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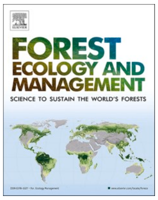

Norway spruce monoculture has lower resilience and carbon sequestration capacity than a more diverse broadleaved forest: A case study in Central Europe

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ABSTRACT

Norway spruce (*Picea abies*, (L.) H.Karst) monocultures have been widely planted across Europe to meet timber demands, yet their resilience to climate change remains uncertain compared to natural mixed broadleaved forests. This study examines the long-term carbon sequestration potential, soil CO_2 and CH_4 fluxes, and microbial community dynamics in a Norway spruce monoculture and a mixed forest under increasing drought and disturbance pressures in Central Europe. Using a three-year monitoring program combined with long-term tree-ring analysis (2000–2023), we quantified seasonal and annual biomass increments, soil respiration patterns, and microbial diversity shifts. The results indicate that the severe drought of 2022 significantly reduced growth rates and soil CO_2 efflux in the monoculture, whereas the mixed forest maintained more stable growth and soil respiration. Thereafter, bark beetle outbreaks in 2023 led to rapid mortality and salvage clear-cutting in the spruce stand, triggering a sharp increase in soil CO_2 emissions and a temporary decline in CH_4 oxidation, though CH_4 uptake recovered within a year—contrary to previous studies suggesting prolonged suppression. Microbial community analysis revealed a higher proportion of ectomycorrhizal fungi in the mixed forest, while saprotrophs dominated in the spruce stand, influencing soil carbon dynamics. These findings highlight the greater resilience of mixed forests to climate stressors and suggest that the increasing vulnerability of spruce monocultures could lead to long-term carbon losses. The study underscores the need for diversified forest management strategies that enhance climate resilience and carbon sequestration stability.

1. Introduction

Climate warming has recently accelerated (IPCC, 2023), and the frequency of extreme climatic events, such as heatwaves and droughts, has increased over the past few decades (Chiang et al., 2021). In the changing climate, forest ecosystems must adapt to new environmental conditions to continue providing essential ecosystem services, including timber production and mitigating climate change by sequestering atmospheric carbon dioxide (CO_2) (Jandl et al., 2019). Globally, forests play a critical role in mitigating warming by absorbing carbon (C) from the atmosphere (Grassi et al., 2017). However, over the past two centuries, forest management has not contributed to cooling the atmosphere (Naudts et al., 2016). Between 1750 and 1850, large areas of Europe's primary forests were converted into agricultural land, with coniferous species predominantly used for subsequent reforestation efforts (Naudts et al., 2016). Norway spruce has been widely planted outside its natural range, often replacing the original broadleaf and mixed forests across Europe (Spiecker et al., 2004). However, in recent years, spruce

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monocultures have been experiencing large-scale bark-beetle outbreaks across Europe (Hlásny et al., 2021; Pirtskhalava-Karpova et al., 2024) and increased climate vulnerability (Arend et al., 2021). Such large-scale disturbances, often followed by salvage-cut logging, can lead to significant C emissions from soils and reduce methane (CH_4) uptake by altering methanotrophic communities (Ho et al., 2016). However, the long-term comparative impact of these forest types on C sequestration and greenhouse gas (GHG) fluxes remains insufficiently quantified, especially under extreme climatic conditions.

Soils store more than 50 % of ecosystem C in temperate forests and are essential to the global forest C sink (Georgiou et al., 2022; Pan et al., 2011). Soil CO_2 emissions from heterotrophic respiration represent the largest terrestrial C source to the atmosphere (Nissan et al., 2023). While the amount of CO_2 released from soils roughly equals the input from vegetation returning to the soil (Lehmann and Kleber, 2015), respiration tends to slightly exceed net primary production due to additional contributions from plant roots and mycorrhizae (Schlesinger and Andrews, 2000). Human activities, such as land use change and forest management, have disrupted the balance between inputs and emissions in soils, leading to increased soil C emissions. Forest disturbances, which have intensified due to climate change in recent centuries (Seidl et al., 2017, 2014), are key contributors to this C cycle imbalance. These disturbances can result in the loss of up to 60 Mg ha^{-1} of soil organic carbon (SOC), with carbon-rich soils experiencing higher losses than carbon-poor soils (Mayer et al., 2024). Soil respiration is primarily driven by soil temperature and water content (Hursh et al., 2017). Extreme weather events, such as droughts and disturbances, can significantly alter these factors, influencing C release from soils (Mayer et al., 2017) by affecting soil microbial diversity and activity (Sáez-Sandino et al., 2023). However, most research focuses on aboveground C storage, while the effects of extreme droughts and disturbances on soil respiration and CH₄ uptake remain unclear (Mayer et al., 2024). Due to the high costs and time requirements, the extent of drought—and disturbance-induced soil C fluxes and emissions remains insufficiently quantified across diverse forest ecosystems and management practices. While microbial diversity influences soil C dynamics, comparative studies on microbial structure in monoculture vs. mixed forests remain limited (Sáez-Sandino et al., 2023).

Methane (CH_4) is the second most important anthropogenic greenhouse gas after CO_2 in terms of radiative forcing (Saunois et al., 2016), with an effect approximately 84 times greater than CO_2 over 20 years (IPCC, 2013). Temperate forest soils are generally net sinks of CH_4 (Dlugokencky et al., 2011), but this uptake has declined over the past three decades (Ni and Groffman, 2018). Although forest soils act as a net sink of CH_4, their uptake capacity may decrease with increasing precipitation and soil moisture (Feng et al., 2020). Methanotrophs in the soil oxidise CH_4 to CO_2 under optimal soil moisture and porosity conditions, but this process is inhibited when soils become waterlogged. Methanogenic organisms can survive extended periods of drought and oxidised soils and become active under damp, anoxic conditions. Several factors influence CH_4 uptake in forest soils, but the combined effects of drought, disturbances, and forest management are not yet fully understood due to their complexity. Moreover, it is well established that clear-cutting reduces CH₄ uptake (Wu et al., 2011), yet previous studies suggest a slow recovery over several years.

Extreme weather events reduce C assimilation, transfer to roots, and input to soil microorganisms, ultimately affecting soil microbial structure and respiration (Kang et al., 2022). However, these impacts have largely been neglected in the national greenhouse gas (GHG) inventories of forest soils (Mäkipää et al., 2023). Therefore, it is crucial to quantify the effects of disturbances, droughts, and forest management on soil CO_2 and CH_4 fluxes to better understand the role of forest soils in C dynamics under rapidly changing environmental conditions.

Many studies have demonstrated the benefits of mixed forests for drought resistance and productivity stability, but fewer studies quantify their long-term C sequestration advantage compared to spruce monocultures. Studies like Pretzsch and Schütze (2009) and Pretzsch et al. (2014) have focused on aboveground biomass production, but the impact on belowground C stocks and soil respiration remains unclear.

In this study, we examine long-term growth trends in tree species and their responses to drought events, exploring how these factors contribute to C sequestration and storage in above-ground biomass in Norway spruce monocultures and mixed stands. We place the seasonal drought responses of tree growth in the context of long-term growth trends and investigate how drought influences soil CO_2 and CH_4 fluxes by altering temperature, water availability, and, indirectly, tree growth. The study utilises data from a three-year root-exclusion experiment conducted as part of the European-wide Horizon 2020 project, HoliSoils (https://holisoils.eu/).

In this study, we specifically seek to:

i) Assess differences in soil C fluxes (CO₂ and CH₄ uptake) between the two forest types, Norway spruce monoculture and mixed forest, particularly under drought conditions. We hypothesise that the total soil CO2 efflux is more resilient in mixed forests during drought, while spruce monocultures show a sharper decline.

ii) Evaluate how drought and disturbances (e.g., bark beetle outbreaks) impact resilience and aboveground C sink in spruce monocultures compared to mixed forests. We hypothesise that mixed forests exhibit more resilience by stable C sequestration rates in aboveground biomass than spruce monocultures, which are expected to be more vulnerable to decline in C sink under climate stress.

iii) Analyse microbial community differences in relation to forest type and management practices, focusing on their role in soil respiration and CH₄ oxidation.

iv) Investigate the post-disturbance effects of a clear-cut in a spruce monoculture on soil CO₂ emissions and CH₄ uptake recovery. We hypothesise that CH₄ uptake is higher in mixed forests and less affected by clear-cutting than spruce stands, where oxidation rates initially decrease.

2. Material and methods

2.1. Site description

The research area was established in central Slovakia (N:48.6737, E: 19.6792) as a test site for the European-scale Horizon Europe project titled Holistic management practices, modelling and monitoring for European forest soils (HoliSoils, https://holisoils.eu/). We established two sites to test the effects of forest management on soil CO_2 and CH_4 fluxes (Fig. 1A, B): 1) The first site was a mixed-species stand in a buffer zone of the Dobroč primary forest declared as a nature reserve in 1913 and with minimal management, it was left to develop without active management thus can be considered as close to nature management; 2) the second site was a Norway spruce monoculture established by planting 70 years ago and regularly managed by thinning from below (one thinning per decade, rotation period of 100 years). The last thinning before the clear-cut was done in 2019, removing approx. 5 % of the growing stock. These two stands are located right next to each other at 950 m a.s.l. on a productive site with deep Dystric Cambisol.

The mixed stand is formed by five species, European beech (*Fagus sylvatica*, L.) – 43 %, silver fir (*Abies alba*, Mill.) – 25 %, Norway spruce (*Picea abies*, (L.) H.Karst) – 14 %, Sycamore maple (*Acer pseudoplatanus*, L.) – 13 %, and Common ash (*Fraxinus excelsior*, L.) – 5 % (the percentage calculated from tree basal area). The monoculture is formed by 100 % of Norway spruce.

In 2023, the Norway spruce monoculture faced a bark-beetle outbreak, and after all the spruce trees died, the trees were harvested by clear-cutting in September (Fig. 1C). The forest residues (tops and branches) were haphazardly left on site.

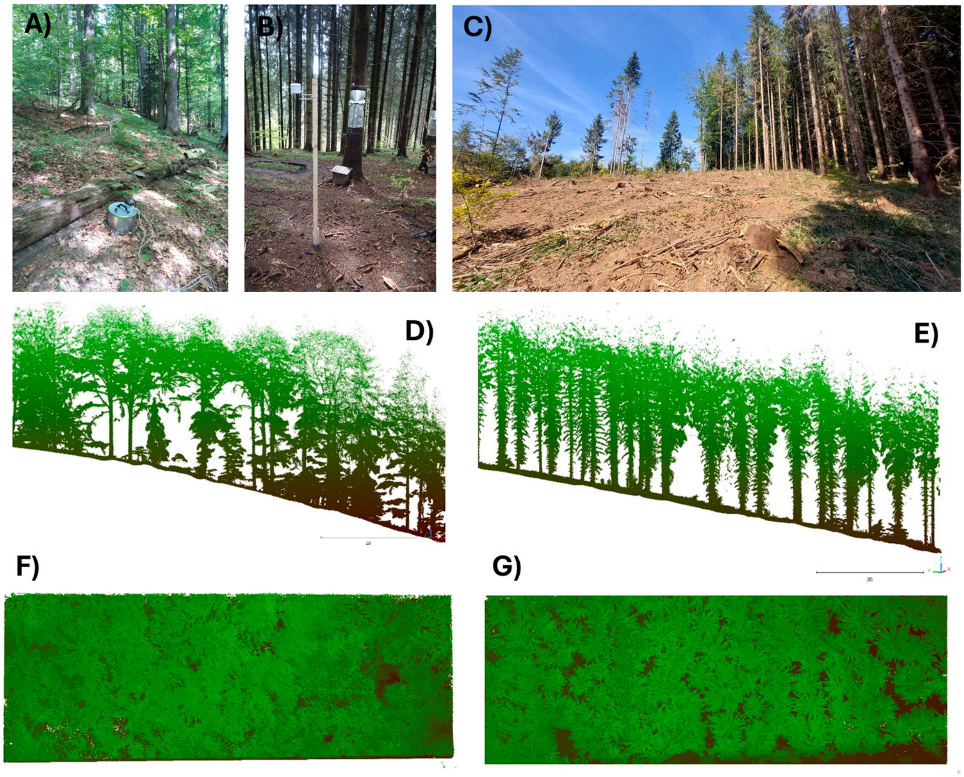

Fig. 1. A) Mixed stand in the buffer zone of Dobroč primary forest; B) 70-years-old spruce monoculture planted next to the mixed stand (before clear-cut); C) the clear-cut in the spruce stand after the bark-beetle invasion in 2023 (dead trees harvested in September 2023); D) and F) vertical and horizontal structure, respectively, of the mixed stand; E) and G) vertical and horizontal structure, respectively, of the spruce stand (before clear-cut).

