher under the closed canopy. Meanwhile, gap saplings exhibited higher tissue nitrogen levels in both roots and needles. Light availability also influenced belowground symbiosis as canopy gaps saplings hosted a more diverse ectomycorrhizal fungal community (higher α-diversity), whereas the long-distance morphotype of *Boletus* occurred under a closed canopy and covaried positively with carbohydrate pools and negatively with SRL. These linked changes illustrate a shift from carbon conservative, symbiont supported growth in shaded patches to more acquisitive root strategies in canopy gaps.

Keywords: *Abies alba*, *Picea abies* decline, mixed montane forests, canopy gaps, resource acquisition strategies, ectomycorrhizal fungi, fine roots

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Introduction

Sustaining naturally regenerated forests requires understanding how trees respond to environmental changes from the canopy down to their belowground partners. This dynamic is crucial for shade tolerant silver fir (*Abies alba* Mill.), which regenerates most effectively under shaded, low competition understories (Robakowski et al., 2003; Orman et al., 2021; Kolisnyk et al., 2025). Even limited light penetration to the forest floor creates favorable microsites for germination and growth, leading to higher abundance of fir individuals in scattered patches compared to spruce (Grassi et al., 2004; Paluch et al., 2019; Kolisnyk et al., 2024). Under such conditions, silver fir trees can survive for decades with minimal height increment until canopy openings occur and increase light availability (Antos et al., 2000; Grassi et al., 2004; Szymura et al., 2007; Szewczyk & Szwagrzyk, 2010; Mauri et al., 2016). In contrast, the light responsive Norway spruce (*Picea abies* L.) H. Karst) rarely occupies low light areas but can rapidly colonize new canopy gaps with high light transmission (Grassi & Bagnaresi, 2001; Grassi et al., 2004).

The recent widespread dieback of Norway spruce in Central European mountain spruce-fir forests, driven by drought and bark beetle outbreaks, creates large canopy gaps, sharply increasing light availability. These disturbances favor the spread of fast-growing spruce and may hinder silver fir regeneration (Grassi & Bagnaresi, 2001; Pretzsch et al., 2015; Dănescu et al., 2018). Sudden exposure to high, direct irradiance can also impose thermal and drought stress on silver fir seedlings, increase frost risk, and increased competition from ground vegetation, reducing their competitiveness relative to spruce (Albanesi et al., 2008; Szuba et al., 2025). Therefore, the balance between canopy cover and light availability is a key driver of silver fir regeneration patterns, i.e., sufficient light is needed for growth, but excessive exposure can reduce its regeneration capacity (Pretzsch et al., 2015; Dănescu et al., 2018). Understanding how fir saplings respond physiologically and morphologically to light gradients, particularly in the context of resource allocation and storage, remains an important research gap.

Light availability resulting from patterns of closed canopy vs. canopy gaps has a strong effect on plant morphology, physiology, and resource-acquisition strategies (Walters & Reich, 1996; Reich et al., 1998a,b). Silver fir shows high morphological and physiological plasticity, forming shade needles optimized for low-light capture and sun needles for light use efficiency and stress protection (Robakowski et al., 2004; Dörken & Lepetit, 2018). In shade, fir seedlings carry out photosynthesis at a low rate sufficient for tissue maintenance but not rapid growth, while light exposed seedlings typically accumulate more sugars and starch to support growth (Cui & Smith, 1991; Johnson & Smith, 2005; Szuba et al., 2025). However, when fir saplings grow in patches under limited light, they may enhance carbon retention. Increased carbohydrate storage can then enhance plant shade and stress tolerance (Mayers & Kitajima, 2007). Moreover, shade-acclimated needles typically have higher N to support photoassimilate production, whereas seedlings in open, high-light conditions invest in protein-rich tissues for rapid growth and higher nutrient uptake (Niinemets, 1997; Robakowski et al., 2004; Wyka et al., 2008). Alternatively, nutrient uptake requirements of shaded firs may be modest and excess N may accumulate in slow growing tissues (Hawkins et al., 1998). To clarify these relationships, we must assess the degree to which individuals that establish in higher light, canopy gaps tend to exhibit belowground traits contributing to higher resource acquisition needed for rapid growth, while in shaded conditions where energy is limited, roots and mycorrhizal characteristics manifest a conservative carbon allocation.

Belowground trait plasticity is a key determinant of plant responses to altered growing conditions. Individuals growing in higher light availability usually allocate more biomass to roots, boosting fine root surface area and specific root length to enhance nutrient and water uptake in environments with higher evaporative demand (Reich et al., 1998a; Reich, 2002; Parasquive et al., 2023; Zhang et al., 2012). These acquisitive strategies involve metabolically costly, short-lived fine roots that are sustainable under high carbon supply. In shaded settings, where carbon is limited, fir seedlings should develop smaller, more persistent root systems with higher tissue density, reflecting a conservative resource use approach (Walters et al., 1993; Poorter et al., 2012; Kuehne et al., 2016). These patterns correspond to the fast–slow conservation gradient in root economics (Roumet et al., 2016; Weemstra et al., 2016), and also interact with mycorrhizal collaboration strategies that focus on nutrient acquisition through

fungal partners (Bergmann et al., 2020; Laughlin et al., 2024). However, it is unknown whether mycorrhizal fungi are filtered by light limiting conditions in a way analogous to selection along a nitrogen availability gradient, favoring only the most effective partners (Kummel & Salant, 2006; Zheng et al., 2015; Neuenkamp et al., 2021; McPherson et al., 2024). This unanswered question is particularly important as the ectomycorrhizal (ECM) symbiosis is a critical component of silver fir regeneration, substantially enhancing nutrient and water uptake and improving seedling survival (Kowalski, 1982; Cremer, 2009; Ważny, 2014; Rudawska et al., 2016). Despite limited carbon availability, even strongly suppressed fir seedlings maintain high levels of ECM colonization (>90% of fine roots), indicating sustained carbon allocation to fungal partners under shade (Ważny, 2014). Regeneration density and light availability are therefore likely to drive shifts in ECM community composition through carbon and nutrient tradeoffs. Dense clusters with overlapping root systems may favor selective, often late-successional fungal taxa shared among neighboring individuals, leading to low within patch fungal diversity (Ważny, 2014). In contrast, seedlings and saplings in high light gaps can supply more carbon to ECM fungi, promoting taxa with long- and medium-distance exploration strategies that support rapid nutrient and water acquisition (Pellitier et al., 2021; Pellitier & Zak, 2021; Baranowska et al., 2023). Such shifts in fungal community structure may strongly influence regeneration success under contrasting canopy conditions.

The aim of this work was to understand how forest disturbances associated with the sudden decline of Norway spruce modulate the growth of silver fir seedings and saplings within a closed canopy and in canopy gaps with higher light availability. We measured the morphology and biochemistry of fine roots and needles and assessed mycorrhizal communities to better understand how juvenile silver firs acquire resources and persist during natural regeneration under a closed canopy and in canopy gaps following spruce dieback. In addressing these issues, we hypothesized that: 1) higher light availability would generally enhance fine root production of higher absorptive potential (thinner roots of longer specific root length) than shaded firs growing in a closed canopy; 2) light limitation would have a negative effect on non-structural carbohydrate concentration (TNC) in fir needles and roots compared with understory trees growing under higher light availability; 3) under higher light availability gaps, silver firs would host a more diverse ECM fungal community and more frequently display long-distance morphotypes relative to individuals subjected to greater light limitation under closed canopies.

Material and Methods

Study site

The study site was located in south-western Poland in the Szczeliniec forest district of the Góry Stołowe National Park (50.503333N, 16.320780E; Central Sudetes). The elevation is 682 m a. s. l. The research site (6.26 ha) occupied a flat area with a uniform slope to reduce potential variation among plots. The climatic characteristics of this site are representative of the Stołowe Mountains region, which is part of the Sudetes Mountains. The soil type is acid brown developed on eroded siliceous and aluminosilicate parent material with relatively homogeneous properties (Polish Forest Data Bank: https://www.bdl.lasy.gov.pl). The annual mean temperature is 5.8 °C for 1970–2000, the mean temperature of the warmest quarter is 14.2 °C, and the minimum temperature of the coldest quarter is −2.8 °C. The annual precipitation is 645 mm, and the precipitation of the warmest quarter is 266 mm (WorldClim database: http://www.worldclim.org).

We also monitored hydrometeorological conditions from 4 April to 30 September 2025, covering the growing season (180 days). Meteorological data were collected with an automatic ATMOS 41 All-in-One station (METER Group, Inc.) installed in a canopy gap within the study area. The station continuously recorded air temperature (°C), relative humidity (%), wind speed and direction (m s^{-1}, °), incoming solar radiation (W m^{-2}), and precipitation (mm) at 60-minute intervals.Data were logged by a ZENTRA ZL6 data logger and automatically transmitted to the ZENTRA Cloud platform for storage and remote access. For analyses, daily and monthly summaries were derived, including precipitation totals and mean values for the remaining parameters. The station was mounted at a height of 2 m above ground level.

