



D4.4 Empirical analysis on the impact of CSF management on soil properties and GHG exchange

Holistic management practices, modelling and monitoring for European forest soils, HoliSoils

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Deliverable D4.4: Empirical analysis on the impact of CSF

This deliverable describes the major findings from the HoliSoils WP4 test sites on forest management. The impact of Climate-Smart Forestry (CSF) on soil processes and functioning has been evaluated through measurements of soil greenhouse gas (GHG) emissions, soil physical and chemical properties, litter inputs, root biomass quantity, soil microbial populations, and soil fauna. The main findings here have been derived from a comparison between control and CSF management on these sites.

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1. Introduction

Project and objective

HoliSoils tackles gaps in knowledge on forest soil processes and aims to harmonise available soil monitoring information to support decision making towards climate and sustainability goals. HoliSoils identifies and tests novel soil management practices aiming to mitigate or adapt to climate change and sustain provision of various ecosystem services essential for human livelihoods and wellbeing in changing climate.

2. HoliSoils test sites

The concept underlying the establishment of HoliSoils test sites in the WP4 entailed the creation of experimental sites where current (business-as-usual, BAU) forest management practices have been modified to respond current knowledge requirements that relate to climate-smart forestry (CSF) practices. HoliSoils established a network of test sites across Europe and in Uruguay, as part of both WP4 and WP5 (Fig. 2.1 and Table 2.1). It is important to note that due to unforeseen technical issues, the setup of the experimental sites 'Buchenhorst' and 'Eisenstraßenmoor' was delayed. Challenges included delays in acquiring and installing in the field the equipment for monitoring of environmental variables and GHG fluxes, as well as unexpected logistical obstacles that disrupted the overall timeline. These setbacks had a cascading effect, delaying both data collection and analysis. As a result, preliminary results from these sites were not included in the Deliverable.

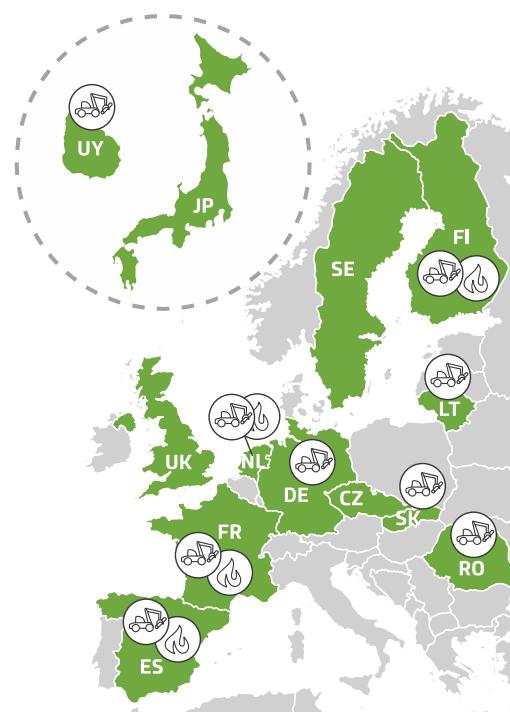


Figure 2.1. Geographical distribution of HoliSoils' consortium participants & test sites for WP4 and WP5.

Table 2.1. HoliSoils' twelve test sites for experimental forest management, updated during the project.