2.2. *Measurements of soil CO_2 and CH_4 fluxes*

Carbon dioxide (CO_2) and methane (CH_4) fluxes from the soil were measured biweekly since August 2021 using the LI-COR LI-7810 CH_4/CO_2/H_2O Trace Gas Analyzer (LI-COR Biosciences GmbH) connected to a dynamic non-transparent chamber (30 cm diameter, 21 cm height) equipped with a small fan for air mixing. The chamber was manually placed airtight on the soil within a pre-cut 2 cm deep ridge along its circumference and connected via tubing to the trace gas analyzer. Gas concentrations were recorded every second for three minutes, with linearity continuously monitored. Flux calculations were based on the stable portion of the data, specifically from 30 seconds after measurement initiation to 15 seconds before completion (Zhao, 2019). We conducted a total of 2800 CO_2 and CH_4 measurements over the three years of the study. Five months prior to the first measurement, we applied a root exclusion method (RE) to separate the heterotrophic (Rh) and autotrophic (Ra) respiration and to quantify their contribution to the total soil respiration (Rt) (Díaz-Pinés et al., 2010). We established 40 measurement points (16 trenched and 24 untrenched/control), i.e. 20 points in each stand. The root exclusion (trenching) was done by inserting a geotextile fabric to a depth of 50 cm to prevent the ingrowth of tree roots (Díaz-Pinés et al., 2010). The size of the trenching plots of 2 m^2 minimised the boundary effects of the root prevention fabric on GHG flux measurements. It allowed the collection of soil samples for microbiology on a 0.3 × 1 m stripe and the placement of sensors to monitor soil microclimate. When establishing trenching plots, the woody roots were cut. In the laboratory, roots were separated into diameter categories, and the root biomass was estimated (Table S1). We installed sensors inside and outside the trenching plots at 5–10 cm soil depths to continually measure soil temperature, soil water potential and volumetric water content (see Section 2.5 for more details). Gas fluxes were measured using a 21.7 L non-transparent dynamic chamber equipped with a small fan for air mixing. The chamber was manually placed airtight on the soil within a pre-cut 2 cm deep ridge along its circumference and connected via Teflon tubing to a LI-COR LI-7810 (CH4/CO2/H2O) or LI-7820 (N2O/H2O) trace gas analyser. Gas concentrations were recorded every second for three minutes, with linearity continuously monitored. Flux calculations were based on the stable portion of the data, specifically from 30 seconds after measurement initiation to 15 seconds before completion (Zhao, 2019).

2.3. *Soil physical and chemical analyses*

Soil samples were collected along the soil profiles in 10-cm intervals. The samples were air-dried, gently crushed, and sieved through a 2 mm mesh in the laboratory. Soil pH was measured potentiometrically in water suspension (1:2.5; w:v) after 24 h. The combustion method was used to determine the concentration of organic C and total nitrogen using the equipment LECO CHN 828. The soil texture was determined through sedimentation analysis, employing the pipette method.

Soil organic C stock (SOC, [Mg ha^{-1}]) was computed by summing up the SOC of the eight 10-cm layers. SOC in the *i*th layer was estimated by the following equation:

$$SOC = \sum_{i=1}^{n} BD_i \times SOCC_i \times d_i \times (1 - cf_i) \times 10000$$

Where BD_i is soil bulk density [Mg m^{-3}], $SOCC_i$ is SOC concentration [%], d_i is the thickness [m], and cf_i is the volumetric fraction of coarse fragments [%] in the *i*th layer from the total of n = 8 soil layers.The constant 10000 is used for conversion of m^2 to hectares.

The soil is a deep Dystric Cambisol, with physicochemical and textural properties similar in both the mixed stand and the Norway spruce monoculture (Table 1).

Table 1
Soil physicochemical properties.

Variable	Mixed	Spruce
pH H2O	4.2	4.0
N (%)	0.24	0.23
C (%)	2.3	2.47
C/N	9.4	9.9
Sand (%)	45	47
Silt (%)	41	39
Clay (%)	13.9	14.2
Stoniness (%)	5–50	5–50
Depth of soil organic layer (cm)	3.7	4.95

2.4. Tree and stand characteristics

We conducted a tree inventory on an area of 2700 m^2 (30 × 90 m) in each stand. We measured tree DBH and top height for all trees. A height-diameter (HD) model was developed using the Weibull growth function (Yang et al., 1978) in the "ForestFit" R package (Teimouri et al., 2020):

$$Height = 1.3 + b_1\left(1 - EXP\left(-b_2 DBH^{b_3}\right)\right) \qquad \text{(Eq. 1)}$$

Where $b_1 - b_3$ are regression parameters to be estimated.

2.4.1. Long-term tree growth

All trees surrounding the soil CO_2 and CH_4 flux measurement points were sampled for tree ring analysis. Tree ring width (TRW) sampling from 11 trees in the mixed stand and 14 trees in the spruce stand was conducted in 2023. One core sample per tree (Bosela et al., 2014) was extracted from trees surrounding trenching plots. The standard dendrochronological procedure was applied to process and analyse the tree-ring data (Cook and Kairiukstis, 2013).

We reconstructed past tree DBH between 2000 and 2022 using the TRW samples and the DBH measured during inventory. We limited the reconstruction period to 22 years to minimise the effects of thinning and tree mortality in the stands. We applied the HD model (Eq. 1) to estimate past tree height and used national volume functions (Gschwantner et al., 2019; Petráš and Pajtík, 1991) to estimate the volume of trees (including tree components such as the stem, branches, and foliage). We further used species-specific wood density (Eggleston et al., 2006) to transform the tree volume into dry biomass.

2.4.2. Seasonal tree growth

In the close vicinity of the soil CO_2 and CH_4 flux measurement points, six trees of spruce in the spruce monoculture (with average DBH of 38.8 cm), and three of beech (average DBH=51.8 cm) and two of silver fir (average DBH=44.9 cm) in the mixed stand were equipped with band dendrometers DRL26D (EMS Brno, s.r.o.) in 2021 to continually monitor changes in stem radius in a half-hourly resolution. Tree water deficit was extracted from the continual measurements using the zero growth (ZG) concept (Zweifel et al., 2016) using the "DendroAnalyst" R package (Aryal et al., 2020). We identified three phases of circumference changes: shrinking, swelling, and growth. Using the growth phase, we calculated annual radial growth by cumulating growth increments.

We generalised the annual growth of trees by using the Richards growth function (Richards, 1959):

$$Cumulative\ growth = A \Big/ \left(1 + B EXP\left(-k(t - M)\right)\right)^{\frac{1}{v}} \qquad \text{(Eq. 2)}$$

Where:

A – is the upper asymptote, representing the maximum annual cumulative growth

B – is a scaling parameter

k – is the growth rate parameter

M – is the DOY at the inflection point, where the growth rate changes

v – is a shape parameter that influences the flexibility of the curve

t – time (day of year - DOY)

To simplify the estimation of so many parameters, we replaced the parameters B and v by 0.5 and estimated the asymptote, growth rate and DOY at the inflection point:

$$Cumulative\ growth = A \Big/ \left(1 + 0.5 EXP\left(-k(t - M)\right)\right)^{\frac{1}{0.5}} \qquad \text{(Eq. 3)}$$

2.4.3. Below-ground biomass

For each trenching plot, we measured the dry matter of all roots by sampling roots from one long side of the plot, i.e., in the space of 200 × 30 × 50 cm. All roots were dried at 60 °C until they reached a constant weight. The roots were then split into size categories: 0–2 mm, 2–5 mm, 5–10 mm, 10–20 mm, and > 20 mm. The dry matter was weighted with a precision of 1/100 g.

2.5. Climate data

2.5.1. Long-term monthly data

Long-term, monthly-resolved climate data for 1961–2023 were sourced from the nearest climatological station in Lom nad Rimavicou (1.8 km from the study site), administrated by the Slovak Hydrometeorological Institute. Data include monthly mean air temperature, precipitation sums, and relative humidity. We further calculated climatic water balance (CWB) by subtracting precipitation and potential evapotranspiration estimated using the Thornthwaite equation (Škvarenina et al., 2004; Thornthwaite, 1948). The long-term climate data were used to analyse the long-term growth trends in the spruce and mixed stands.

2.5.2. Hourly climate data

In June 2021, a weather station (Minikin RTHi and precipitation gauge with small event recorder Minikin ERi, manufactured by EMS Brno) was placed in the vicinity of the test site to continually monitor air temperature, humidity, precipitation, and solar radiation at an hourly resolution. Moreover, Minikin THi sensors were installed in each stand to monitor forest microclimate (air temperature and humidity). Air vapour pressure deficit (VPD) was calculated based on minimum and maximum daily temperature and relative humidity.

2.5.3. Soil water potential and moisture content

Soil water potential (SWP) was continually measured inside and outside the trenching plots using a MicroLog SP3 system with sensors embedded in Delmhorst Gypsum blocks (EMS Brno). Similarly, volumetric soil water content was measured using a MicroLog V3A system with sensors SM100 (EMS Brno).

2.6. Soil microbial diversity

Soil samples were collected inside and outside the four trenches (two in spruce monoculture and two in the mixed forest), one year after the trenches were established. In each trenching plot, a transect of five soil cores was established inside and outside of the trenching plot. Soil corers of 4 cm diameter were used. Soil cores in a transect were spaced 15 cm apart, resulting in a transect of soil cores approximately 80 cm long. Material from five soil cores representing the top 10 cm of soil after litter removal was combined to obtain a composite sample. The soil was thoroughly mixed and sieved through a 5-mm sieve.

2.6.1. DNA extraction and sequencing

Total genomic DNA was extracted from 8 soil samples using a modified Miller method (Sagova-Mareckova et al., 2008). DNA was extracted in duplicates from 0.25 g of freeze-dried soil. The duplicates were cleaned using Geneclean Turbo Kit (MP Biomedicals) and pooled before subsequent PCR.

PCR amplification of the fungal ITS2 region from DNA was performed using primers fITS7 and ITS4 (Ihrmark et al., 2012), and the V4 region of bacterial 16 S rRNA was amplified using the primers 515 F and 806 R (Caporaso et al., 2012); both the forward and reverse primers were barcoded. PCR was performed in triplicate for each sample. 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 bacterial 16S rRNA region were: initial denaturation for 4 min at 94°C; 25 cycles of 45 s at 94°C, 60 s at 50°C, 75 s at 72°C, followed by an extension at 72°C for 10 min. The resulting amplicons were purified using MinElute Kit (Qiagen), pooled, and libraries prepared with the TruSeq DNA PCR-Free Kit (Illumina) were sequenced in-house on the Illumina MiSeq (2 × 250-base reads).

2.6.2. *Microbial data analysis*

Sequence data were processed using the SEED 2.0 pipeline (Větrovský et al., 2018). A fastq-join (Aronesty, 2013) was used to join the pair-end reads. Quality filtering was performed with a mean quality score of 30 as a cutoff. Before further processing, the ITS2 region was extracted using ITSx v1.0.8 (Bengtsson-Palme et al., 2013). 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 as the representative sequence, and BLASTn was used for identifications against relevant databases: UNITE v10 for fungi (Abarenkov et al., 2024) and Silva v138.1 (Quast et al., 2013) for bacteria. Sequences identified as non-fungal or non-bacterial were discarded. Putative ecology categories were assigned to fungal OTUs based on their genus identification using FungalTraits (Põlme et al., 2020).

2.6.3. *Soil enzymatic activities*

Enzyme assays (MUFC – Cellobiohydrolase, MUFG - β-Glucosidase, MUFL - β-Galactosidase, MUFN – Chitinase, MUFP - Acid Phosphatase, MUFX - β-Xylosidase, MUFY – Lipase, MUFαG - α-Glucosidase) were performed in soil homogenates as previously described (Štursová and Baldrian, 2011). A 0.25 g of freeze-dried soil was homogenised in sodium acetate buffer (pH 5.0) for total enzyme activity assays. Specific substrates were added to the soil slurry, and the enzyme activities were assessed by measuring fluorescence. The reactions were incubated in multiwell plates at 40°C, with fluorescence measurements taken from 5 to 125 min using a microplate reader (Infinite, TECAN, Austria) at excitation and emission wavelengths of 355 nm and 460 nm, respectively. Enzymatic activities, after blank subtraction, were quantified using MUF standard curves.

2.6.4. *Microbial biomass*

Approximately 2 g of each sample were extracted using a chloroform-methanol-phosphate buffer mixture (1:2:0.8) following the methods of (Bligh and Dyer, 1959) and (Šnajdr et al., 2008). Internal standards were added before extraction. The chloroform fraction was filtered and evaporated, and the total lipid extracts were fractionated into neutral, glyco-, and phospholipid fractions. The phospholipid fraction was further processed using trimethylchlorosilane-methanol derivatisation. Phospholipid fatty acid (PLFA) methylesters were analysed by gas chromatography, identifying different fatty acids by comparing retention times with standards. Specific fatty acids were used as proxies for fungal biomass (18:2ω6,9) and bacterial biomass (sum of various fatty acids). Total PLFA content represented total microbial biomass (Frostegård et al., 1993; Joergensen, 2022; Welc et al., 2012).

2.7. *Above-ground litter collection*

In 2023, six circle-shaped litter collectors with a size of 0.28 m^2 were installed in the spruce and mixed stands to estimate the annual litter fall (including foliage and branches). However, the bark-beetle disturbance prevented us from estimating the yearly litter fall for the spruce stand. The litter in the mixed stand was collected three times throughout the year, and the samples were dried at 60 °C until they reached a constant weight. The dry matter was weighted with a precision of 1/100. The total above-ground annual litterfall dry matter (DM) in 2023 amounted to 7.4 ± 2.3 Mg ha^{-1} yr^{-1} in the mixed stand. We then considered half of the dry matter to be carbon.