Experimental design

A multigenerational mountain mixed forest stand (*Luzulo luzuloidis-Fagetum typicum*) is composed predominantly of 85-year-old *Picea abies* (300 m^3 ha^{-1} on average) and *Abies alba* (70 m^3 ha^{-1} on average), with admixtures of *Larix decidua* Mill., *Pinus sylvestris* L., and *Betula pendula* Roth. of comparable age. Locally occurring older silver fir and Norway spruce trees (~115 years) are a continuous seed source across the plot. The site is fenced and has experienced the absence of silvicultural interventions for 20 years, resulting in a heterogeneous spatial structure and substantial accumulation of coarse woody debris and standing stumps. These unmanaged mortality and regeneration dynamics provide a valuable context

for analyzing forest regeneration processes in the absence of recent silvicultural treatments.

Within the site we selected ten study plots (c. 5 ares each): five with patches of fir saplings growing under closed fir–spruce canopy, and five where fir seedings and saplings have experienced greater canopy opening and light transparency since 2022. Gaps of c. 3 ares resulted from spruce decline caused by the spruce bark beetle, *Ips typographus* (L.) (A. Mazur, R. Witkowski, K. Sankowski – personal observation). In 2025 alone, their infestations destroyed over 1,600 ha of forest in the Wrocław Regional Directorate of State Forests, with the majority of losses occurring in the Sudetes Mountains (RDSF Wrocław 2026). All individuals classified as seedings or saplings in this study were naturally regenerated (no artificial planting of silver fir or spruce occurred in the past 20 years). To test whether spruce decline in the overstory alters soil moisture and light availability in the understory, we monitored moisture and light throughout the growing season. Within each plot, soil moisture and temperature were measured using 30 cm long Drill & Drop Bluetooth probes (Sentek Technologies) (Fig. S1). Probes were installed beneath the organic soil horizon and recorded volumetric soil moisture (m^3 m^{-3}) and temperature (°C) at three depths (5, 15, and 25 cm), representing the 0–30 cm soil layer. Measurements were logged at 60-minute intervals and time synchronized with the meteorological station. The data collected by the probes were pulled into daily (mean, maximum, and minimum) and monthly averages.

Soil water reserve in the 0–30 cm soil layer (Z, mm) was calculated as the total amount of water stored within this layer, expressed as a sum across the three depth intervals represented by the sensors. Depth-averaged soil temperature for the 0–30 cm layer was calculated as the mean of the three temperature measurements. For each plot, the seasonal water reserve amplitude was computed as Zmax − Zmin over the monitoring period.

Photosynthetically active photon flux density (PPFD) was measured during sunny, full-light conditions on the same plots using a quantum meter (Apogee Instruments Inc. Logan, USA) calibrated in quantum units (μmol photons m^{-2} s^{-1}). At each of the ten plots, we sampled three positions within the patch: 15 cm inside the southern edge, one locations in the central area, and 15 cm inside the northern edge. The sensor was moved along the patch from south to north, and the three readings were averaged to give a single PPFD value per plot. Two light meters were used simultaneously to record PPFD at heights of 0.5 m, 1.0 m, and 2.0 m above the ground at each of the three positions and at an adjacent open reference site; these paired measurements were used to calculate relative PPFD for the regeneration patches. Briefly, for each measurement point and height, we first averaged the three within patch PPFD measurements, then calculated the relative PPFD by dividing that mean by the simultaneous PPFD measured in the open reference and expressing the result as a percentage. This procedure enabled calculation of relative PPFD under each canopy variant, reported as the percentage of incident irradiance that reaches the understory. The calculation is:

$$\text{Relative PPFD (\%)} = (\text{PPFD}_{\text{patch}} / \text{PPFD}_{\text{open}}) \times 100$$

where $\text{PPFD}_{\text{patch}}$ is the mean of the three readings at a given height and $\text{PPFD}_{\text{open}}$ is the concurrent open-site value.

Apical dominance ratio

The effect of light availability on shoot growth was evaluated using the light factor, defined as the ratio of apical shoot length to the lateral shoot length of the last whorl (Parent & Messier, 1995; Robakowski et al., 2004). Measurements were taken on silver fir in the fall (end of September), when seasonal shoot extension was completed. Measurements were conducted on seedings as well as small and high understory saplings growing within the same patch. At each of the ten measurement patches (i.e. 5 closed canopy areas and 5 canopy gaps), three seedings, three small and three high saplings were randomly selected. For each selected individuals, we measured the length of the apical and the twigs forming the upper whorl with a ruler. Values ≥ 1 indicate favorable light conditions and high tree vigor, while values < 1 suggest light limitation and reduced apical dominance.

Evaluation of site structure and tree spatial patterns

Stand structure was surveyed at permanent control points (PCPs) corresponding to the measurement plots described above. FieldMap technology (www.fieldmap.cz) was used to precisely locate and map individual stand components by recording distances and azimuths from each plot center. Ten circular measurement plots (radius = 12.61 m; area = 500 m^2) were established, and their centers also served as installation points for the monitoring equipment.

All trees with a diameter at breast height (DBH) ≥ 12 cm were inventoried. DBH was measured for every tree and height was measured for a subsample within each plot. Following standard dendrometric principles, we selected the trees closest to each plot center for height (h) measurements. Within each

circular sample plot, seedings and saplings were classified by spatial pattern as patches or scattered groups. Additionally, three concentric subplots were established to assess seedings and sapling layers. All tree species except current year seedlings were identified and measured within the 10 m^2 subplot (r = 1.78 m). All trees with a height (h) ≥ 0.5 m were recorded within the 20 m^2 subplot (r = 2.52 m). In the 50 m^2 subplot (r = 3.99 m), all trees with a DBH ≥ 2 cm were measured (Fig. S2). Based on the data collected from the internal circular subplots, we determined the density of seedings (h ≤ 0.5 m), low saplings (0.5 m > h ≤ 1.3 m), and high saplings (h > 1.3 m; DBH < 7 cm).

Based on the collected data, the following stand structural parameters were determined for live trees with a diameter at breast height (DBH) ≥ 12 cm: mean DBH of living trees (cm), total basal area (G, m^2 ha^{-1}), and tree density (N ha^{-1}). Based on height measurements of sample trees, we constructed height-DBH curves (species-specific for fir and spruce) according to the Näslund function (1936): where h refers to tree height, DBH is the diameter at breast height (1.3 m), a and b are model parameters. Then following standard dendrometric procedures (Bruchwald, 1995) we calculated tree volume (v, m^3) using the measured DBH, modeled height, and species-specific form factors (f) obtained from empirical dendrometric tables (Szymkiewicz, 2001). Finally, individual tree volumes were summed in order to determine the total stand volume (V, m^3 ha^{-1}).

Root sampling

Root samples of silver fir were collected for trait characterization in May and late September 2025 from each plot. These dates were chosen to coincide with spring needle emergence and the end of growing season. In each of the 10 plots we selected five individuals from each size class: seedings, low saplings, and high saplings with no visible symptoms of damage caused by insects or pathogens. Samples from five trees were taken within each size class from a single patch and then were pooled as a single sample. Thus, we analyzed 30 tree samples from each canopy variant and understory size class, for a total of 300 sampled trees when both seasons were pooled.

From each silver fir, we collected one sample of intact root branches containing at least six individual root orders. The roots were sampled at depths of c. 0–10 cm. To sample root branches, we carefully removed plant litter to locate main lateral roots emerging from the trunk, then followed subsequent root branches to exposed distal root tips. The root branches were removed from lateral roots using pruning scissors. We did not immediately separate roots from adhered organic and inorganic particles in the forest to preserve intact terminal orders. Roots were transported back to the lab, where soil and adhering materials were carefully removed with gentle washing in a water bath. The root branches generally represented all roots < 1mm in diameter. Roots were kept in plastic bags and stored in the refrigerator (at c. 5 °C) until further analysis which was typically completed within 14 days. During the storage period, the bags were opened and fresh moistened towels were changed daily.

Needle sampling

One-year-old c. 30 needles were collected from the same trees used for root sampling to determine specific leaf area (SLA; needle surface area to dry weight ratio, cm^2 g^{-1}) and for subsequent nitrogen and carbohydrate analyses. Needles were taken from the exposed section of the one-year-old vascular shoot growth in the central part of the crown.

Morphological and chemical analysis

Once removed from the bags, the roots were scanned with the Epson Perfection V800 PHOTO desktop scanner (transmitting light system) in gray scale at 600 dpi. Images of individual root orders were analyzed for length and diameter for each subplot separately using WinRHIZO software (Regent Instruments, Inc., Québec, QC, Canada). Root average diameter, fine root tip number, and root length in defined diameter classes (0–0.2 mm, 0.2–0.4 mm, 0.4–0.6 mm, 0.6–0.8 mm, 0.8–1.0 mm) were estimated in intact fresh branches. The roots were dried at 65 °C for 3 days and weighed to enable the determination of SRL (fresh root length per unit root dry mass, m g^{-1}), RTD (root dry mass per unit fresh root volume, g cm^{-3}), and SRA (fresh root area per unit of root dry mass, cm^2 g^{-1}).