Test site (TS) ¹	Country	Location & host organisation	Tree species	Soil type	Climate	Current management	Experimentation (treatment)
A	FI	Ränskälänkorpi (Luke)	<i>P. abies, P. sylvestris, B. pubescens</i>	Drained fen	Dfc	Drained peatland forestry	Manipulating water table depth with continuous cover thinning
B	DE	Buchenhorst (TI)	<i>A. glutinosa</i>		Dfb	Even-aged management	Rewetting vs. control
C	DE	Eisenstraßenmoor (TI)	<i>P. abies</i>	Drained peat bog	Dfc		
D	SK	Dobroc Municipality (TUZVO)	<i>F. sylvatica, P. abies, A. alba</i>	Deep cambisols	Dfb	Spruce monoculture as a control	Mixed stand w/ broadleaved species
E	DE	Three locations along precipitation gradient (TUM)	<i>P. abies, F. sylvatica</i>	Cambisols, luvisols	Dfb		Mixed stands broadleaved species, experimental drought, (Kranzberg)
F	NL	Ugchelen, Kroondomein, NP Hoge Veluwe, Buunderkamp, Zwolse Bos, Weerterheide, Bergeijk, Mastbos, Dorst (WR)	<i>P. sylvestris, P. menziesii, F. sylvatica</i>	Poor sandy	Cfb	Management on water-limited soils	Different thinning intensities
G	FR	St. Mitre (AMU)	<i>P. halepensis</i>	Sandy loamy calcareous	Csa		
H	ES	Three locations (CTFC)	<i>P. nigra</i>	Loamy carbonate	Cfa	Management on fire-prone ecosystem	Debris management using prescribed fire
I	ES	Three locations (CTFC)			Cfa		
J	FI	Karstula (Luke)	<i>P. sylvestris</i>	Sandy – sandy loamy	Dfc	Even-aged management	Fertilization since 1960 w/ NPK
K	LT	Kacergine (VMU)	<i>P. sylvestris</i>	Poor sandy	Dfb		Fertilization w/ ash
L	UY	Paysandu, Rio Negro & Tacuarembo Regions (INIA)	<i>E. grandis, E. dunnii</i>	Sandy loam, fine	Cfa	Degraded native grassland	Afforestation w/ eucalyptus

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3. Methods

The evaluation of BAU versus CSF management has been facilitated by the implementation of a comprehensive set of metrics, encompassing forest inventory, meteorology (e.g. soil moisture and temperature), soil greenhouse gas (GHG) fluxes, organic matter quality, litter, root biomass, soil microbial community and activity analysis, and soil fauna mapping.

A detailed description of soil GHG flux, organic matter quality, soil microbiology, and soil fauna can be found on the HoliSoils webpage (<https://holisoils.eu>) and in the Deliverables 4.1. "Protocol of site, soil property & GHG measurements at management test sites". For soil microbial measurements detailed description of the methods is included in Richy et al. (2024).

4. Ränskälänkorpi, Finland,

Assessment of alternative harvesting methods in boreal drained peatland forests – study design and measurements

Rotation forestry, which involves clearcutting, soil preparation, planting, and intermediate thinnings is currently the dominant harvesting method in intensively managed Fennoscandian forests. However, continuous-cover forestry with selective harvesting may provide a more sustainable alternative to conventional rotation forestry. Specifically, continuous-cover forestry may offer economic savings by avoiding the costs associated with conventional rotation forestry (e.g. ditch maintenance), while concomitantly inducing environmental benefits. It is crucial to determine which alternative emits less GHGs, given a considerable proportion of the boreal forestry-drained peatland forests in Finland are reaching maturity, which will result in their imminent harvest. However, the effects of these harvesting methods on the dynamics of vegetation recovery, soil greenhouse gas (GHG) fluxes, and soil microbial biomass and community in these boreal drained forested peatlands remain poorly understood.

The study was conducted at 'Ränskälänkorpi' study site, which is a mature forestry-drained boreal peatland forest dominated by Norway Spruce and located in southern Finland ($61^{\circ}11'N$, $25^{\circ}16'E$, 144 m a.s.l.). Two harvesting methods clearcutting (CC) and continuous-cover forestry with selection harvesting (CCF, 57% of basal area removed) were compared to a non-harvested control (C). Harvesting was conducted during March to June 2021. Logging residues (comprising foliage, branches, stumps) were left on the ground in both the CCF and CC sites. Tree growth, water table levels, soil environmental conditions, and GHG fluxes were measured to examine the effect of implemented harvesting.