2.8. *Statistical analyses*

2.8.1. *Long-term growth trends*

The long-term growth trends were analysed using the stand above-ground biomass chronologies estimated from TRW samples (see Section 2.4 – Long-term tree growth). The trends in AGB increments since 2000 were tested using the linear least-squares regression.

2.8.2. *Effects of drought on seasonal tree growth*

To quantify the effects of soil microclimate and air vapour pressure deficit on daily tree water deficit, we used linear mixed-effects models employing the R function “lmer” in the “lme4” package (Bates et al., 2015). To account for trees’ different responses to SWP at varying levels of VPD, we used the interaction between the two in the model. In addition, Species (Norway spruce, European beech, and silver fir) were added to the interaction term to account for varying SWP × VPD terms among species. Considering varying responses of individual trees to the explanatory factors due to factors not measured in the study, we added the random intercept and slope of the effect of SWP in the model (Eq. 4).

$$\begin{aligned} TWD_{\min,i} = {} & \beta_0 + \beta_1 SWP_{\max,i} + \beta_2 VPD_i + \beta_3 Species_i + \beta_4 \big(SWP_{\max,i} \\ & \times VPD_i\big) + \beta_5 \big(SWP_{\max,i} \times Species_i\big) + \beta_6 \big(VPD_i \\ & \times Species_i\big) + \beta_7 \big(SWP_{\max,i} \times VPD_i \\ & \times Species_i\big) + b_{0,j} + b_{1,j} SWP_{\max,i} + \varepsilon_i \end{aligned} \tag{Eq. 4}$$

Where:

$TWD_{min,i}$– the minimum stem water deficit in mm for observation i
β_0 – the fixed intercept
$\beta 1, \beta 2, \beta 3, \beta 4, \beta 5, \beta 6, \beta 7$ – fixed-effect coefficients
$SWP_{max,i}$ – the maximum soil water potential for observation i (Mpa).
VPD_i – the vapour pressure deficit for observation i
$Species_i$ – the categorical species variable
$b_{0,j}$ and $b_{1,j}$ – the random intercept and random slope associated with tree ID j (ID_{Tree}), accounting for within-tree correlation
ε_i – the residual error term

2.8.3. *Effects of soil temperature and moisture on CO_2 and CH_4 fluxes*

The Wilcoxon test was used to compare mean CO_2 and CH_4 fluxes between the mixed and spruce stands during the study period.

A mixed-effects generalised additive model was used to quantify the effects of soil temperature (T_{soil}) and soil moisture (SWC) on the CO_2 and CH_4 fluxes and to estimate the annual C and methane emissions. The “*gam*” function in “*mgcv*” R package (Wood, 2003; Wood et al., 2016) was used to model the effect of the interaction between soil temperature and moisture separately in spruce and mixed stands. We added the ID of points (IDpoint) as a random effect to account for repeated measurements at individual measurement points.

$$CO_{2,i} = f\big(T_{soil,i}, \quad SWC_i, Site_i\big) + b_j + \varepsilon_i \tag{Eq. 5}$$

Where:

$CO_{2,i}$ – the response variable, representing soil CO₂ flux at observation *i*

$f(T_{soil,i}, SWC_i, Site_i)$ – a smooth function of soil temperature (T_{soil}) and soil water content (*SWC*), with smoothing varying by site (*Site*)

b_j – a random effect associated with measurement point ID_{point}, capturing repeated measurements at the same location

The same model structure was applied to test the difference between trenched and untrenched plots (Treatment) separately in mixed and spruce stands:

$$CO_{2,i} = f\left(T_{soil,i}, \quad SWC_i, Treatment_i\right) + b_j + \varepsilon_i \qquad \text{(Eq. 6)}$$

The “plot_diff” function in the “itsadug” R package (van Rij et al., 2022) was used to plot the differences along the soil temperature gradient.

2.8.4. *Analysis of microbial community structure*

The "vegan" (Oksanen et al., 2024) and "phyloseq" (McMurdie and Holmes, 2013) packages were used for fungal and bacterial diversity and community composition analyses. Fungal and bacterial community compositions were visualised by nonmetric multidimensional scaling (NMDS) based on Bray–Curtis distance using the “metaMDS” function from the vegan package on the Hellinger-transformed OTU matrices.

3. Results

3.1. *Quantification of forest stand characteristics*

Stand density, average tree DBH and height, stand basal area, and the above- and below-ground stand biomass were estimated (Table 2). Despite the stand basal area being larger in the spruce compared to the mixed stand, the above-ground biomass (AGB) is more prominent in the mixed stand because of the higher wood density of the broadleaved species and the occurrence of large broadleaved trees with DBH larger than 60 cm which at lower density have large branches.

3.2. *The climate change of the site*

Climate warming on the study site has been pronounced since the 1990s compared to the reference period of 1961–1990, exceeding the reference mean by more than 2°C in the recent decade (Fig. 2). Although annual precipitation totals have not changed since 1961, climatic water balance due to increased evapotranspiration shows continually drying climate limiting available water to plants. There have been multiple years of extreme drought with CWB dropping below −250 mm, such as 2000, 2003, 2008, and 2022. An extreme drought, such as that in 2022, occurred after more than ten years (Fig. 2).

3.3. *Effects of warming and drought events on long-term and seasonal tree and stand growth*

The long-term changes in the above-ground tree biomass suggest that spruce growth significantly decreased since 2000 (Fig. 3). In contrast, the mixed stand did not experience long-term growth changes in the same period. Spruce trees significantly reduced their perimetral growth during the dry years since 2000, with 2022, the year before the bark beetle outbreak, being the worst year for spruce growth ever. Seasonal measurements of circumference changes showed that spruce trees reduced the stem water due to drought (especially the 2022 drought) the most (Fig. 4A). Despite the drought being most potent in 2022, the cumulative annual growth of fir and beech trees didn’t differ between the study years (Fig. 4B). This was, however, not the case for spruce, when the cumulative perimetral growth decreased in 2022 compared to 2021.

The linear mixed-effects model of tree water deficit showed the soil water potential and air vapour pressure deficit to be the main factors driving tree water transport (Table 3). These two variables and their interaction explained 54 % of the total variability in TWD. The total variability explained by the model was 66 %, suggesting that individual responses of trees to spatial variability in microclimate are likely due to varying competition among trees. Of the three species, spruce experienced the strongest response to SWP and VPD, suggesting its higher vulnerability to drought than fir and beech (Fig. 5).

3.4. *Soil C stocks and effects of drought and disturbances on soil CO_2 and CH_4 fluxes*

The 2022 drought significantly reduced total soil CO_2 fluxes in the Norway spruce stand, reaching a quarter to half of that in the mixed stand during the late spring and early summer period (Fig. 6A). The differences were found insignificant for the heterotrophic respiration, which suggests that the total respiration was affected by the significantly reduced growth and root respiration of Norway spruce trees during the drought period (Fig. 4A). The oxidisation of CH_4 in the soil was strongly reduced during the same drought period in 2022, however, the differences between the spruce and mixed stands were much lower (insignificant in most cases).

After the clear-cut in September 2023 following the bark-beetle invasion, the total soil CO_2 respiration significantly increased in the Norway spruce stand and remained significantly higher up to the last measurement. The higher CO_2 fluxes result from an increased soil temperature and moisture, and an increased decomposition of roots. SWP remained close to zero values (soil saturated with water) in the spruce stand after clear-cut, but went up in the mixed stand, indicating a decrease in available water. The heterotrophic respiration was not significantly different between the forest types before the clear-cut, suggesting trees’ vital role in total soil respiration (Fig S1). However, after trees were removed, the heterotrophic respiration increased in the clear-cut. While oxidation of CH_4 was reduced considerably immediately after the clear-cut in 2023, it fully recovered in 2024.

The mixed GAM model of CO_2 fluxes suggests that temperature is the most important explanatory variable (Fig. 7). The significant interaction term of soil temperature and moisture indicated that soil moisture affected CO_2 fluxes, but only at higher temperatures and much lesser intensity compared to temperature (Fig. S1).

The mixed GAM model was used to extrapolate the CO_2 fluxes and, after conversion to C mass, estimate total annual soil C emission in the mixed stand and spruce monoculture in 2022 and 2023 (for these years, we had full-year measurements of CO_2 and soil microclimate) (Fig. 9). Despite the 2022 drought reduced the soil respiration at a higher intensity in the spruce than mixed stand, the total annual C emissions were not significantly different, reaching between 7 and 8 Mg $ha^{-1}yr^{-1}$. We estimated the yearly above-ground litter biomass input (including foliage and branches) to the soil to be around 3.65 ± 0.6 Mg C $ha^{-1}yr^{-1}$ in the mixed stand (not available for the spruce stand due to the bark-beetle outbreak and clear-cut). Thus, the litter input to the soil is half

Table 2
Stand characteristics (DBH – median tree breast-height diameter, H – median tree height, BA – stand basal area, AGB – above-ground biomass (including stem, stem top, and branches, without foliage)).

Site	Age	Stand density	DBH	H	Merchantable volume	BA	AGB	Branches	Foliage
	yr	N ha^{-1}	cm	m	m^3 ha^{-1}	m^2 ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}
mix	150	278	42	32	794	48	467	86	9
spruce	70	433	42	37	983	60	361	16	20

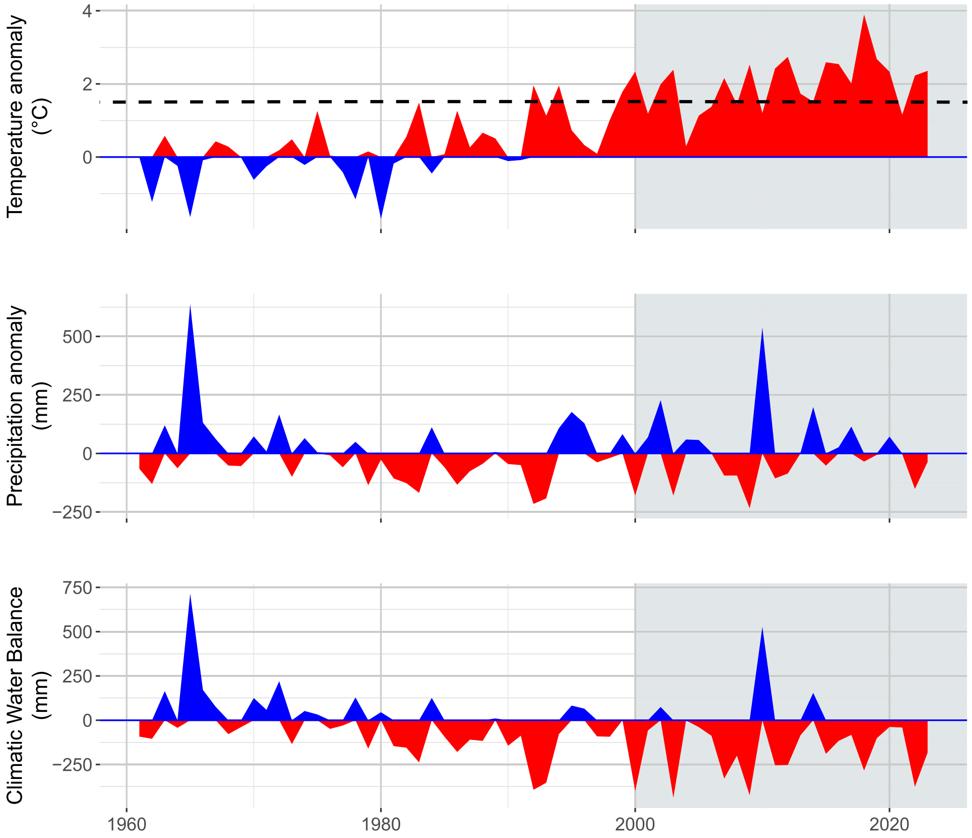

Fig. 2. Long-term variation in climate variables using data from a nearby climate station (Lom nad Rimavicou, 1.8 km from the Dobroc site). Temperature and precipitation anomalies were calculated to vary around the mean of the reference period of 1961–1990. Climate water balance (CWB) was estimated using the Thornthwaite equation to compute potential evapotranspiration. The grey shaded area highlights the period after 2000, which was used to analyse mean tree AGB increment trends.

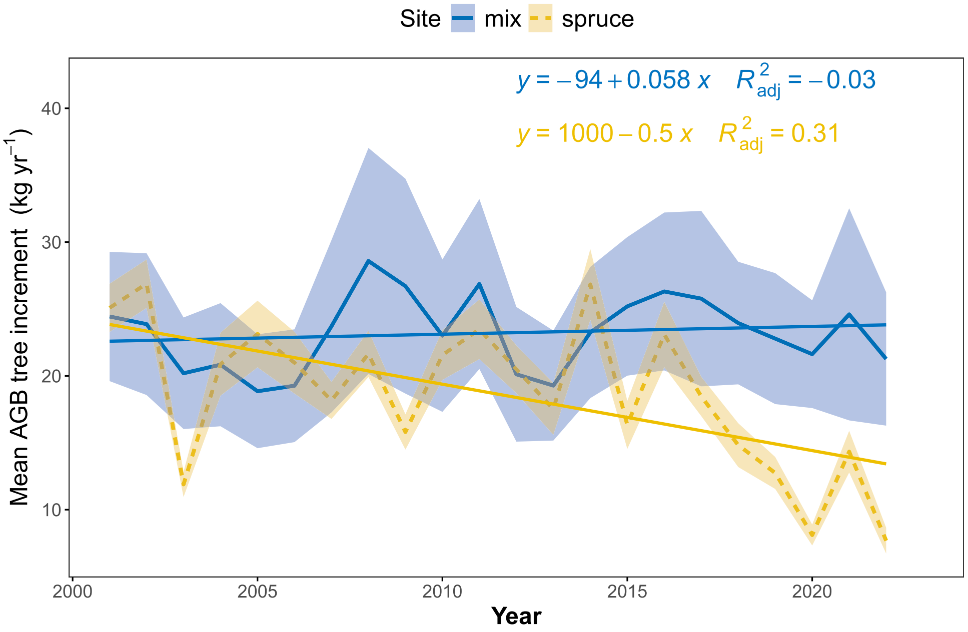

Fig. 3. Long-term changes in the average tree above-ground biomass increment in the spruce monoculture and mixed stand. The regression lines indicate changes after 2000, similar to our earlier study (Bosela et al., 2021).

of that emitted from the soil. The increased C emission in 2023 was mainly due to the bark-beetle outbreak and clear-cut in September, but also to more favourable climate conditions for decomposition than in 2022.