Additionally, specific leaf area (SLA) was determined using the same Epson Perfection V800 scanner, and needle surface area was calculated with WinFOLIA software (Regent Instruments, Inc., Québec, QC, Canada). Needles were then dried at 65 °C for 3 days to obtain dry mass.

The dried root segments and needles were ground into powder and analyzed for C and N concentrations using gas chromatography. To explore patterns of carbohydrate allocation within canopy variant types and across seasons, concentrations of total non- structural carbohydrates (TNC) were determined. TNC, defined as the sum of soluble carbohydrates and starch, was determined using a two-step analytical procedure (Łukowski et al., 2022). Soluble carbohydrates were first extracted in a methanol–chloroform–water solution and assayed spectrophotometrically following a color reaction with anthrone. The remaining

precipitate was subsequently used for starch determination. This process involved the enzymatic hydrolysis of starch into glucose using amyloglucosidase, followed by oxidation with a peroxidase–glucose oxidase complex. Starch concentrations were measured after reacting with dianisidine. All concentrations were calculated against a glucose standard and expressed as a percentage of dry mass (% d.m.).

Analysis of fungal communities colonizing root during the growing season

For analysis of fungal communities colonizing roots the same individuals, timing, and procedure as the samples for root morphology/chemistry was used.

Roots were gently cleaned of adhering soil particles while preserving root structure. Surface sterilization was performed following Maitra et al. (2024) by a single immersion in 70% ethanol for 1 min, followed by immersion in 15% hydrogen peroxide for 1 min. After each sterilization step, roots were rinsed three times with distilled water. Sterilized samples were stored at −20 °C until further analyses. Frozen roots were ground in liquid nitrogen using a sterile mortar and pestle. DNA was extracted from 0.1 g of homogenized tissue using the GeneMatrix Plant & Fungi DNA Purification Kit (EURx Ltd.). DNA quantity and quality were assessed using a NanoDrop ND-1000 spectrophotometer and by electrophoresis on a 1% agarose gel.

DNA quality control, amplification, and sequencing of the fungal ITS region (ITS2) were performed by Genomed. Prior to library preparation, genomic DNA concentration was quantified fluorometrically using the PicoGreen reagent. Libraries were prepared using a two-step PCR protocol with primers specific to the targeted region: ITS3/GCATCGATGAAGAACGCAGC and ITS4/TCCTCCGCTTATTGATATGC. The first PCR amplified the target fragment and appended adapter sequences for subsequent indexing. Despite repeated attempts and modifications of PCR conditions for samples representing silver fir seedlings in the studied variants, libraries could not be obtained for these samples. The most likely reason was PCR inhibition, which persisted despite dilution of the reaction mixture. After each PCR reaction, libraries were purified using magnetic beads, and library concentration was measured fluorometrically with PicoGreen. Sequencing was conducted on an AVITI platform using paired-end (PE) chemistry. Sample demultiplexing and FASTQ file generation were performed using MiSeq Reporter (MSR) v2.6.

Bioinformatic analyses were carried out in QIIME 2 (v2024.5) (Bolyen et al., 2019) using the UNITE_10 reference database. The analytical workflow is described below. Adapters and primers were removed using Cutadapt (v4.7) (Martin, 2011). At this stage, quality trimming was applied using a Q30 threshold and a minimum sequence length of 30 bp. Quality-filtered reads were denoised using DADA2 (Callahan et al., 2016) implemented in QIIME 2. Paired reads were merged into longer sequences, and reads that could not be merged were discarded. Chimeric sequences were removed, and sequences were resolved into amplicon sequence variants (ASVs). ASVs were clustered based on sequence similarity using a 99% threshold.

Taxonomy was assigned to ASVs using the UNITE_10 reference database (Quast et al., 2013) with a hybrid classification approach consisting of: (i) preliminary ASV classification using VSEARCH (Rognes et al., 2016) in sequence-alignment (similarity search) mode; taxonomy was assigned based on the best hit meeting the selected criteria (minimum sequence identity 50% and minimum query coverage 80%); and (ii) classification of sequences that remained unassigned after step (i) using a Naive Bayes classifier with a minimum confidence threshold of 0.7.

ASV sequences resulting from denoising were aligned using MAFFT (Katoh et al., 2019). Phylogenetic trees were then inferred using FastTree (Price et al., 2009). Both tools were run within the QIIME 2 environment. In addition, functional assignment was conducted using the FungalTraits database (Põlme et al., 2020) to evaluate whether fungal communities associated with different age classes comprised analogous functional groups of ectomycorrhizal fungi.

Raw read data from sequencing have been submitted to the NCBI Sequence Read Archive (SRA) under the BioProject with accession number PRJNA1475843.

Statistical analyses

Morphological and biochemistry data were log-transformed to ensure normal data distribution across treatments and analyze data expressed as %. Figures, however, show non-transformed data for clarity. Outliers were excluded from analyses when they were 2 times higher/lower than the values comprising 75% of observations. The main and interaction effects of study variant (closed canopy and canopy gaps), season (spring and fall), and silver fir size classes (seedings, low and high saplings) were determined using a three-way ANOVA. Differences between mean values were then analyzed using Student's *t*-test or we used Tukey's HSD as a post-hoc test. For tree density and volume, only two groups were compared, so the data were analyzed using a t-test.

Analyses were conducted using JMP (SAS Institute Inc., Cary, NC, USA).

To minimize the impact of potential contaminants to fungal communities colonizing roots, fungal amplicon sequence variants (ASVs) with a total abundance below 10 reads were removed from the dataset. To account for varying sequencing depths, samples were normalized to a consistent depth of 50,000 reads via 1000-fold rarefaction using the rarefy function (GNuniFrac package; Chen et al., 2012), with subsequent averaging of the iterations. Alpha diversity indices, including Shannon–Wiener and Simpson's indices, were calculated using the diversity function, while species richness was determined via specnumber (package vegan v2.5-7; Oksanen et al., 2021). Pielou's evenness was derived as the ratio of the Shannon index to the logarithm of the observed species richness.

The normality of data distribution was assessed using the Shapiro–Wilk test. Given that the assumptions for parametric testing were violated, differences between groups were evaluated using the non-parametric Kruskal–Wallis H test. Post-hoc comparisons were conducted using Dunn's test with the dunn.test package to identify specific significant differences between groups. Furthermore, the relationships between plant physiological traits and Shannon diversity were analyzed using Spearman's rank correlation coefficient (rho).

To detect notable differences in the relative abundance of fungal taxa among the studied groups, differential abundance analysis was conducted using the DESeq2 package (v1.x.x). Although initially developed for RNA-seq, DESeq2 is also a reliable tool for marker-gene survey data, such as ITS amplicon sequencing, thanks to its advanced management of overdispersion.

Beta diversity was assessed based on Bray–Curtis dissimilarity matrices. The influence of experimental factors (tree root zone, treatment, and time points) and edaphic parameters on fungal community structure was tested using Permutational Multivariate Analysis of Variance (PERMANOVA, adonis2 function in vegan). Variations in community composition and were visualized through Non-metric Multidimensional Scaling (NMDS) ordinations. Relative abundance plots were generated using the phyloseq framework (McMurdie & Holmes, 2013).

Computational procedures were executed in R (v4.1.2) and RStudio (v2021.09.2-382). Statistical significance for all analyses was defined at a threshold of $P \leq 0.05$.

Results

Weather conditions

Total precipitation during the observation period (April–September) was 389 mm, averaging 2.2 mm d^{-1}. Monthly totals were highest in September (91.3 mm) and July (82.3 mm) and lowest in April (40.6 mm) and June (52.3 mm). The mean relative humidity over the study period was 84.1%, with the lowest monthly averages in April (78.4%) and June (81.1%), and the highest in September (93.7%). Monthly averages for the remaining months ranged from 82.2% to 86.1%. Air temperature ranged from −4.9 °C (6 April) to 28.3 °C (15 August), with a daily mean of 12.4 °C. The lowest monthly mean temperatures were recorded in April (8.3 °C), May (8.2 °C), and September (11.9 °C); summer monthly means (June–August) ranged from 15.0 °C to 15.4 °C (Table S1).

Soil moisture and temperature

Soil water reserve in the 0–30 cm soil layer showed a consistent seasonal pattern. It was highest in spring, then declined steadily through summer despite occasional rainfall (evapotranspiration > precipitation), reaching a minimum at the end of August, and partially recovered in September, when lower temperatures and higher precipitation totals led to a clear recovery across the study area (Fig. S3). The lowest mean reserves was observed at the canopy gap plot P9 (Table S2). Soil temperature in the 0–30 cm soil layer (depth-averaged) varied aslo among plots (Table S2). Most plots recorded mean temperatures between 9.1 and 9.8 °C. Minimum temperatures ranged from 1.5 °C (P8) to 3.7 °C (P6) at the closed canopy plots, and maximum temperatures ranged from 12 °C (P8) to 14.6 °C at the canopy gap plot (P9).

Light availability

Compared with the closed canopy, gaps created by spruce dieback were characterized by higher photosynthetically active radiation availability. Midday photosynthetically active radiation in canopy gap plots was 1.5 times greater than under the closed canopy (Fig. S4, $P<0.001$).

Site structure and tree spatial patterns

We found a nonsignificant trend in tree density across the study plots ($P = 0.055$). In plots classified as the fir–spruce canopy variant, the number of mature trees was more than twice (c. 250 tree ha^{-1}) that observed in plots categorized as canopy gaps (Fig. 1)

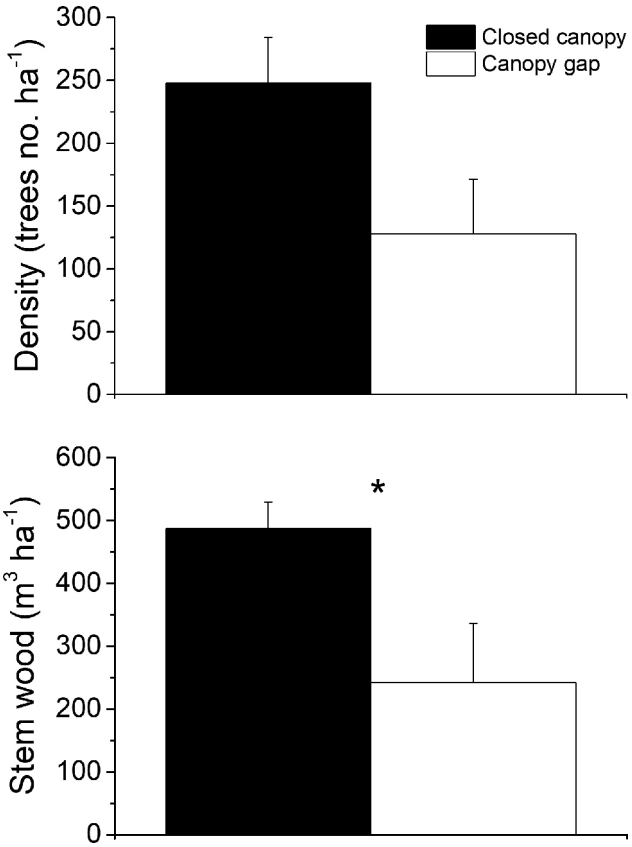

Fig. 1. Tree density (a) and total stand volume (b) in closed canopy and canopy gap plots; values are shown as mean ± SE across plots. Values were transformed but charts show non-transformed data for clarity. Asterisks indicate significantly different means between closed canopy and canopy gaps at P <0.05 (*), at P < 0.01 (**), and at P < 0.001 (***) according to Student's t-test

with relatively high variability within each study variant. Differences in volume were significantly related to canopy variant, with values in the fir–spruce canopy plots being approximately double (almost 500 m^3 ha^{-1}) those in the canopy gap plots (Fig. 1, *P<0.05*).

For the understory size classes (seedings, low saplings, high saplings), silver fir density under closed canopy was approximately twice that of Norway spruce in the first two size categories: seedings 53% and low saplings 57%. High Norway spruce saplings were generally absent (Fig. 2). In canopy gaps (i.e. under higher light availability) densities of low saplings did not differ between silver fir and Norway spruce, but spruce had about four times higher density of seedings when compared with silver fir, while the opposite pattern occurred for high saplings (Fig. 2). Comparing size classes within species revealed that nearly half of all small young trees were in high sapling class (46%) under canopy gaps and the smallest percent be for seedlings (c. 18%). This indicates a marked reduction of silver fir individuals as light availability increases.

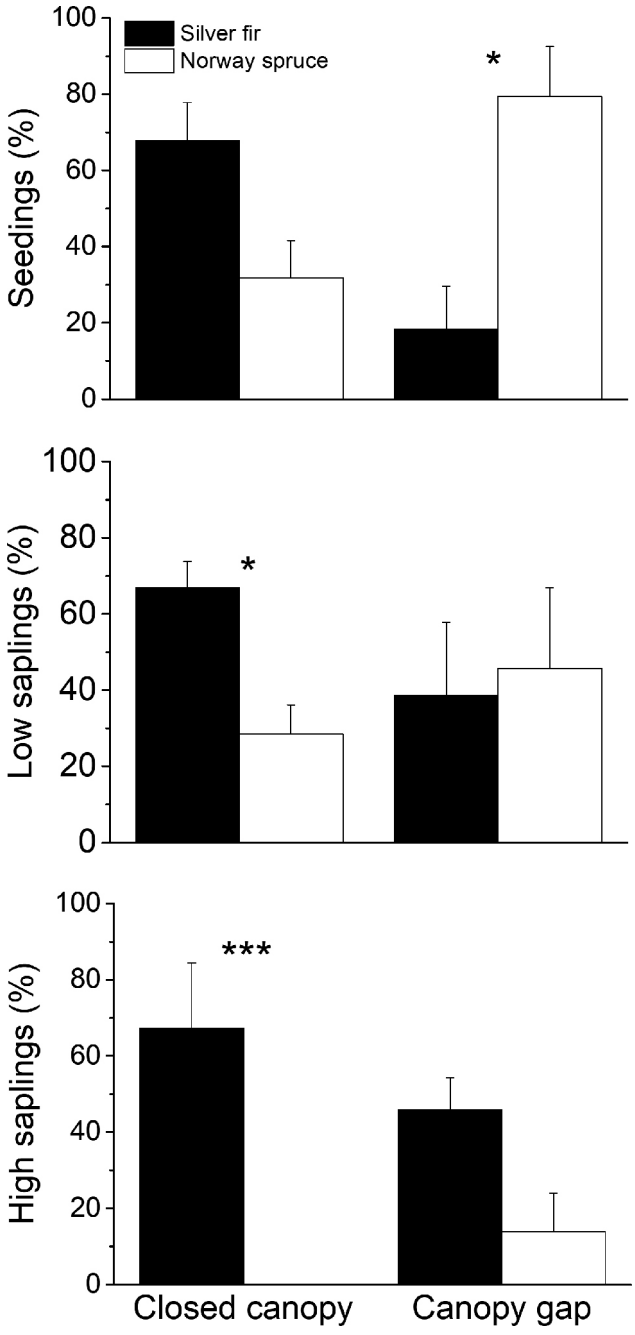

Fig. 2. Densities of silver fir (black columns) and Norway spruce (open columns) for seedings (upper panel), low saplings (middle panel), and high saplings (lower panel) in closed canopy and canopy gap plots, given as percent of total understory trees per hectare (numbers per hectare). Values were transformed but charts show non-transformed data for clarity. Asterisks indicate significantly different means between species within closed canopy and canopy gaps at P <0.05 (*), at P < 0.01 (**), and at P < 0.001 (***) according to Student's t-test

Shoot and root morphological traits

The apical dominance ratio differed between canopy variants, with saplings in canopy gaps developing longer apical shoots relative to lateral twigs

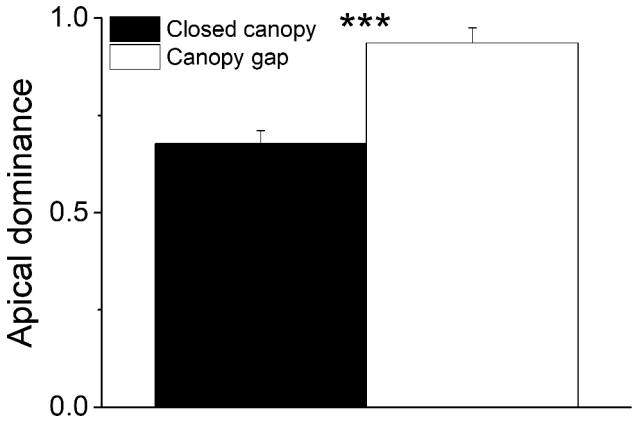

Fig. 3. Apical dominance ratios of silver fir compared between closed canopy and canopy gap plots. Values are shown as an average means of seedings and low and high saplings ± SE. Values were transformed but chart shows non-transformed data for clarity. Asterisks indicate significantly different means between closed canopy and canopy gaps at P <0.05 (*), at P < 0.01 (**), and at P < 0.001 (***) according to Student's t-test

(Fig. 3; *P<0.001*). The pattern of variation in apical dominance values across seedings and saplings was consistent in both canopy variants (Fig. S5).