We estimated the total C stock in the upper 80 cm organic soil layer to be 125 Mg ha^{-1} in the spruce and 135 Mg ha^{-1} in the mixed stand,

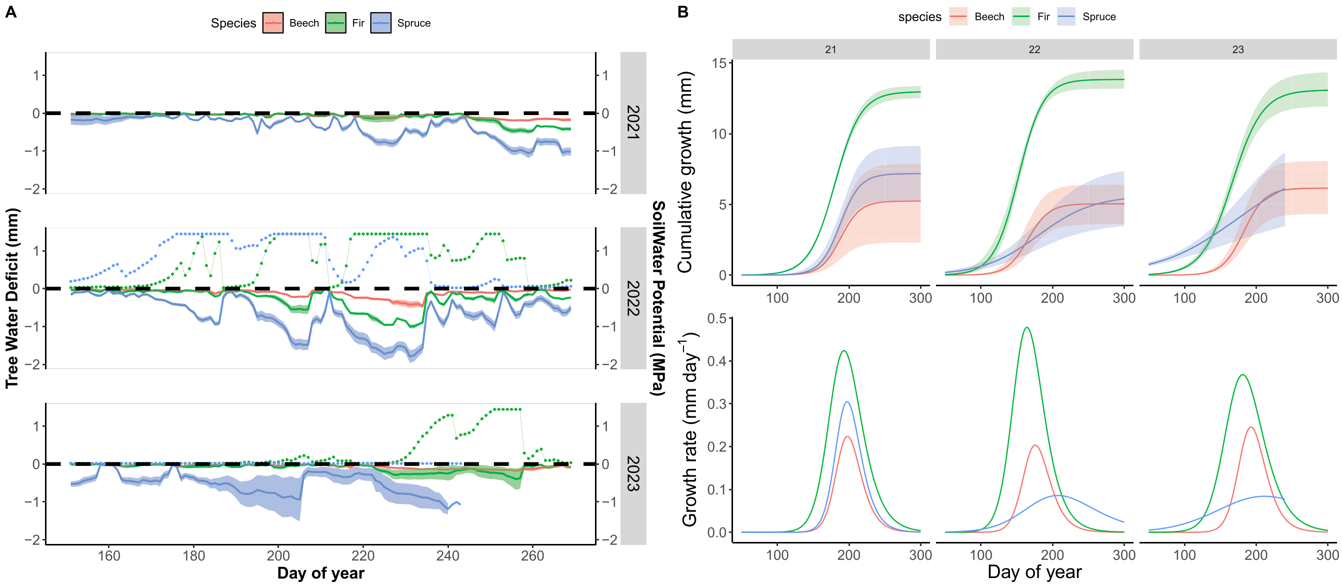

Fig. 4. A) Tree water deficit (TWD, solid lines with confidence intervals) and soil water potential (SWP, solid lines with dots); and B) annual perimetral growth and growth rate of beech, fir and spruce trees in 2021, 2022, and 2023.

Table 3
Results of the linear mixed-effects model with tree water deficit (TWD) as the dependent variable and soil water potential (SWP), air vapour pressure deficit (VPD) and species as explanatory variables.

Model parameters	Estimate	Std. Error	df	t value	p-value
(Intercept)	−0.0320	0.0717	11	−0.446	0.665
SWPmax	−0.0778	0.0727	11	−1.07	0.307
VPD	−0.0267	0.0452	3530	−0.59	0.555
species(Fir)	−0.0194	0.1140	11	−0.17	0.868
species(Spruce)	−0.2040	0.0880	11	−2.313	**0.042**
SWPmax: VPD	−0.0676	0.0633	3530	−1.069	0.285
SWPmax: species (Fir)	−0.1060	0.1160	11	−0.918	0.378
SWPmax: species (Spruce)	−0.4150	0.0897	12	−4.629	**< 0.001**
VPD: species(Fir)	0.0091	0.0742	3530	0.122	0.903
VPD: species (Spruce)	−0.1510	0.0565	3530	−2.681	**0.007**
SWPmax: VPD: species(Fir)	−0.1590	0.1010	3530	−1.568	0.117
SWPmax: VPD: species(Spruce)	−0.3020	0.0816	3530	−3.697	**< 0.001**
Random effects:					
Groups	**Name**	**Variance**	**Std. Dev.**	**Corr**	
ID Tree	(Intercept)	0.01318	0.1148		
	SWPmax	0.0126	0.1123	0.14	
Residual		0.05894	0.2428		
R-squared marginal = 0.54, R-squared conditional = 0.66					

representing 35 % of the above-ground biomass in the spruce and 29 % in the mixed stand.

The below-ground biomass in the 50-cm depth was similar in both stands, and it was estimated to be 12.3 Mg ha-1 in the spruce and 13.8 Mg ha-1 in the mixed stand. However, the share of fine roots from the total root biomass differed between the stands, amounting to 15 % in the spruce and 29 % in the mixed stand.

The species richness of microbial communities seemed not to differ between the mixed old-growth forest and Norway spruce monoculture (Fig. S2). However, the species composition of microbial organisms differed, with ectomycorrhizal fungi (*EcM*) being dominant in the mixed and saprotrophic fungi prevailing in the spruce stand. Expectedly, root exclusion treatment changed the species composition of fungi, with *EcM* fungi being dominant in the plots where roots were present. Saprotrophs prevailed inside the trenching plots from where roots were excluded (Fig. 8). The bacteria community structure did not differ between spruce and mixed stands, either between non-trenched and trenched (roots and autotrophic respiration excluded) plots. However, Actinobacteriota, more drought-sensitive, tended to be more frequent in the spruce stand.

4. Discussion

4.1. Comparison of C sequestration and resilience between monocultures and mixed forests

Norway spruce was the most adversely affected by drought in 2022, exhibiting the highest tree water deficit and the lowest annual cumulative growth among the three studied species. This aligns with its known isohydric behaviour, where it minimises hydraulic failure risks by closing stomata and reducing sap flow earlier than European beech and silver fir (Ulrich and Grossiord, 2023). An irrigation experiment in Germany revealed that both spruce and beech responded similarly to drought conditions, but spruce displayed a significantly delayed recovery after irrigation, indicating better acclimation of beech to drought (Motte et al., 2023). Beech, unlike spruce, quickly restores its turgor loss point after watering, leading to faster recovery of stomatal conductance and resilience (Hesse et al., 2023).

In our study, the earlier decline in soil water content in the spruce monoculture during the 2022 drought (Fig. S3) supports the hypothesis that beech and beech-dominated mixed forests are less water-intensive than spruce. Additionally, our results indicated a long-term decline in spruce growth rates since 2000, consistent with previous large-scale studies across the Carpathian Arc (Bosela et al., 2021) and other regions (Yue et al., 2011). A drought experiment demonstrated that repeated drought severely reduced xylem sap flow in spruce, with incomplete recovery even two years after drought exposure, a pattern not observed in beech (Gebhardt et al., 2023). This is likely due to beech's anisohydric nature, which favours C gain over water conservation. Despite its anisohydric tendencies, beech exhibits a moderate transpiration rate and conservative water use compared to spruce (Leuschner, 2020). The declining growth in spruce monocultures could lead to significant net C losses over multiple decades.

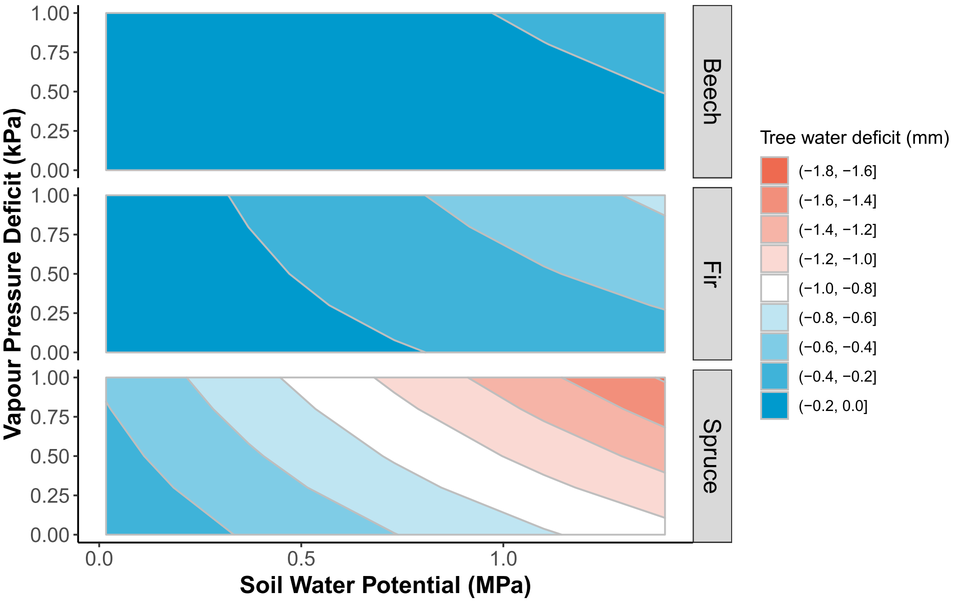

Fig. 5. Change in tree water deficit (TWD) in response to soil water potential and air vapour pressure deficit in beech, fir, and spruce trees as the result of the linear mixed-effects model.

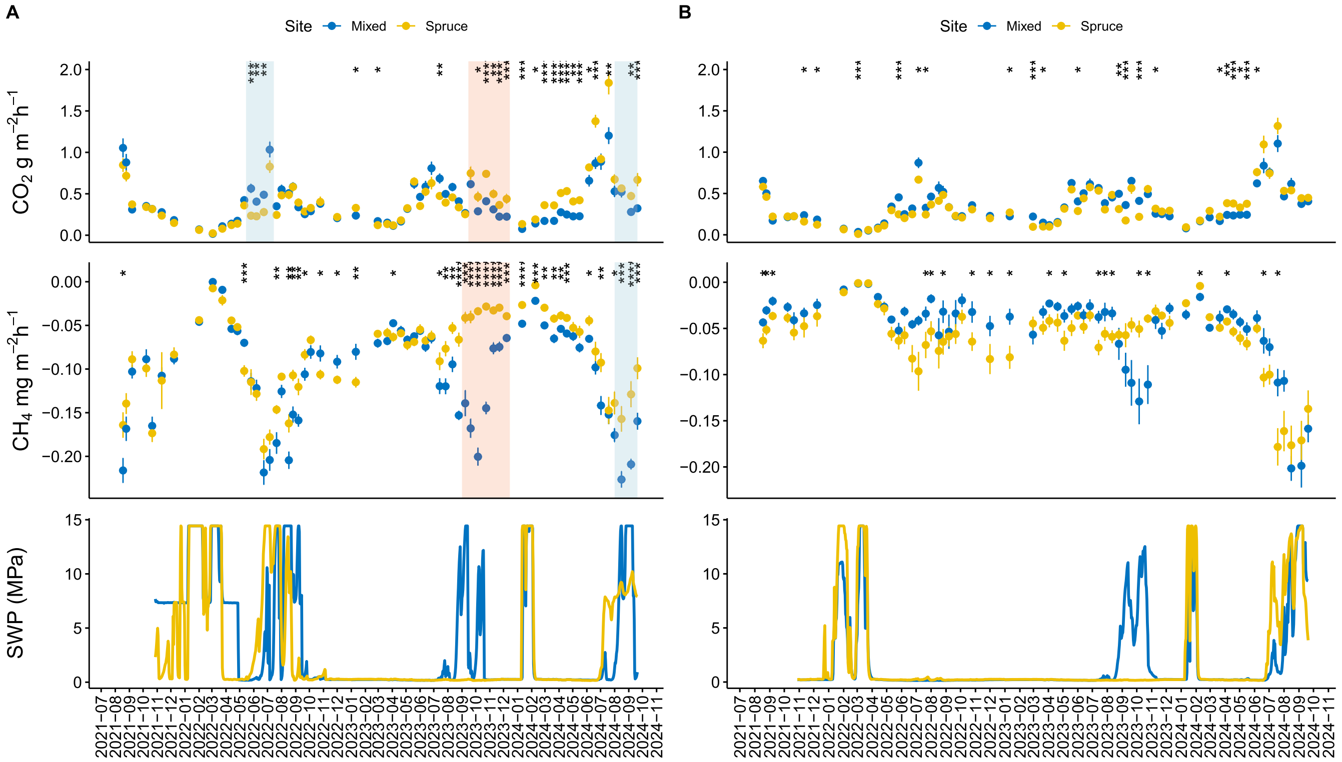

Fig. 6. **A)** CO_2 and CH_4 fluxes in the study period with soil water potential (SWP) in the control plots (total soil respiration) and **B)** in the trenching plots (heterotrophic soil respiration). The whiskers represent the 95% confidence intervals of the point estimates. Wilcoxon test was used to test the differences between mixed and spruce stands, and the asterisks indicate the significance level (* - 0.05, ** - <0.01, *** - <0.001). Light blue rectangles indicate drought periods in 2022 and 2024, and the red rectangles indicate the period after the clear-cut in 2023.