Examination of fine root morphology revealed differences in selected traits between closed canopy and canopy gap growth conditions (Table 1). In canopy gaps, silver fir individuals exhibited clear shifts toward resource-acquisitive morphology. Fine roots of canopy gaps understory individuals were significantly thinner than those of the closed canopy (on average by 9% smaller diameter; Fig. 4, *P = 0.008*). This reduction in fine root diameter corresponded with longer specific root length (on average by 24%) and specific root area (on average by 14%), indicating a greater length of fine roots per unit biomass in case of individuals growing in brighter gaps (Fig. 4, *P<0.01*). This resulted from individual root orders becoming thinner as the branching ratio (number of root tips divided by total length reported) did not differ significantly (data not shown). In contrast, individuals under the closed canopy had thicker fine roots of shorter SRL. No other differences were observed for RTD which highlights fine root diameter, SRL and SRA as the primary divergent traits (Table 1). Furthermore, there were no significant changes in most diameter classes, with only the 0.2–0.4 mm category decreasing significantly indicating that higher order roots did not change significantly, but rather the finest order roots were responsible for most changes in diameter and subsequently SRL. Moreover, a trend of greater specific leaf area (on average by ~9.5%), although not statistical significant, under closed canopy was observed in the one-year-old needles compared with those growing in plots with greater light transmission (Table 1, P = 0.074).

Carbohydrate and nitrogen status

Marked differences in carbohydrate concentration were observed between canopy variants, but surprisingly, the expected reduction in sugar concentration with reduced light availability was not observed (Table 2, Fig. 5). Contrary to initial assumptions, understory individuals growing under closed canopy accumulated significantly more total non-structural carbohydrates (TNC) in their tissues compared

Table 1. Results of analysis of variance with a combination of canopy variant (C), season (S) and understory fir size classes (U) on plant traits: *A)* root diameter, specific root length (SRL), specific root area (SRA), root tissue density (RTD), specific leaf area (SLA); *B)* fine root length (L) in five diameter category (0–0.2 mm; 0.2–0.4 mm, 0.4–0.6 mm, 0.6–0.8 mm, 0.8–1 mm)

Characteristic		d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
			Root diameter		SRL		SRA		RTD		SLA	
A) Morphological traits	C	1	7.06	*0.008*	9.87	*0.002*	10.17	*0.002*	1.7	0.198	3.33	0.074
	S	1	23.42	*<0.001*	1.58	0.213	42.07	*<0.001*	326.6	*<0.001*	0.53	0.466
	U	2	4.67	*0.014*	8.26	*<0.001*	11.07	*<0.001*	8.54	*<0.001*	3.42	*0.04*
	C×S	1	0.85	0.359	1.50	0.225	1.90	0.174	0.91	0.324	1.74	0.192
	C×U	2	1.18	0.313	0.98	0.384	0.80	0.450	0.97	0.386	0.22	0.801
	S×U	2	3.59	*0.035*	8.81	*<0.001*	13.40	*<0.001*	12.08	<0.001	0.32	0.725
	C×S×U	2	0.81	0.448	1.48	0.256	2.18	0.123	2.52	0.09	0.6	0.55
			0<L>0.2 mm		0.2<L>0.4 mm		0.4<L>0.6 mm		0.6<L>0.8 mm		0.8<L>1 mm	
B) Length category	C	1	1.33	0.253	5.77	*0.020*	2.80	0.100	0.58	0.449	4.44	*0.04*
	S	1	24.95	*<0.001*	0.22	0.640	20.98	*<0.001*	58.65	*<0.001*	26.25	*<0.001*
	U	2	4.64	*0.014*	2.74	0.074	4.90	*0.011*	8.99	*<0.001*	12.48	*<0.001*
	C×S	1	0.10	0.904	1.58	0.214	1.47	0.230	0.0	0.951	0.04	0.833
	C×U	2	1.65	0.201	2.81	0.070	0.71	0.494	0.75	0.477	0.65	0.524
	S×U	2	3.06	0.056	2.76	0.073	8.24	*<0.001*	14.59	*<0.001*	16.27	*<0.001*
	C×S×U	2	2.28	0.112	2.75	0.073	2.60	0.084	1.94	0.154	1.48	0.23

Italics indicate significant effect at *P*≤0.05.

with canopy gap conditions, irrespective of seasons. Significant variability in soluble carbohydrates and TNC concentrations existed with respect to canopy variant with sugar concentrations ($P<0.05$) being respectively 30%, 21% and 24% higher in the fine roots of shaded seedings, low saplings and high saplings growing in close canopy conditions (Fig. 5). We also observed a consistent trend of lower soluble carbohydrates, starch and TNC concentrations in

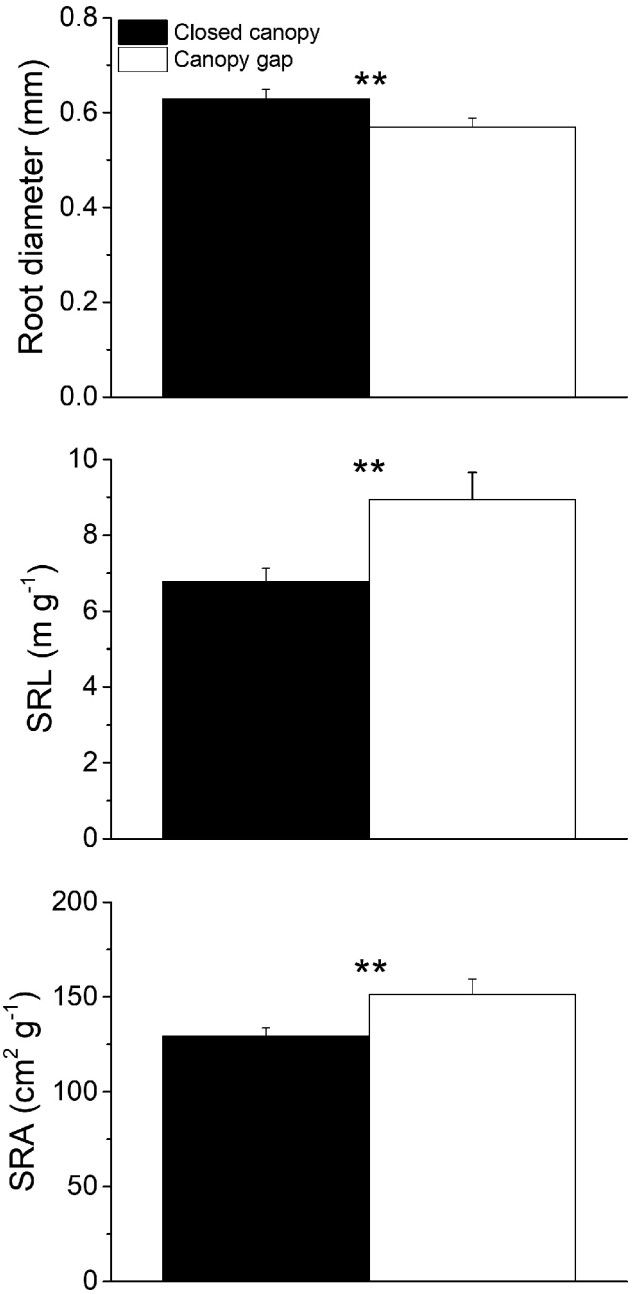

Fig. 4. Fine root diameter (upper panel), specific root length (SRL, middle panel) and specific root area (SRA, lower panel) of understory individuals of silver fir in closed canopy (black columns) and canopy gap plots (open columns); values are means ± SE for seedings, low saplings, and high saplings. Values were transformed but charts show non-transformed data for clarity. Asterisks indicate significantly different means between closed canopy and canopy gaps at P<0.05 (*), at P<0.01 (**), and at P<0.001 (***) according to Student's t-test

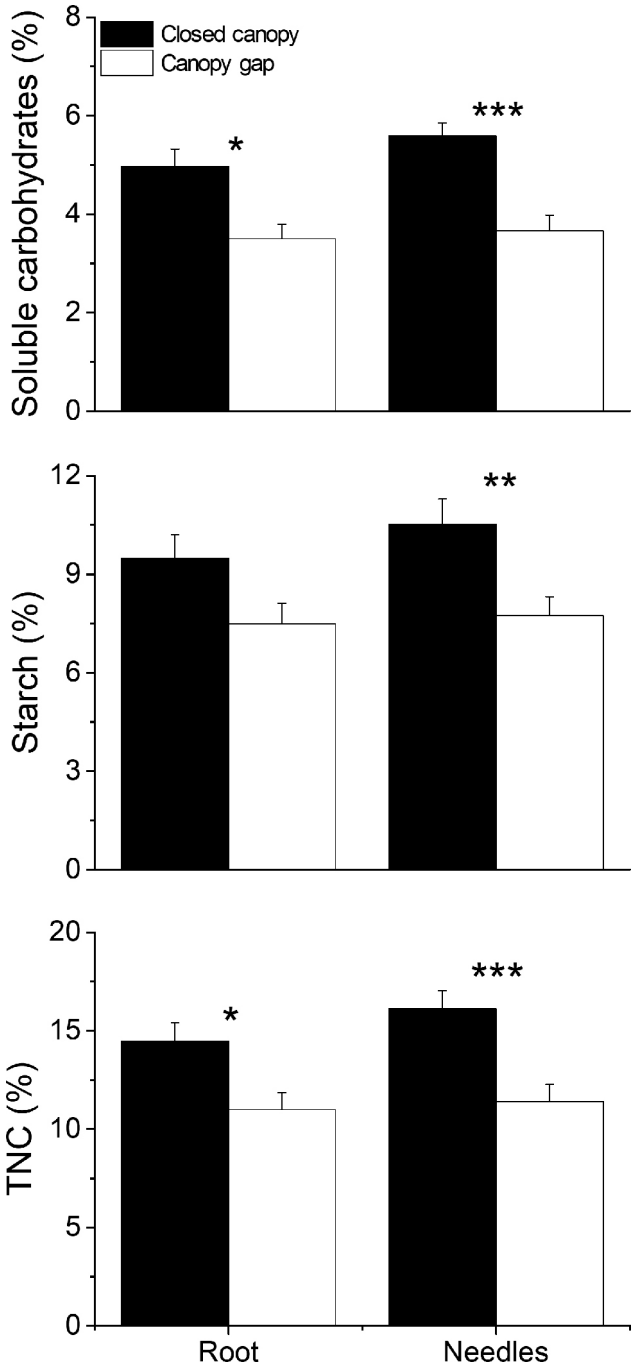

Fig. 5. Soluble carbohydrates (upper panel), starch (middle panel) and total non-structural carbohydrates (TNC) concentrations (lower panel) in silver fir fine roots (left side) or needles (right side) of understory individuals in closed canopy and canopy gap plots; values are means ± SE for seedings, low saplings, and high saplings. Values were transformed but charts show non-transformed data for clarity. Asterisks indicate significantly different means between species within closed canopy and canopy gaps at P<0.05 (*), at P<0.01 (**), and at P<0.001 (***) according to Student's t-test

Table 2. Results of analysis of variance with a combination of canopy variant (C), season (S) and understory fir size classes (U) on plant traits: *A*) SS = soluble sugars concentration, Starch concentration, TNC = total non-structural carbohydrates in fine roots or needles; *B)* nitrogen concentration, C/N = carbon:nitrogen ratio in fine roots or needles

Characteristic		d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
			Fine root SS		Fine root Starch		Fine root TNC		Needle SS		Needle Starch		Needle TNC	
A) Nonstructural carbohydrate concentrations	C	1	5.62	*0.021*	3.42	0.070	5.10	*0.028*	15.17	*<0.001*	7.22	*0.009*	10.97	*0.001*
	S	1	0.27	0.605	0.21	0.648	0.21	0.645	0.300	0.581	1.08	0.302	1.02	0.316
	U	2	0.0	0.994	0.24	0.780	0.13	0.874	0.060	0.934	0.31	0.729	0.16	0.850
	C×S	1	0.0	0.962	0.20	0.651	0.10	0.749	0.190	0.664	0.10	0.742	0.23	0.631
	C×U	2	0.1	0.896	0.13	0.871	0.09	0.911	0.132	0.876	0.28	0.753	0.26	0.765
	S×U	2	0.03	0.969	0.33	0.720	0.15	0.858	0.191	0.826	0.01	0.982	0.07	0.930
	C×S×U	2	0.2	0.871	0.68	0.511	0.44	0.646	0.163	0.849	1.47	0.239	0.979	0.383
			Fine root N		Fine root C/N		Needle N		Needle C/N					
B) Root nutrient concentration	C	1	20.08	*<0.001*	22.03	*<0.001*	22.39	*<0.001*	24.34	*<0.001*				
	S	1	3.05	0.087	8.56	*0.005*	6.55	*0.013*	2.29	0.136				
	U	2	4.25	*0.019*	5.25	*0.008*	1.00	0.374	1.05	0.355				
	C×S	1	0.21	0.646	1.22	0.270	0.10	0.749	0.08	0.770				
	C×U	2	4.19	*0.021*	3.45	*0.039*	0.28	0.753	0.20	0.819				
	S×U	2	0.18	0.835	0.22	0.800	4.02	*0.024*	4.11	*0.022*				
	C×S×U	2	3.05	0.056	2.91	0.063	3.34	*0.043*	3.77	*0.030*				

Italics indicate significant effect at $P \leq 0.05$.

needles of firs growing in canopy gaps (Fig. 5). By contrast, root N concentration in canopy gap saplings was about 11% greater than in close canopy variant (*P<0.001*), and needle N showed a similar trend (Table 2, Fig. 6). Consequently, individuals in canopy gaps had a lower C:N ratio, whereas shade-grown seedings and saplings growing under closed canopy held more carbon relative to nitrogen. These results support a trade-off: shaded firs retained more stored carbon, while seedings and saplings growing in greater light transmission invested more in N-rich tissues for growth.

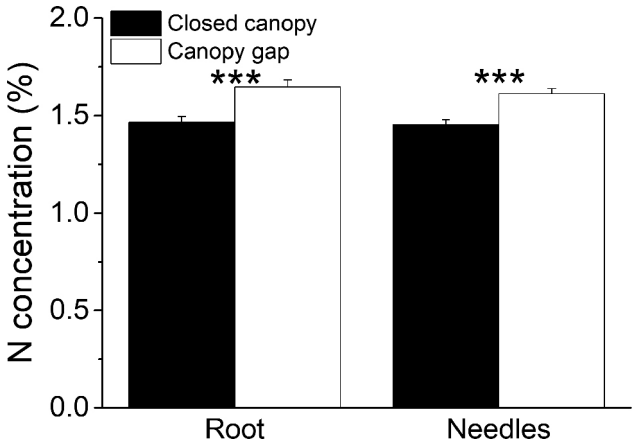

Fig. 6. Nitrogen concentration as a percentage of dry mass (N%) in fine roots (left side) and needles (right side) of understory individuals of silver fir in closed canopy and canopy gap plots; values are means ± SE for seedings, low saplings, and high saplings. Values were transformed but chart shows non-transformed data for clarity. Asterisks indicate significantly different means between closed canopy and canopy gaps at P<0.05 (*), at P<0.01 (**), and at P<0.001 (***) according to Student's t-test

Fungal diversity and community composition in silver fir roots

Distinct differences in root-associated fungal communities point to a potential regeneration niche partitioning via mycorrhizal symbiosis. In total, the sequencing identified 5968 fungal amplicon sequence variants (ASVs) on fir seedling fine roots, spanning seven fungal phyla. The communities were overwhelmingly dominated by ectomycorrhizal (ECM) fungi in the phyla *Basidiomycota* and *Ascomycota*, with their abundances strongly influenced by light availability (Fig. S6).

Season and size classes had no effect on the α-diversity indices of all the community and Ectomycorrhizal community (Table S3). However, canopy variant (closed canopy vs. canopy gap) significantly affected all α-diversity indices for all community (Shannon – *P<0.001*; Simpson – *P<0.001*) and ectomycorrhizal community (Shannon – *P<0.001*; Simpson – *P<0.001*; Pielou evenness – *P<0.001*) (Table S3). Fungal communities from canopy gaps were characterized by the highest Shannon index in contrast to the closed canopy, and this index was positively correlated with PPFD, and number of tips, but negatively with carbohydrates in needles (Table S4).