The productivity of the spruce monoculture in our study dropped below that of the mixed stand, signalling a reduction in C sequestration potential for spruce-dominated forests under future climate warming and drying scenarios. While drought reduces the leaf area and water demand for both spruce and beech (Jacobs et al., 2021), beech and fir can recover fully when conditions improve. Spruce, on the other hand, has a needle longevity of 5–6 years (Reich et al., 1996), meaning foliage recovery takes longer (Zweifel et al., 2020). This lower recovery capacity likely contributed to the long-term growth decline observed in our study. The delayed recovery of spruce may also be linked to its lack of vessels, which restricts water transport velocity in the xylem (Hesse et al., 2023).

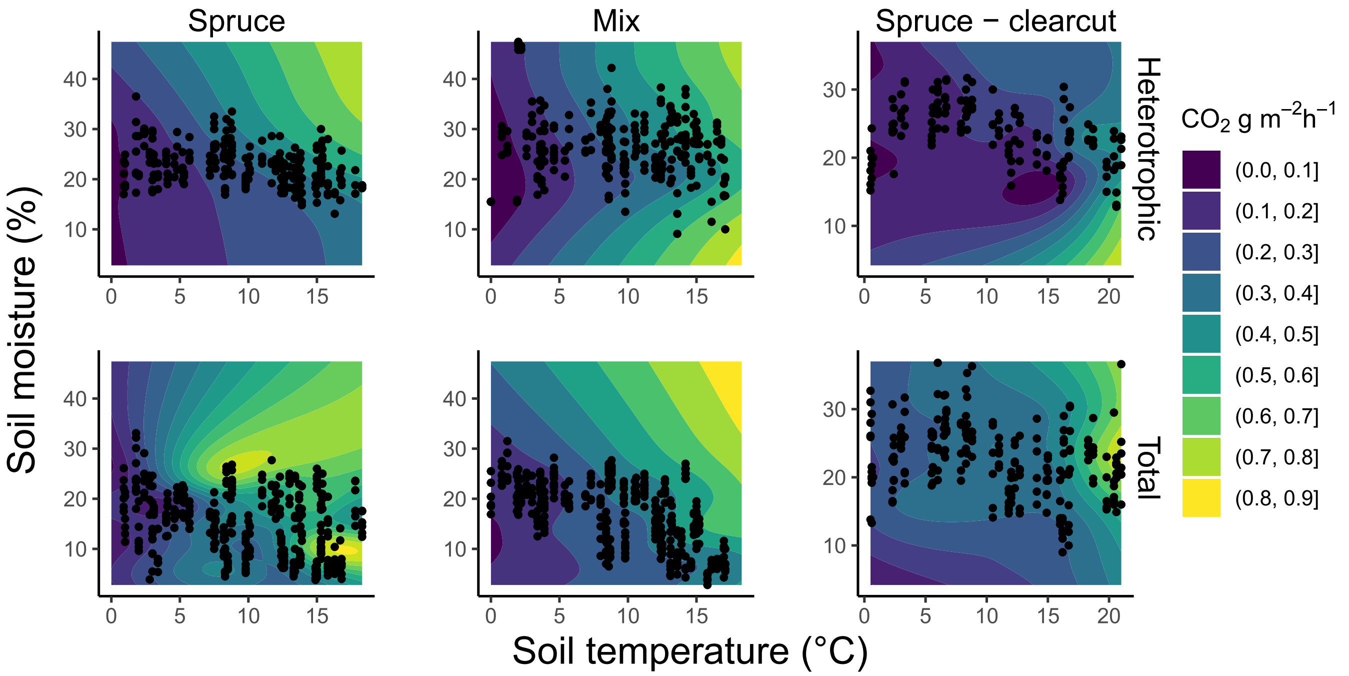

Fig. 7. Effects of soil temperature and moisture on heterotrophic and total CO_2 fluxes in the mixed and spruce stands as modelled by the mixed-effects GAM.

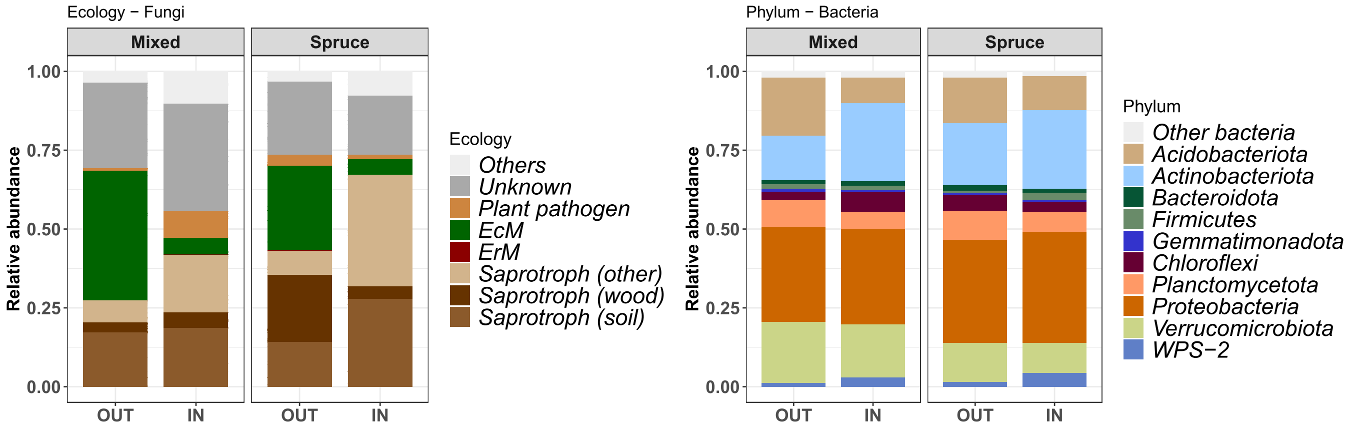

Fig. 8. The composition of fungi and bacteria in the mixed stand and spruce monoculture inside (IN, roots excluded, heterotrophic respiration only) and outside (OUT) the trenched plots.

In 2023, following the severe drought of 2022, the spruce monoculture was invaded by bark beetles, leading to a clear-cut in September 2023. The beetle outbreak was likely exacerbated by the reduced vitality and resin exudation of spruce caused by water stress (Netherer et al., 2015).

Silver fir exhibited a less intense response to the 2022 drought than spruce, consistent with recent findings (Bottero et al., 2021). Fir trees in our study even showed slightly higher annual growth in 2022 compared to other years, suggesting their ability to balance the dry period from June to August (Fig. S3) or that the drought was not severe for fir, which may have benefited from warmer conditions (Bottero et al., 2021; Vitasse et al., 2019). As an evergreen species, fir likely extends its growing season, starting photosynthesis as early as February or March and continuing until late autumn (Bošeľa et al., 2014). Norway spruce's shallower roots and more vulnerable xylem can lead to an earlier drop in gas exchange, cutting short the effective "extra" time (Lévesque et al., 2013).

Sycamore maple and common ash, present in the mixed stand, have been less studied regarding drought resistance and resilience. However, recent research suggests these species exhibit low climate vulnerability and long-term growth stability, contributing to the stable growth observed in the mixed stand throughout the study period (Leuschner et al., 2024). The limited sample size of the tree-ring series did not allow us to analyse these species' long-term growth and drought resistance in detail.

All species in this study responded significantly to vapour pressure deficit (VPD) in interaction with soil water potential (SWP), becoming more sensitive to VPD at higher SWP levels. Increasing VPD typically reduces stomatal aperture and conductance, curbing water loss and limiting growth (Grossiord et al., 2020; Yuan et al., 2019). Spruce was most vulnerable to this effect, a concern given the global rise in VPD and its potential impact on vegetation growth (Yuan et al., 2019). If VPD continues to rise under future warming, Norway spruce faces heightened mortality risks and large-scale disturbances, especially near or beyond its current distribution range (Honkaniemi et al., 2020).

Our findings demonstrate that while Norway spruce monocultures initially provide high productivity, long-term C sequestration in mixed forests is more stable, mitigating climate-related C losses. This suggests

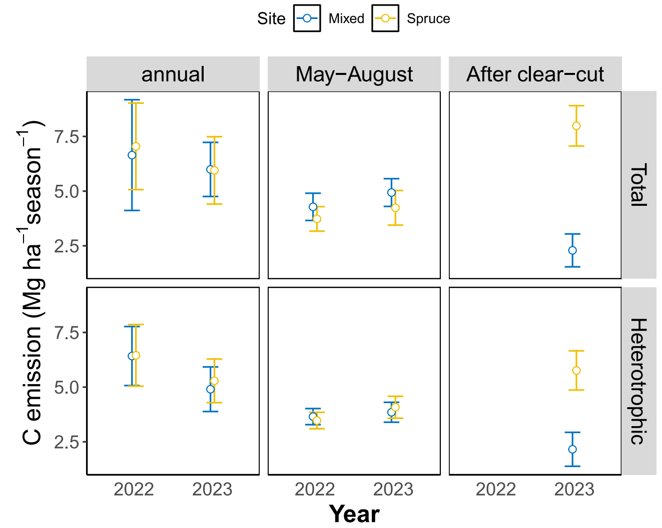

Fig. 9. Annual, seasonal (May-August), and post-disturbance total floor and heterotrophic C emissions from soil based on interpolation by the mixed GAM model. The estimation of C emitted after the clear-cut sums emissions from September (when the clear-cut was realised) to December. The whiskers represent the 95 % confidence intervals of the point estimates. The bars represent 95 % confidence intervals.

that forest management strategies favouring mixed compositions could enhance long-term C storage.

4.2. Drought and disturbance impacts on soil CO_2 and CH_4 fluxes

Heterotrophic respiration was the primary source of soil CO_2 emissions during the study period (Fig. S4), consistent with earlier findings (Mayer et al., 2017). Notably, the extreme drought of 2022 (particularly in May and June) reduced heterotrophic respiration's contribution to total forest floor respiration. In the spruce monoculture, heterotrophic respiration accounted for nearly 100 % of total respiration due to severely diminished tree growth (Fig. 4), which reduced autotrophic respiration by roots—a trend not observed in the mixed stand.

Drought significantly decreased total and heterotrophic respiration in both the mixed stand and spruce monoculture. This can be attributed to reduced soil moisture, tree growth, and microbial activity (Akande et al., 2023). Severe drought alters microbial community composition and function, shifting the balance between autotrophic and heterotrophic respiration (Meng et al., 2021). However, our study only conducted soil sampling for DNA analysis once in 2022, limiting our understanding of microbial community shifts due to drought. Microbial communities may tolerate periodic drought, with their activity being more affected than species composition (Schimel, 2018).

Soil respiration significantly increased following the clear-cut caused by the bark-beetle invasion, leading to a doubling of C emissions compared to the mixed stand or the same period in the previous year. This increase was likely driven by higher soil temperatures, soil moisture, and root decomposition, which stimulated microbial activity and litter breakdown (Mayer et al., 2024; Morehouse et al., 2008). While we found soil moisture to strongly influence soil respiration, the effect was more pronounced in the spruce monoculture, where soil drying began as early as April (falling below 10 % of the soil's full water capacity), whereas it remained stable at around 20 % in the mixed stand until late May, indicating faster and earlier transpiration of evergreen spruce trees than species in the mixed stand (Rötzer et al., 2017). The earlier start of transpiration in the spruce stand might be because spruce is evergreen and thus can start photosynthesis earlier than broadleaved species dominating the mixed stand. Moreover, the shallow root system of Norway spruce cannot access water in the deeper layers of the soil later in spring, particularly following a mild winter.

Our findings contrast with those of a recent study in a temperate forest in Germany, which found no significant contribution of soil moisture to soil respiration variability (Jevon et al., 2023). The discrepancy may be due to the extreme drought conditions during our measurement period, which were absent in the German study.

We expected that CH_4 uptake would be much lower after clear-cutting than during the pre-harvest period (Wu et al., 2011) and lower when compared to the mixed stand. We found that the uptake was strongly reduced immediately after the clear-cut; however, it returned to the same rates as in the mixed stand by the second year, 2024. The total recovery of CH_4 uptake in the second year does not align with other studies from European temperate forests, which suggest that reduced CH_4 consumption may persist for a decade or longer (Wu et al., 2011). However, it corroborates more recent post-harvest and post-fire studies (Halim et al., 2024). Another study in Norway spruce stands reported full recovery of CH_4 oxidation within 15–19 months (Saari et al., 2004), which is consistent with our findings. The discrepancy in the duration of CH_4 uptake recovery compared to earlier studies may be attributed to site-specific microbial adaptation, rapid changes in nitrogen cycling, or improved soil aeration following clear-cutting, warranting further investigation.