In contrast to season and size classes, canopy variant significantly affected the fungal community composition (Adonis – R2 = 0.03, P = *0.0142*). NMDS indicated that groupings according to this

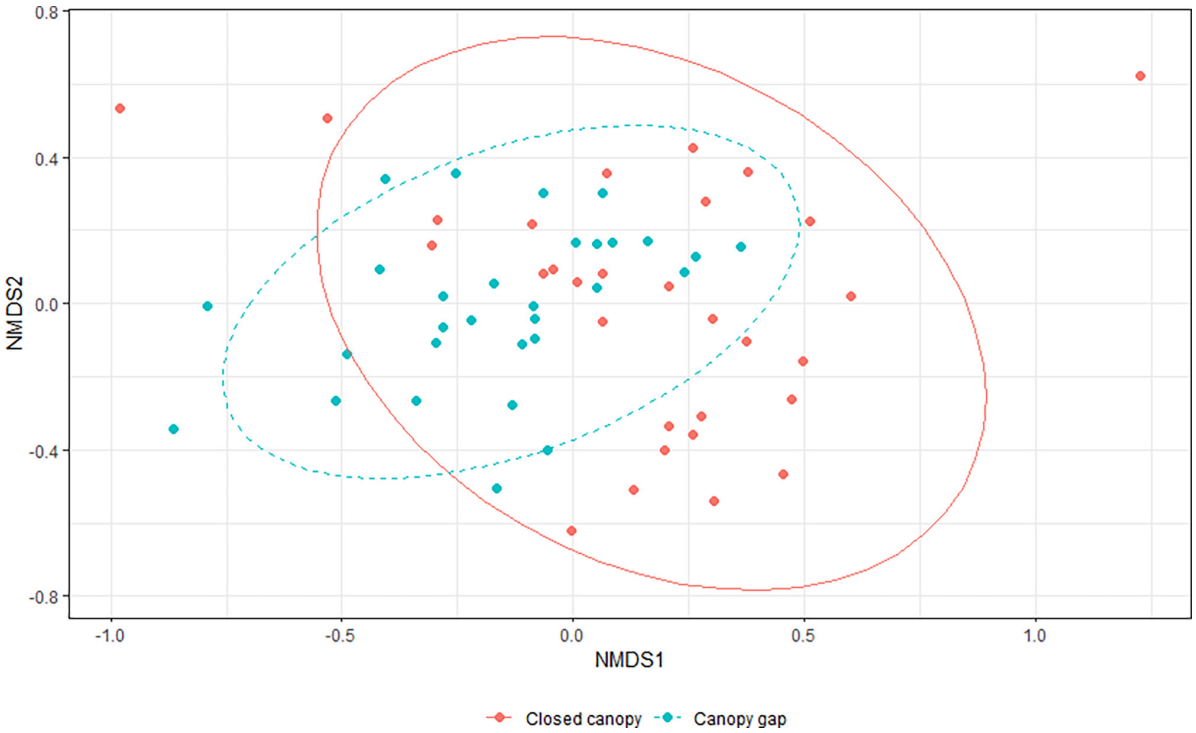

Fig. 7. NMDS on fungal community dissimilarity (Bray-Curtis). Each point represents the root fungal community of one sample. Ellipses: 95 % confidence interval for samples from canopy gaps (solid line, blue points) and closed (dotted line, red points) canopy

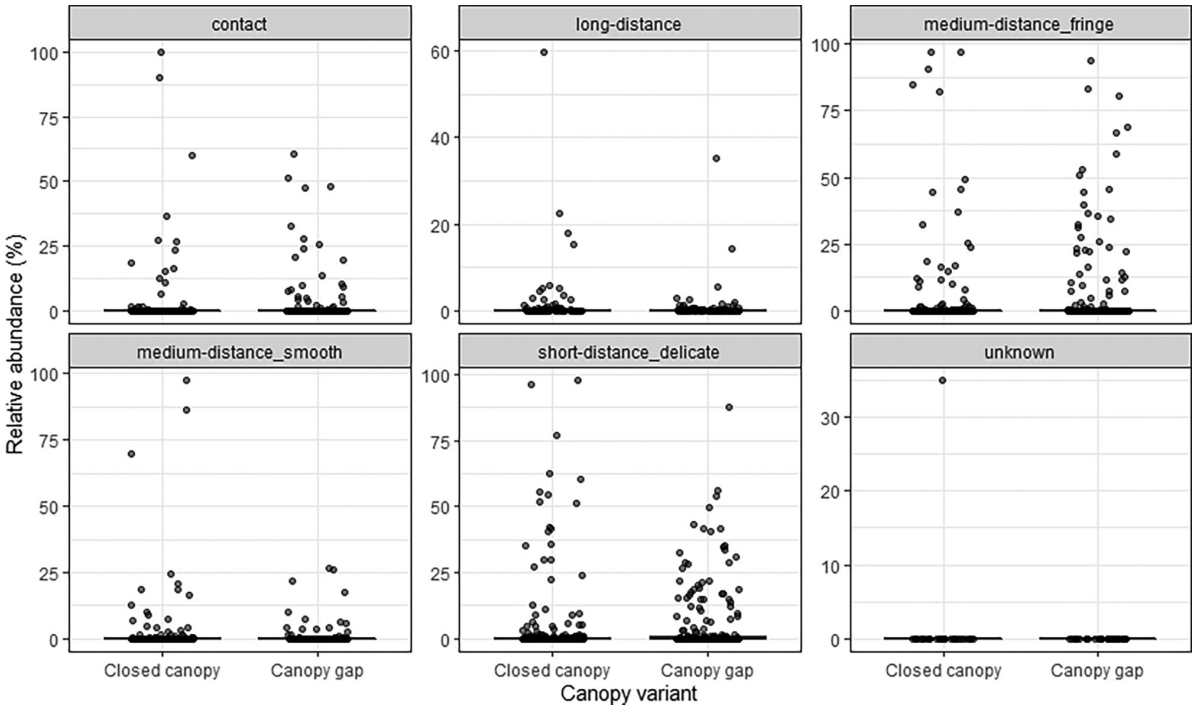

Fig. 8. Relative abundance of ectomycorrhizal exploration types in silver fir roots across canopy variants. Data points represent the relative abundance (%) of functional exploration strategies identified in root samples under closed canopy and canopy gap conditions. Exploration types are categorized into: Contact – direct contact between the mantle and the substrate, Long-distance – characterized by highly differentiated rhizomorphs, Medium-distance fringe and smooth – representing intermediate mycelial extension strategies, Short-distance delicate – indicating minimal mycelial outgrowth into the surrounding soil. The individual points denote biological replicates, with horizontal bars indicating the mean values. Statistical analysis revealed that while taxonomic composition underwent significant shifts (as seen in DESeq2 results), the overall functional distribution of exploration types remained relatively stable between the canopy gaps and closed canopy forest interior

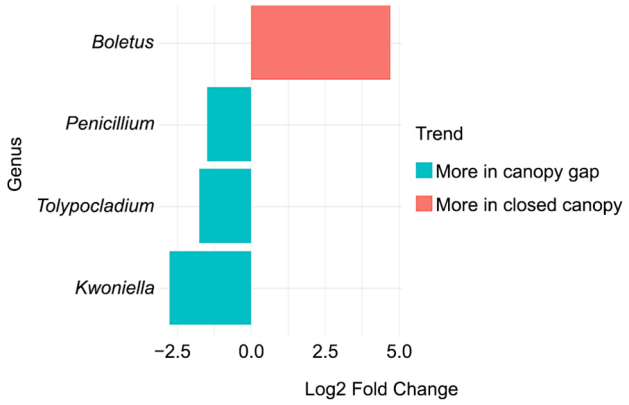

Fig. 9. Differential abundance of fungal genera between canopy variants. The chart displays log2 fold changes of significantly enriched fungal taxa ($P_{adj} < 0.05$). Positive values indicate enrichment in closed canopy plots, whereas negative values indicate enrichment in canopy gap plots

factor were evident (Fig. 7). While absolute abundance values suggested a proliferation of contact, short-, and medium-distance exploration types under canopy gap conditions, these trends were not maintained when analyzing relative abundance data, which showed no significant differences between the groups (Fig. 8, S7). Differential abundance analysis using DESeq2 revealed that canopy openness significantly influenced only four fungal genera ($P_{adj}<0.05$). *Boletus*, representing long-distance exploration type, was significantly enriched in the closed canopy variant (Fig. 9), showing the strongest positive response (Log2FC>5). Relative abundance of *Boletus* was positively correlated with carbohydrate concentrations in needles and roots and negatively with PPFD and SRL (Table S5). In contrast, *Penicillum*, *Typocladium* and *Kwoniella* were significantly associated with the canopy gap conditions (Log2FC<0).

Discussion

Our findings showed that canopy structure strongly shapes regeneration patterns and resource-acquisition strategies. In plots where the decline of mature spruce trees created canopy gaps and increased light availability, regeneration was more spatially dispersed due to increasing competition with spruce seedlings, whereas under closed canopies seedings and saplings occurred in more aggregated patches. These conditions were associated with shifts in belowground traits and symbiotic associations. In more open plots, seedlings produced thinner roots, indicating greater direct soil foraging capacity than those growing in more shaded patches. Shaded individuals developed thicker roots and stored more carbohydrates in both fine roots and needles. Mycorrhizal fungal communities also changed in diversity and functional composition with some taxa becoming more common in closed canopy areas, especially *Boletus* that form long-distance exploration types and are linked to high extraradical biomass and thus high carbon costs (Agerer, 2001; Lilleskov et al., 2011; Jörgensen et al., 2023). These patterns indicate that silver fir modifies root traits in response to canopy light availability to optimize belowground resource acquisition with greater light penetration.