The reduced CH_4 oxidation shortly after the clear-cut can be attributed to various confounding factors. Clearcutting may increase soil mineral N contents, and NH_4^+ and NO_3^- could inhibit methane oxidation. This reaction is usually short-term, which may explain why CH_4 consumption recovered in the second year after the clear-cut at our site (Saari et al., 2004). However, increased N content does not explain the long-term reduction in CH_4 oxidation found in other studies (Wu et al., 2011). Another factor affecting CH_4 oxidation could be soil CH_4 diffusivity and O_2 availability, which is influenced by soil water content (Guckland et al., 2009). Higher soil moisture limits the activity of methanotrophic microorganisms, thus reducing CH_4 oxidation. Therefore, the recovery of CH_4 uptake following disturbances will depend on soil moisture conditions in subsequent years.

CH_4 uptake was found to be more sensitive to tree species than CO_2 fluxes and tends to be higher in deciduous forests (Jevon et al., 2023). Our study revealed that CO_2 fluxes and CH_4 uptake were higher in the mixed stand than the spruce stand, but the differences were only significant during drought periods. Estimates of annual and seasonal totals of soil C emissions did not show significant differences between the mixed stand and spruce monoculture. Microbial activity and decomposition strongly depend on soil organic C content, which, when low, can limit enzyme production (Sáez-Sandino et al., 2023). The relatively high and similar soil organic C content in both the mixed and spruce stands may explain the non-significant differences in soil respiration. The significant differences observed during drought can likely be attributed to soil moisture. Immediately after the clearcut, C emissions (from CO_2 effluxes) rose higher than in the mixed stand and remained significantly higher the following year, while sequestration dropped to nearly zero. In contrast, an empirical study showed that windthrow did not affect total soil CO_2 efflux because an increase in heterotrophic respiration offset reduced autotrophic respiration (Mayer et al., 2017).

In our study, increased respiration due to elevated soil temperature and moisture, coupled with the decomposition of dead fine and coarse roots after the clear-cut, significantly raised total soil respiration. Furthermore, the enhanced growth of ground vegetation, due to increased solar radiation and soil moisture on un-trenched plots, may have contributed to the elevated soil respiration after the clear-cut (Zhao et al., 2022). A decrease in the proportion of heterotrophic respiration in total CO_2 effluxes immediately following the clear-cut (Fig. S4) supports this hypothesis.

Unexpectedly, we found non-significant differences in annual and seasonal totals of CO_2 effluxes and CH_4 oxidation between the mixed stand and spruce monoculture, which contrasts with findings from other studies (Jevon et al., 2023). However, species identity was not found to be the primary driver of soil respiration; rather, different soil properties and microbial communities shaped under various tree species played a role (Lang et al., 2020; Sáez-Sandino et al., 2023). We found no significant difference in soil acidity, C:N, soil depth, and soil texture (percentage of sand, silt and clay) between the spruce and mixed stands, which allowed us to test the species effect rather than soil property effects. This may partly explain the similar CO_2 efflux and CH_4 uptake.

A higher proportion of saprotrophic fungi was present in the spruce stand, while the dominance of EcM fungi species in the mixed stand did not lead to differences in soil respiration. The slightly higher C stored in the mixed stand's soil may have contributed to the marginally higher (though not statistically significant) CO_2 fluxes and CH_4 uptake compared to the spruce monoculture, as the amount of C influences soil microbial biomass and, consequently, soil respiration (Sáez-Sandino et al., 2023). Our results indicated higher microbial biomass in the mixed forest compared to the spruce stand (Fig. S5), which may explain the elevated CO_2 fluxes at higher temperatures in the mixed forest (Fig. S1). The spruce stand, being 70 years old, may not have had sufficient time to substantially alter soil properties after replacing the originally broadleaf-dominated forest. The dominance of ectomycorrhizal fungi in mixed forests suggests a more stable soil microbial network, which may help sustain CH_4 oxidation and mitigate soil CO_2 efflux under drought conditions (Sáez-Sandino et al., 2023). In contrast, the higher saprotrophic activity in spruce stands may accelerate organic matter decomposition, potentially leading to higher C losses post-disturbance.

4.3. Study limitations and future research directions

Several methods have been used to separate autotrophic and heterotrophic soil respiration, with the root exclusion method being one of the most widely employed due to its relatively easy application (Chin et al., 2023). However, soil disturbance, root decay following root cutting, and changes in soil moisture and microbial community can increase CO_2 fluxes within the trenching plots (Díaz-Pinés et al., 2010). Many studies have reported higher soil respiration inside trenching plots even after long periods following their installation (Chin et al., 2023), limiting the accurate quantification of heterotrophic soil respiration.

In our study, we installed the trenching plots in April 2021 and began measurements in August, after five months. The first measurement revealed significantly lower soil CO_2 respiration within the trenching plots compared to the control plots, suggesting that the effects of soil disturbance and root decay had diminished after five months. Microbial analyses conducted during the study provide firm support, indicating a significantly reduced proportion of EcM fungi and an increased proportion of saprotrophs inside the trenching plots in both spruce and mixed stands. To further minimise the effects of plot installation, we quantified annual Rh and Rt for 2022 and 2023, i.e., one and two years after root exclusion (RE) installation. However, repeated soil sampling for DNA analyses would be necessary to understand how microbial community changes impact soil CO_2 and CH_4 fluxes.

Furthermore, root cutting and inserting a textile fabric to prevent root regrowth into the trenching plot can lead to waterlogging, which reduces heterotrophic respiration (Chin et al., 2023; Díaz-Pinés et al., 2010). We used water-permeable textile fabrics to limit waterlogging effects, allowing water to enter and exit while preventing root ingrowth. Additionally, we avoided using permanent PVC collars. Instead, we employed PVC collars with a larger diameter than the chamber to avoid gas leaching out of or air entering the chamber. Since we used a LI-COR gas analyser for CO_2 and CH_4 flux measurements, we could continuously monitor for any gas leaching during the measurements. In cases where leaching occurred, we repeated the measurements.

While this study provides valuable insights into the resilience of mixed forests and spruce monocultures, its findings are based on a single site in Central Europe. However, a study comparing CO_2 effluxes between temperate coniferous and deciduous forests in Central Europe showed that site characteristics had little impact on soil respiration, with soil temperature, moisture, and forest type being the dominant drivers (Wunderlich et al., 2012). Our study's long-term, continuous CO_2 and CH_4 effluxes monitoring using the root exclusion method was possible only at a smaller spatial scale. Nonetheless, establishing a monitoring network across diverse forest and soil types would be crucial to validate our results in the future. Further, future research should expand across different climatic regions and incorporate long-term post-clearcut CH_4 monitoring to better understand methane oxidation recovery mechanisms.

4.4. Conclusions and implications for forest management and climate mitigation

Our study examined the effects of tree species composition and Norway spruce clear-cutting on soil CO_2 and CH_4 fluxes. First, we compared drought resilience and soil GHG fluxes of a mixed unmanaged and a managed Norway spruce monoculture. The mixed stands consistently showed higher CO_2 effluxes from the soil and CH_4 uptake, especially during droughts. However, the differences in annual totals between the mixed and spruce stands were not statistically significant. This suggests that soil properties, microbial communities, and site-specific conditions, rather than tree species alone, drive soil C dynamics. The tree growth of the mixed stand was not strongly affected by drought periods, while the growth of Norway spruce showed a declining trend and was vulnerable to bark-beetle invasion after an extremely dry year.

Second, we analysed soil GHG fluxes after clear-cutting a Norway spruce stand after a drought period and bark-beetle invasion. CO_2 emissions were initially elevated post-clearcut due to increased soil temperatures, moisture, dead roots and vegetation decomposition. We also found that CH_4 uptake was significantly reduced immediately after clear-cutting but recovered within two years, contrary to previous studies suggesting more extended recovery periods.

The increasing vulnerability of spruce monocultures to climate stressors such as drought and bark beetle outbreaks accelerates C losses and highlights the need for forest management strategies that favour mixed and resilient species compositions to maintain ecosystem services like C sequestration. Thinning monocultures and transitioning toward mixed stands may not only stabilise biomass production but also prevent catastrophic C losses due to drought and bark beetle outbreaks. Future research should focus on expanding long-term monitoring across different soil types and forest ecosystems to better understand the complex interactions between forest management, soil properties, and greenhouse gas fluxes. Moreover, incorporating long-term post-clearcut CH_4 monitoring will help better understand methane oxidation recovery mechanisms.

CRediT authorship contribution statement

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Declaration of Competing 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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2025.122829.

Data availability

Data are available on Zenodo under the following DOI: 10.5281/zenodo.13906771

References

Abarenkov, K., Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., May, T.W., Frøslev, T.G., Pawlowska, J., Lindahl, B., Põldmaa, K., Truong, C., Vu, D., Hosoya, T., Niskanen, T., Piirmann, T., Ivanov, F., Zirk, A., Peterson, M., Cheeke, T.E., Ishigami, Y., Jansson, A.T., Jeppesen, T.S., Kristiansson, E., Mikryukov, V., Miller, J.T., Oono, R., Ossandon, F.J., Paupério, J., Saar, I., Schigel, D., Suija, A., Tedersoo, L., Kõljalg, U., 2024. The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. Nucleic Acids Res. 52, D791–D797. https://doi.org/10.1093/nar/gkad1039.

Akande, O.J., Ma, Z., Huang, C., He, F., Chang, S.X., 2023. Meta-analysis shows forest soil CO effluxes are dependent on the disturbance regime and biome type. Ecol. Lett. 26, 765–777. https://doi.org/10.1111/ele.14201.

Arend, M., Link, R.M., Patthey, R., Hoch, G., Schuldt, B., Kahmen, A., 2021. Rapid hydraulic collapse as cause of drought-induced mortality in conifers. Proc. Natl. Acad. Sci. 118, e2025251118. https://doi.org/10.1073/pnas.2025251118.

Aronesty, E., 2013. Comparison of sequencing utility programs. Open Bioinforma. J. 7, 1–8.

Aryal, S., Häusser, M., Grießinger, J., Fan, Z., Bräuning, A., 2020. dendRoAnalyst": A tool for processing and analysing dendrometer data. Dendrochronologia (Verona) 64, 125772. https://doi.org/10.1016/j.dendro.2020.125772.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss v067.i01.

Bengtsson-Palme, J., Veldre, V., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., Bertrand, Y., De Wit, P., Sanchez, M., Ebersberger, I., Sanli, K., de Souza, F., Kristiansson, E., Abarenkov, K., Eriksson, K.M., Nilsson, R.H., 2013. ITSx: Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for use in environmental sequencing. Methods Ecol. Evol. 4 (10), 914–919.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. https://doi.org/10.1139/o59-099.

Bosela, M., Sedmak, R., Marusak, R., Sedmakova, D., Petras, R., Barna, M., Bošeľa, M., Sedmák, R., Marušák, R., Sedmáková, D., Petráš, R., Barna, M., Bošel'a, M., Sedmák, R., Marušák, R., Sedmáková, D., Petráš, R., Barna, M., 2014. Evaluating similarity of radial increments around tree stem circumference of European beech and Norway spruce from Central Europe. Geochronometria 41, 136–146. https://doi.org/10.2478/s13386-013-0152-3.

Bosela, M., Tumajer, J., Cienciala, E., Dobor, L., Kulla, L., Marčiš, P., Popa, I., Sedmák, R., Sedmáková, D., Sitko, R., Šebeň, V., Štěpánek, P., Büntgen, U., 2021. Climate warming induced synchronous growth decline in Norway spruce populations across biogeographical gradients since 2000. Sci. Total Environ. 752, 141794. https://doi.org/10.1016/j.scitotenv.2020.141794.

Bošeľa, M., Petráš, R., Sitková, Z., Priwitzer, T., Pajtík, J., Hlavatá, H., Sedmák, R., Tobin, B., 2014. Possible causes of the recent rapid increase in the radial increment of silver fir in the Western Carpathians. Environ. Pollut. 184, 211–221. https://doi.org/10.1016/j.envpol.2013.08.036.

Bottero, A., Forrester, D.I., Cailleret, M., Kohnle, U., Gessler, A., Michel, D., Bose, A.K., Bauhus, J., Bugmann, H., Cuntz, M., Gillerot, L., Hanewinkel, M., Lévesque, M., Ryder, J., Sainte-Marie, J., Schwarz, J., Yousefpour, R., Zamora-Pereira, J.C., Rigling, A., 2021. Growth resistance and resilience of mixed silver fir and Norway spruce forests in central Europe: Contrasting responses to mild and severe droughts. Glob. Chang Biol. 27, 4403–4419. https://doi.org/10.1111/gcb.15737.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Chiang, F., Mazdiyasni, O., AghaKouchak, A., 2021. Evidence of anthropogenic impacts on global drought frequency, duration, and intensity. Nat. Commun. 12, 2754. https://doi.org/10.1038/s41467-021-22314-w.

Chin, M.-Y., Lau, S.Y.L., Midot, F., Jee, M.S., Lo, M.L., Sangok, F.E., Melling, L., 2023. Root exclusion methods for partitioning of soil respiration: Review and methodological considerations. Pedosphere 33, 683–699. https://doi.org/10.1016/j.pedsph.2023.01.015.

Cook, E., Kairiukstis, L., 2013. Methods of Dendrochronology: Applications in the Environmental Sciences. Springer Netherlands.

Díaz-Pinés, E., Schindlbacher, A., Pfeffer, M., Jandl, R., Zechmeister-Boltenstern, S., Rubio, A., 2010. Root trenching: a useful tool to estimate autotrophic soil respiration? A case study in an Austrian mountain forest. Eur. J. Res 129, 101–109. https://doi.org/10.1007/s10342-008-0250-6.

Dlugokencky, E.J., Nisbet, E.G., Fisher, R., Lowry, D., 2011. Global atmospheric methane: budget, changes and dangers. Philos. Trans. R. Soc. A: Math., Phys. Eng. Sci. 369, 2058–2072. https://doi.org/10.1098/rsta.2010.0341.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. https://doi.org/10.1038/nmeth.2604.

Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K., 2006. 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Japan. https://doi.org/4-88788-032-4.

Feng, H., Guo, J., Han, M., Wang, W., Peng, C., Jin, J., Song, X., Yu, S., 2020. A review of the mechanisms and controlling factors of methane dynamics in forest ecosystems. Ecol. Manag. 455, 117702. https://doi.org/10.1016/j.foreco.2019.117702.

Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. Environ. Microbiol 59, 3605–3617. https://doi.org/10.1128/aem.59.11.3605-3617.1993.

Gebhardt, T., Hesse, B.D., Hikino, K., Kolovrat, K., Hafner, B.D., Grams, T.E.E., Häberle, K.-H., 2023. Repeated summer drought changes the radial xylem sap flow profile in mature Norway spruce but not in European beech. Agric. Meteor. 329, 109285. https://doi.org/10.1016/j.agrformet.2022.109285.

Georgiou, K., Jackson, R.B., Vindušková, O., Abramoff, R.Z., Ahlström, A., Feng, W., Harden, J.W., Pellegrini, A.F.A., Polley, H.W., Soong, J.L., Riley, W.J., Torn, M.S., 2022. Global stocks and capacity of mineral-associated soil organic carbon. Nat. Commun. 13, 3797. https://doi.org/10.1038/s41467-022-31540-9.

Grassi, G., House, J., Dentener, F., Federici, S., den Elzen, M., Penman, J., 2017. The key role of forests in meeting climate targets requires science for credible mitigation. Nat. Clim. Chang 7, 220–226. https://doi.org/10.1038/nclimate3227.

Grossiord, C., Buckley, T.N., Cernusak, L.A., Novick, K.A., Poulter, B., Siegwolf, R.T.W., Sperry, J.S., McDowell, N.G., 2020. Plant responses to rising vapor pressure deficit. N. Phytol. 226, 1550–1566. https://doi.org/10.1111/nph.16485.

Gschwantner, T., Alberdi, I., Balázs, A., Bauwens, S., Bender, S., Borota, D., Bosela, M., Bouriaud, O., Cañellas, I., Donis, J., Freudenschuß, A., Hervé, J.-C., Hladnik, D., Jansons, J., Kolozs, L., Korhonen, K.T., Kucera, M., Kulbokas, G., Kuliešis, A., Lanz, A., Lejeune, P., Lind, T., Marin, G., Morneau, F., Nagy, D., Nord-Larsen, T., Nunes, L., Pantić, D., Paulo, J.A., Pikula, T., Redmond, J., Rego, F.C., Riedel, T., Saint-André, L., Šebeň, V., Sims, A., Skudnik, M., Solti, G., Tomter, S.M., Twomey, M., Westerlund, B., Zell, J., 2019. Harmonisation of stem volume estimates in European National Forest Inventories. Ann. Sci. 76. https://doi.org/10.1007/s13595-019-0800-8.

Guckland, A., Flessa, H., Prenzel, J., 2009. Controls of temporal and spatial variability of methane uptake in soils of a temperate deciduous forest with different abundance of European beech (Fagus sylvatica L.). Soil Biol. Biochem 41, 1659–1667. https://doi.org/10.1016/j.soilbio.2009.05.006.

Halim, M.A., Bieser, J.M.H., Thomas, S.C., 2024. Large, sustained soil CO2 efflux but rapid recovery of CH4 oxidation in post-harvest and post-fire stands in a mixedwood boreal forest. Sci. Total Environ. 930, 172666. https://doi.org/10.1016/j.scitotenv.2024.172666.

Hesse, B.D., Gebhardt, T., Hafner, B.D., Hikino, K., Reitsam, A., Gigl, M., Dawid, C., Häberle, K.-H., Grams, T.E.E., 2023. Physiological recovery of tree water relations upon drought release—response of mature beech and spruce after five years of recurrent summer drought. Tree Physiol. 43, 522–538. https://doi.org/10.1093/treephys/tpac135.

Hlásny, T., König, L., Krokene, P., Lindner, M., Montagné-Huck, C., Müller, J., Qin, H., Raffa, K.F., Schelhaas, M.-J., Svoboda, M., Viiri, H., Seidl, R., 2021. Bark Beetle Outbreaks in Europe: State of Knowledge and Ways Forward for Management. Curr. For. Rep. 7, 138–165. https://doi.org/10.1007/s40725-021-00142-x.

Ho, A., van den Brink, E., Reim, A., Krause, S.M.B., Bodelier, P.L.E., 2016. Recurrence and frequency of disturbance have cumulative effect on methanotrophic activity, abundance, and community structure. Front Microbiol 6.

Honkaniemi, J., Rammer, W., Seidl, R., 2020. Norway spruce at the trailing edge: the effect of landscape configuration and composition on climate resilience. Land. Ecol. 35, 591–606. https://doi.org/10.1007/s10980-019-00964-y.

Hursh, A., Ballantyne, A., Cooper, L., Maneta, M., Kimball, J., Watts, J., 2017. The sensitivity of soil respiration to soil temperature, moisture, and carbon supply at the global scale. Glob. Chang Biol. 23, 2090–2103. https://doi.org/10.1111/gcb.13489.

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol Ecol. 82, 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x.

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge, United Kingdom and New York, NY, US.

IPCC, 2023. Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.)]. Geneva, Switzerland.

Jacobs, M., Rais, A., Pretzsch, H., 2021. How drought stress becomes visible upon detecting tree shape using terrestrial laser scanning (TLS). Ecol. Manag. 489, 118975. https://doi.org/10.1016/j.foreco.2021.118975.

Jandl, R., Spathelf, P., Bolte, A., Prescott, C.E., 2019. Forest adaptation to climate change—is non-management an option? Ann. Sci. 76, 48. https://doi.org/10.1007/s13595-019-0827-x.

Jevon, F.V., Gewirtzman, J., Lang, A.K., Ayres, M.P., Matthes, J.H., 2023. Tree species effects on soil CO2 and CH4 fluxes in a mixed temperate forest. Ecosystems 26, 1587–1602. https://doi.org/10.1007/s10021-023-00852-2.

Joergensen, R.G., 2022. Phospholipid fatty acids in soil—drawbacks and future prospects. Biol. Fertil. Soils 58, 1–6. https://doi.org/10.1007/s00374-021-01613-w.

Kang, E., Li, Y., Zhang, X., Yan, Z., Zhang, W., Zhang, K., Yan, L., Wu, H., Li, M., Niu, Y., Yang, A., Wang, J., Kang, X., 2022. Extreme drought decreases soil heterotrophic respiration but not methane flux by modifying the abundance of soil microbial functional groups in alpine peatland. Catena (Amst.) 212, 106043. https://doi.org/10.1016/j.catena.2022.106043.

Lang, A.K., Jevon, F.V., Ayres, M.P., Hatala Matthes, J., 2020. Higher Soil Respiration Rate Beneath Arbuscular Mycorrhizal Trees in a Northern Hardwood Forest is Driven by Associated Soil Properties. Ecosystems 23, 1243–1253. https://doi.org/10.1007/s10021-019-00466-7.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68. https://doi.org/10.1038/nature16069.

Leuschner, C., 2020. Drought response of European beech (Fagus sylvatica L.)—A review. Perspect. Plant Ecol. Evol. Syst. 47, 125576. https://doi.org/10.1016/j.ppees.2020.125576.

Leuschner, C., Fuchs, S., Wedde, P., Rüther, E., Schuldt, B., 2024. A multi-criteria drought resistance assessment of temperate Acer, Carpinus, Fraxinus, Quercus, and Tilia species. Perspect. Plant Ecol. Evol. Syst. 62, 125777. https://doi.org/10.1016/j.ppees.2023.125777.

Lévesque, M., Saurer, M., Siegwolf, R., Eilmann, B., Brang, P., Bugmann, H., Rigling, A., 2013. Drought response of five conifer species under contrasting water availability suggests high vulnerability of Norway spruce and European larch. Glob. Chang Biol. 19, 3184–3199. https://doi.org/10.1111/gcb.12268.

Mäkipää, R., Abramoff, R., Adamczyk, B., Baldy, V., Biryol, C., Bosela, M., Casals, P., Curiel Yuste, J., Dondini, M., Filipek, S., Garcia-Pausas, J., Gros, R., Gömöryová, E., Hashimoto, S., Hassegawa, M., Immonen, P., Laiho, R., Li, H., Li, Q., Luyssaert, S., Menival, C., Mori, T., Naudts, K., Santonja, M., Smolander, A., Toriyama, J., Tupek, B., Ubeda, X., Johannes Verkerk, P., Lehtonen, A., 2023. How does management affect soil C sequestration and greenhouse gas fluxes in boreal and temperate forests? – A review. Ecol. Manag. 529, 120637. https://doi.org/10.1016/j.foreco.2022.120637.

Mayer, M., Sandén, H., Rewald, B., Godbold, D.L., Katzensteiner, K., 2017. Increase in heterotrophic soil respiration by temperature drives decline in soil organic carbon stocks after forest windthrow in a mountainous ecosystem. Funct. Ecol. 31, 1163–1172. https://doi.org/10.1111/1365-2435.12805.

Mayer, M., Baltensweiler, A., James, J., Rigling, A., Hagedorn, F., 2024. A global synthesis and conceptualization of the magnitude and duration of soil carbon losses in response to forest disturbances. Glob. Ecol. Biogeogr. 33, 141–150. https://doi.org/10.1111/geb.13779.

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8, e61217.

Meng, B., Ochoa-Hueso, R., Li, J., Zhong, S., Yao, Y., Yang, X., Collins, S.L., Sun, W., 2021. Nonlinear decoupling of autotrophic and heterotrophic soil respiration in response to drought duration and N addition in a meadow steppe. Biol. Fertil. Soils 57, 281–291. https://doi.org/10.1007/s00374-020-01524-2.

Morehouse, K., Johns, T., Kaye, J., Kaye, M., 2008. Carbon and nitrogen cycling immediately following bark beetle outbreaks in southwestern ponderosa pine forests. Ecol. Manag. 255, 2698–2708. https://doi.org/10.1016/j.foreco.2008.01.050.

Motte, F., Rötzer, T., Biber, P., Uhl, E., Pritsch, K., Pretzsch, H., 2023. Growth of European beech recovered faster than that of Norway spruce after a five-year experimental drought in a mixed forest stand. Trees 37, 1695–1715. https://doi.org/10.1007/s00468-023-02453-x.

Naudts, K., Chen, Y., McGrath, M.J., Ryder, J., Valade, A., Otto, J., Luyssaert, S., 2016. Europe's forest management did not mitigate climate warming. Science 351 (1979), 597–599. https://doi.org/10.1126/science.aad7270.

Netherer, S., Matthews, B., Katzensteiner, K., Blackwell, E., Henschke, P., Hietz, P., Pennerstorfer, J., Rosner, S., Kikuta, S., Schume, H., Schopf, A., 2015. Do water-limiting conditions predispose Norway spruce to bark beetle attack? N. Phytol. 205, 1128–1141. https://doi.org/10.1111/nph.13166.

Ni, X., Groffman, P.M., 2018. Declines in methane uptake in forest soils. Proc. Natl. Acad. Sci. 115, 8587–8590. https://doi.org/10.1073/pnas.1807377115.

Nissan, A., Alcolombri, U., Peleg, N., Galili, N., Jimenez-Martinez, J., Molnar, P., Holzner, M., 2023. Global warming accelerates soil heterotrophic respiration. Nat. Commun. 14, 3452. https://doi.org/10.1038/s41467-023-38981-w.

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2024. vegan: Community Ecology Package.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science 333, 988–993. https://doi.org/10.1126/science.1201609.

Petráš, R., Pajtík, J., 1991. Sústava česko-slovenských objemových tabuliek drevín [Tree volume equations for main tree species in Czechoslovakia]. Lesnícky časopis 37, 49–56.

Pirtskhalava-Karpova, N., Trubin, A., Karpov, A., Jakuš, R., 2024. Drought initialised bark beetle outbreak in Central Europe: Meteorological factors and infestation dynamic. Ecol. Manag. 554, 121666. https://doi.org/10.1016/j.foreco.2023.121666.