Our results partially supported the first hypothesis that increased light availability would promote more acquisitive root traits. Canopy gap grown individuals produced thinner fine roots (by 9%) and showed longer specific root length (24%) and greater specific root area (14%). These traits reflect an acquisitive strategy that enhances absorptive surface area relative to biomass (Reich et al., 1998a; Roumet et al., 2016; Weemstra et al., 2016). Such shifts are expected when seedlings must intensify soil exploration to meet higher nutrient and water demand in warmer microsites (Paz, 2003; Poorter et al., 2012; Reich, 2014). In contrast, thicker roots and lower SRL in closed canopy patches align with a conservative strategy that limits construction and turnover costs under energy limitation (Walters et al., 1993; Kuehne et al., 2016). Unexpectedly, the specific leaf area (SLA) of fir individuals was found to be relatively similar regardless of light conditions. Thus, while root traits clearly responded to canopy openness, the lack of change in needle morphology may reflect insufficient contrast in light conditions to induce morphological acclimation or a divergence in organ level strategies (Weemstra et al., 2022).

Unexpectedly, carbohydrate concentration opposed our second hypothesis. Under denser shade, seedings and saplings had higher soluble sugars and TNC in fine roots, with needles showing the same trend. This likely indicates sink limitation, as growth and structural investments are constrained by shade, leading to accumulation as TNC reserves (Körner, 2003). Reserve carbohydrates improve low-light tolerance and stress resilience (Mayers & Kitajima, 2007), especially for fir near the carbon balance threshold (Walters & Reich, 1996). In canopy gaps, lower carbohydrate levels may result from rapid carbon use for growth, higher respiration in warmer conditions, and increased costs for water regulation and competition. Indeed, canopy gap individuals had higher N concentrations in roots and needles and consequently lower C:N ratios. High N accompanied by low TNC and more acquisitive traits i.e., longer SRL and thinner AD suggests that fir in canopy gaps prioritizes growth and resource acquisition strategy over carbon buffering when N availability may increase in canopy gaps, as increased SRL together with mycorrhizae may provide a parallel pathway for N foraging (Chen et al., 2016, 2018; Schaffer-Morrison & Zak, 2023).

On the other hand, the higher carbohydrate concentration and thicker AD and shorter SRL of roots that had lower N concentration under closed canopy conditions simultaneously imply more storage function than direct nutrient uptake (Hermans et al., 2006; Kobe et al., 2010; Chen et al., 2016). Therefore, firs growing in closed canopy clumps exhibited lower tissue nitrogen, higher carbon reserves, and thicker absorptive roots hosting mycorrhizae, suggesting that nitrogen acquisition may be regulated by mycorrhizal community composition.

Associations between root traits and fungal diversity may help explain how plants and their symbiotic partners jointly regulate resource acquisition (Chen et al., 2016; Pellitier & Zak, 2021; Schaffer-Morrison & Zak, 2023). In our study, canopy structure significantly influenced fungal α-diversity and produced a small but significant shift in community composition (PERMANOVA $R^2 = 0.03$, $P = 0.0142$), whereas seasonal variation and size class had negligible effect. Fungal community assembly was driven by multiple factors including host genetics, small-scale environmental variations, soil properties, dispersal, successful colonization of hosts or soil, and historical legacies. Importantly, many of these factors operate at scales smaller than canopy treatments. Consequently, canopy structure is only one of several environmental filters rather than the main factor determining fungal composition. According to our third hypothesis, ectomycorrhizal (ECM) diversity was higher under a canopy gaps than in closed canopy. These results are broadly consistent with Ważny (2014) and Ważny and Kowalski (2017), who reported that fir seedlings growing beneath non-fir canopies, such as Scots pine or larch, had significantly reduced ectomycorrhizal diversity and a distinct set of dominant fungi when compared to seedlings growing beneath a mature fir overstory. Although light availability was not directly assessed in these studies, differences in canopy structure and, consequently, in light conditions were also indirectly observed between the different overstory types, along with other environmental characteristics that may influence ECM community assembly. In our study, lower light availability and greater individual aggregation in shaded patches were associated with thicker roots, lower SRL, a lower number of root tips and higher carbohydrate reserves, which favors associations with mycorrhizal partners, particularly long-distance exploration-type, for nutrient acquisition when direct soil exploration by roots is limited as in dense mountain forests (Guo et al., 2008; Koide et al., 2014; Stuart & Plett, 2020; Schaffer-Morrison & Zak, 2023).

Although ECM taxa varied between canopy types, their exploration type abundances remained largely similar, indicating functional redundancy. The minimal variance explained by canopy differences supports this redundancy. While opening the canopy affected the abundance of certain taxa, most fungal genera were found in both conditions, reflecting shifts in relative abundance rather than complete community replacement. However, even small changes in community composition can influence symbiotic functions if they involve taxa with different carbon or nutrient strategies. Consistent with this, only a small set of genera responded strongly to canopy variants. *Boletus,* representing a long-distance exploration type, was more abundant under a closed canopy ($\log2FC > 5$) and positively correlated with carbohydrate pools, while negatively correlated with PPFD and SRL. These fungi develop an extensive rhizomorphic network that facilitates optimal exploration of soil resources but requires significant carbon from their hosts (Agerer, 2001; Lilleskov et al., 2011; Jörgensen et al., 2023). Their higher abundance under shaded canopies indicates that silver fir may allocate stored carbon preferentially to these fungi, which can forage beyond the nearest root zone. In contrast, canopy gaps with higher soil temperatures favor stress-tolerant fungi such as *Penicillium* (Barnard et al., 2013; Birnbaum et al., 2019), which can mobilize poorly available phosphorus in dry soils (Richardson et al., 2009). The greater abundance of *Penicillium*, *Tolypocladium*, and *Kwoniella* under canopy gaps indicates a shift toward stress-tolerant, opportunistic endophytes. While canopy structure had limited impact on overall community composition, the patterns observed imply that canopy opening acts as a selective filter for some fungal groups rather than reshaping the entire root associated fungal community (Porras-Alfaro & Bayman, 2011; Courty et al., 2016). Such an acclimation may help firs persist where abiotic stress weakens classic mycorrhizal symbioses and alters mutualistic outcomes (Johnson et al., 2010). This observed pattern supports a refined view of the root-fungal "collaboration" gradient (Bergmann et al., 2020; Laughlin et al., 2024). In shaded patches, thicker roots with higher carbohydrate reserves and increased prevalence of a long-distance ECM partner are consistent with selective symbiotic outsourcing that reduces the need for carbon-expensive proliferation of thin absorptive roots. In canopy gaps, fir shifts toward a more autonomous, root-driven foraging strategy (thin roots, longer SRL and higher SRA) and hosts a more diverse fungal community. The negative association between fungal diversity and needle carbohydrates is consistent with a higher cumulative carbon demand of a richer mycobiome contributing to reduced reserve pools. Future work should directly quantify ECM colonization intensity, nutrient uptake, and fungal functional traits (e.g., enzymatic capacity) to test whether the observed taxonomic shifts translate into predictable changes in resource acquisition.

Canopy structure created a strong filter on fir regeneration (Hofmeister et al., 2008; Orman et al., 2021). The environmental contrasts appear to favour spruce in gaps but fir under shaded canopy, highlighting the role of small-scale canopy heterogeneity in shaping species composition (Albanesi et al., 2008; Pretzsch et al., 2015; Kolisnyk et al., 2024). Accordingly, canopy opening following spruce dieback drastically reduced fir seeding regeneration, making the maintenance or enhancement of fir abundance a key silvicultural challenge (Drozdowski et al., 2014; Bebre et al., 2021). Therefore, under these conditions, care and maintenance are crucial including a focus on removing spruce individuals that directly compete with fir. Consequently, these maintenance activities during the regeneration phase are likely to be important for shaping species composition, spatial mixing, and regeneration density.

Conclusions

Our findings indicate that silver fir regeneration is governed by a coordinated interaction among canopy structure, carbon allocation, root traits, and ectomycorrhizal symbiosis. Under a closed canopy, seedings and saplings typically adopt a conservative strategy, retaining carbon, developing thicker roots, and forming robust associations with ectomycorrhizal fungi. This approach enables them to persist as advanced regeneration until moderate increases in light are available. Conversely, individuals growing under higher light availability tend to adopt a more acquisitive strategy, developing thinner roots and investing more in nitrogen rich tissues to support rapid growth while also maintaining more diverse fungal associations, even if it means reduced carbohydrate reserves. However, a limitation of this finding is that the observed patterns may be partly result of the local, site-specific conditions. We cannot rule out that the unique history of this single study site may not be representative of other silver fir regeneration systems or disturbance contexts. In any case, a general understanding how canopy structure regulates carbon allocation to roots and fungal partners will improve predictions of fir regeneration dynamics under ongoing spruce dieback and management strategies that maintain stands for advanced regeneration.

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Author contribution

R.K. conceptualized the study, drafted the manuscript and interpreted the results with contribution from M.B., J.M., M.Z.; R.K., M.B., B.B., S.C., M.H-K., A.I., W.K., A.Ł., J.M., M.Z. performed analyses; all authors contributed further to the development of the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data that support the findings of this study are available from the corresponding author upon request. Raw read data from sequencing have been submitted to the NCBI Sequence Read Archive (SRA) under the BioProject with accession number PRJNA1475843.

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