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B.D., Clemmensen, K.E., Kauserud, H., Nguyen, N., Kjøller, R., Bates, S.T., Baldrian, P., Frøslev, T.G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järv, H., Madrid, H., Nordén, J., Liu, J.-K., Pawlowska, J., Põldmaa, K., Pärtel, K., Runnel, K., Hansen, K., Larsson, K.-H., Hyde, K.D., Sandoval-Denis, M., Smith, M.E., Toome-Heller, M., Wijayawardene, N.N., Menolli, N., Reynolds, N.K., Drenkhan, R., Maharachchikumbura, S.S.N., Gibertoni, T.B., Læssøe, T., Davis, W., Tokarev, Y., Corrales, A., Soares, A.M., Agan, A., Machado, A.R., Argüelles-Moyao, A., Detheridge, A., de Meiras-Ottoni, A., Verbeken, A., Dutta, A.K., Cui, B.-K., Pradeep, C.K., Marín, C., Stanton, D., Gohar, D., Wanasinghe, D.N., Otsing, E., Aslani, F., Griffith, G.W., Lumbsch, T.H., Grossart, H.-P., Masigol, H., Timling, I., Hiiesalu, I., Oja, J., Kupagme, J.Y., Geml, J., Alvarez-Manjarrez, J., Ilves, K., Loit, K., Adamson, K., Nara, K., Küngas, K., Rojas-Jimenez, K., Bitenieks, K., Irinyi, L., Nagy, L.G., Soonvald, L., Zhou, L.-W., Wagner, L., Aime, M.C., Öpik, M., Mujica, M.I., Metsoja, M., Ryberg, M., Vasar, M., Murata, M., Nelsen, M.P., Cleary, M., Samarakoon, M.C., Doilom, M., Bahram, M., Hagh-Doust, N., Dulya, O., Johnston, P., Kohout, P., Chen, Q., Tian, Q., Nandi, R., Amiri, R., Perera, R.H., dos Santos Chikowski, R., Mendes-Alvarenga, R.L., Garibay-Orijel, R., Gielen, R., Phookamsak, R., Jayawardena, R.S., Rahimlou, S., Karunarathna, S.C., Tibpromma, S., Brown, S.P., Sepp, S.-K., Mundra, S., Luo, Z.-H., Bose, T., Vahter, T., Netherway, T., Yang, T., May, T., Varga, T., Li, W., Coimbra, V.R.M., de Oliveira, V.R.T., de Lima, V.X., Mikryukov, V.S., Lu, Y., Matsuda, Y., Miyamoto, Y., Kõljalg, U., Tedersoo, L., 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Divers 105, 1–16. https://doi.org/10.1007/s13225-020-00466-2.

Pretzsch, H., Rötzer, T., Matyssek, R., Grams, T.E.E., Häberle, K.-H., Pritsch, K., Kerner, R., Munch, J.-C., 2014. Mixed Norway spruce (Picea abies [L.] Karst) and European beech (Fagus sylvatica [L.]) stands under drought: from reaction pattern to mechanism. Trees 28 (5), 1305–1321. https://doi.org/10.1007/s00468-014-1035-9.

Pretzsch, H., Schütze, G., 2009. Transgressive overyielding in mixed compared with pure stands of Norway spruce and European beech in Central Europe: Evidence on stand level and explanation on individual tree level. Eur. J. For. Res. 128 (2), 183–204. https://doi.org/10.1007/s10342-008-0215-9.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

Reich, P.B., Oleksyn, J., Modrzynski, J., Tjoelker, M.G., 1996. Evidence that longer needle retention of spruce and pine populations at high elevations and high latitudes is largely a phenotypic response. Tree Physiol. 16, 643–647. https://doi.org/10.1093/treephys/16.7.643.

Richards, F.J., 1959. A Flexible Growth Function for Empirical Use. J. Exp. Bot. 10, 290–300.

van Rij, J., Wieling, M., Harald Baayen, R., van Rijn, H., 2022. itsadug: Interpreting Time Series and Autocorrelated Data Using GAMMs.

Rötzer, T., Häberle, K.H., Kallenbach, C., Matyssek, R., Schütze, G., Pretzsch, H., 2017. Tree species and size drive water consumption of beech/spruce forests - a simulation study highlighting growth under water limitation. Plant Soil 418, 337–356. https://doi.org/10.1007/s11104-017-3306-x.

Saari, A., Smolander, A., Martikainen, P.J., 2004. Methane consumption in a frequently nitrogen-fertilized and limed spruce forest soil after clear-cutting. Soil Use Manag 20, 65–73. https://doi.org/10.1111/j.1475-2743.2004.tb00338.x.

Sáez-Sandino, T., García-Palacios, P., Maestre, F.T., Plaza, C., Guirado, E., Singh, B.K., Wang, J., Cano-Díaz, C., Eisenhauer, N., Gallardo, A., Delgado-Baquerizo, M., 2023. The soil microbiome governs the response of microbial respiration to warming across the globe. Nat. Clim. Chang 13, 1382–1387. https://doi.org/10.1038/s41558-023-01868-1.

Sagova-Mareckova, M., Cermak, L., Novotna, J., Plhackova, K., Forstova, J., Kopecky, J., 2008. Innovative Methods for Soil DNA Purification Tested in Soils with Widely Differing Characteristics. Appl. Environ. Microbiol 74, 2902–2907. https://doi.org/10.1128/AEM.02161-07.

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J.G., Dlugokencky, E.J., Etiope, G., Bastviken, D., Houweling, S., Janssens-Maenhout, G., Tubiello, F.N., Castaldi, S., Jackson, R.B., Alexe, M., Arora, V.K., Beerling, D.J., Bergamaschi, P., Blake, D.R., Brailsford, G., Brovkin, V., Bruhwiler, L., Crevoisier, C., Crill, P., Covey, K., Curry, C., Frankenberg, C., Gedney, N., Höglund-Isaksson, L., Ishizawa, M., Ito, A., Joos, F., Kim, H.-S., Kleinen, T., Krummel, P., Lamarque, J.-F., Langenfelds, R., Locatelli, R., Machida, T., Maksyutov, S., McDonald, K.C., Marshall, J., Melton, J.R., Morino, I., Naik, V., O'Doherty, S., Parmentier, F.-J.W., Patra, P.K., Peng, C., Peng, S., Peters, G.P., Pison, I., Prigent, C., Prinn, R., Ramonet, M., Riley, W.J., Saito, M., Santini, M., Schroeder, R., Simpson, I.J., Spahni, R., Steele, P., Takizawa, A., Thornton, B.F., Tian, H., Tohjima, Y., Viovy, N., Voulgarakis, A., van Weele, M., van der Werf, G.R., Weiss, R., Wiedinmyer, C., Wilton, D.J., Wiltshire, A., Worthy, D., Wunch, D., Xu, X., Yoshida, Y., Zhang, B., Zhang, Z., Zhu, Q., 2016. The global methane budget 2000–2012. Earth Syst. Sci. Data 8, 697–751. https://doi.org/10.5194/essd-8-697-2016.

Schimel, J.P., 2018. Life in dry soils: Effects of drought on soil microbial communities and processes. Annu Rev Ecol Evol Syst 49, 409–432. https://doi.org/10.1146/ANNUREV-ECOLSYS-110617-062614/CITE/REFWORKS.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20. https://doi.org/10.1023/A:1006247623877.

Seidl, R., Schelhaas, M.-J., Rammer, W., Verkerk, P.J., 2014. Increasing forest disturbances in Europe and their impact on carbon storage. Nat. Clim. Chang 4, 806–810. https://doi.org/10.1038/nclimate2318.

Seidl, R., Thom, D., Kautz, M., Martin-Benito, D., Peltoniemi, M., Vacchiano, G., Wild, J., Ascoli, D., Petr, M., Honkaniemi, J., Lexer, M.J., Trotsiuk, V., Mairota, P., Svoboda, M., Fabrika, M., Nagel, T.A., Reyer, C.P.O., 2017. Forest disturbances under climate change. Nat. Clim. Chang 7, 395–402. https://doi.org/10.1038/nclimate3303.

Škvarenina, J., Križová, E., Tomlain, J., 2004. Impact of the climate change on the water balance of altitudinal vegetation stages in Slovakia. Ekológia. Bratislava 23, 13–29.

Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biol. Biochem 40, 2068–2075. https://doi.org/10.1016/j.soilbio.2008.01.015.

Spiecker, H., Hansen, J., Klimo, E., Skovsgaard, J.P., Sterba, H., von Teuffel, K., 2004. Norway spruce conversion - Options and consequences. Koninklijke Brill NV, Leiden, The Netherlands.

Štursová, M., Baldrian, P., 2011. Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. Plant Soil 338, 99–110. https://doi.org/10.1007/s11104-010-0296-3.

Teimouri, M., Doser, J.W., Finley, A.O., 2020. ForestFit: an R package for modeling plant size distributions. Environ. Model. Softw. 131, 104668. https://doi.org/10.1016/j.envsoft.2020.104668.

Thornthwaite, C.W., 1948. An approach toward a rational classification of climate. Geogr. Rev. 38, 55–94. https://doi.org/10.2307/210739.

Ulrich, D.E.M., Grossiord, C., 2023. Faster drought recovery in anisohydric beech compared with isohydric spruce. Tree Physiol. 43, 517–521. https://doi.org/10.1093/treephys/tpad009.

Větrovský, T., Baldrian, P., Morais, D., 2018. SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. Bioinformatics 34, 2292–2294. https://doi.org/10.1093/bioinformatics/bty071.

Vitasse, Y., Bottero, A., Cailleret, M., Bigler, C., Fonti, P., Gessler, A., Lévesque, M., Rohner, B., Weber, P., Rigling, A., Wohlgemuth, T., 2019. Contrasting resistance and resilience to extreme drought and late spring frost in five major European tree species. Glob. Chang Biol., gcb.14803 https://doi.org/10.1111/gcb.14803.

Welc, M., Bünemann, E.K., Fließbach, A., Frossard, E., Jansa, J., 2012. Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. Soil Biol. Biochem. 49, 184–192. https://doi.org/10.1016/j.soilbio.2012.01.032.

Wood, S.N., 2003. Thin plate regression splines. J. R. Stat. Soc. Ser. B Stat. Method. 65, 95–114. https://doi.org/10.1111/1467-9868.00374.

Wood, S.N., Pya, N., Säfken, B., 2016. Smoothing parameter and model selection for general smooth models. J. Am. Stat. Assoc. 111, 1548–1563. https://doi.org/10.1080/01621459.2016.1180986.

Wu, X., Brüggemann, N., Gasche, R., Papen, H., Willibald, G., Butterbach-Bahl, K., 2011. Long-term effects of clear-cutting and selective cutting on soil methane fluxes in a temperate spruce forest in southern Germany. Environ. Pollut. 159, 2467–2475. https://doi.org/10.1016/j.envpol.2011.06.025.

Wunderlich, S., Schulz, C., Grimmeisen, W., Borken, W., 2012. Carbon fluxes in coniferous and deciduous forest soils. Plant Soil 357, 355–368. https://doi.org/10.1007/s11104-012-1158-y.

Yang, R.C., Kozak, A., Smith, J.H.G., 1978. The potential of Weibull-type functions as flexible growth curves. Can. J. For. Res. 8, 424–431. https://doi.org/10.1139/x78-062.

Yuan, W., Zheng, Y., Piao, S., Ciais, P., Lombardozzi, D., Wang, Y., Ryu, Y., Chen, G., Dong, W., Hu, Z., Jain, A.K., Jiang, C., Kato, E., Li, S., Lienert, S., Liu, S., Nabel, J.E.M.S., Qin, Z., Quine, T., Sitch, S., Smith, W.K., Wang, F., Wu, C., Xiao, Z., Yang, S., 2019. Increased atmospheric vapor pressure deficit reduces global vegetation growth. Sci. Adv. 5, eaax1396. https://doi.org/10.1126/sciadv.aax1396.

Yue, C., Kohnle, U., Hanewinkel, M., Klädtke, J., 2011. Extracting environmentally driven growth trends from diameter increment series based on a multiplicative decomposition model. Can. J. For. Res. 41, 1577–1589. https://doi.org/10.1139/x11-056.

Zhao, B., Ballantyne, A.P., Meng, S., Zhao, G., Zheng, Z., Zhu, J., Cao, J., Zhang, Y., Zhao, X., 2022. Understory plant removal counteracts tree thinning effect on soil respiration in a temperate forest. Glob. Chang Biol. 28, 6102–6113. https://doi.org/10.1111/gcb.16337.

Zhao, J., 2019. FluxCalR: a R package for calculating CO2 and CH4 fluxes from static chambers. J. Open Source Softw. 4, 1751. https://doi.org/10.21105/joss.01751.

Zweifel, R., Haeni, M., Buchmann, N., Eugster, W., 2016. Are trees able to grow in periods of stem shrinkage? New Phytol. 211, 839–849. https://doi.org/10.1111/nph.13995.

Zweifel, R., Etzold, S., Sterck, F., Gessler, A., Anfodillo, T., Mencuccini, M., von Arx, G., Lazzarin, M., Haeni, M., Feichtinger, L., Meusburger, K., Knuesel, S., Walthert, L., Salmon, Y., Bose, A.K., Schoenbeck, L., Hug, C., De Girardi, N., Giuggiola, A., Schaub, M., Rigling, A., 2020. Determinants of legacy effects in pine trees – implications from an irrigation-stop experiment. New Phytol. 227, 1081–1096. https://doi.org/10.1111/nph.16582.