The CC site underwent stand regeneration in the summer of 2021. This was accomplished using ditch mounding and the planting of Norway spruce seedlings, with an approximate density of $1800-2000$ seedlings ha^{-1} . The growth of the seedlings is to be monitored over the coming years at the CC site.

Successive stand measurements were conducted in different forest inventory plots located at each study site, during which diameter at breast height (DBH, 1.3 m above ground), total tree height (Ht) and tree crown base height (Hcb) were recorded for each tree. Specifically, the CC

site was measured in 2020, the C site in 2020 and 2024, while the CCF site was measured in 2020, 2021 and 2024.

In June 2024, 48 healthy trees were randomly selected from a total of 8 plots located within the C and CCF sites (i.e., 96 trees in total). These trees ranged in diameter at breast height (DBH, 1.3 m above ground) from 13 to 38 cm, ensuring a diverse representation of tree dimensions. One 5-mm diameter increment core was extracted at DBH using an increment borer for each tree. The cores were then prepared in the laboratory and scanned at a resolution of 800 dpi using a colour scanner (Epson perfection v700 Photo). Finally, ring widths were measured with a precision of 0.001 mm using the WinDendroTM software (WinDendro Reg 2022a; Regent Instruments Inc., Quebec, Canada). Tree ring widths were subsequently converted to basal area increments (BAI, $\text{mm}^2 \text{ tree}^{-1} \text{ y}^{-1}$).

Manual chamber-based measurements of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) fluxes from the soil were conducted biweekly between May and November during the period 2020-2024 in different locations within the C, CC, and CCF sites using different portable gas analysers (Licor-7810, Licor-7820). In addition, CO_2 and N_2O fluxes were measured at high temporal (hourly) resolution using three automated chambers at each site. CH_4 fluxes were also measured with these automated chambers at the CCF site. For CO_2 , CH_4 , and N_2O concentration measurements inside the automated chambers, we used different gas analysers, i.e. LGR (Los Gatos Research Inc., Mountain View, CA, USA) at the C and CC sites and Picarro G2308 (Picarro Inc., Santa Clara, CA, USA) at the CCF site.

Ecosystem-atmosphere CO_2 , CH_4 , and N_2O exchange was measured since December 2021 with an eddy-covariance (EC) tower (3.1 m height; 3-D sonic anemometer uSonic-3, METEK GmbH, Elmshorn, Germany; $\text{CO}_2\text{-H}_2\text{O}$ gas concentration analyser LI-7200RS, LICOR Biosciences, NE, USA; $\text{CH}_4\text{-N}_2\text{O}$ gas concentration analyser TILDAS, Aerodyne Research Inc., MA, USA) located in the central part of the CC site. In collaboration with the Finnish Meteorological Institute, CO_2 exchange was monitored since August 2019 with an additional EC tower (29.0 m height; 3-D sonic anemometer uSonic-3, METEK GmbH, Elmshorn, Germany; $\text{CO}_2\text{-H}_2\text{O}$ gas concentration analyser LI-COR LI-7200RS) located in the eastern border of the CCF site, providing information for both the C and CCF sites (Laurila et al., 2021).

Soil samples were collected from 5 replicated plots in each site (C, CC and CCF) every year in September during 2020 to 2023 (3 sites x 5 plots = 15 samples). In each plot, 5 soil cores (0.5 m away from each other) were sampled then pooled as a composite sample. During each sampling, the topsoil moss layer was removed first from the soil sampler. Afterwards, a soil core of 5 cm x 5 cm x 10 cm depth was cut from the soil sampler using a kitchen knife then carefully collected by hand. Samples were sieved on site with 0.5 cm sieve and transferred to lab in cold condition. The microbial biomass was determined by phospholipid fatty acid (PLFA) extraction protocol (Šnajdr et al., 2008). Lipids were extracted from approximately 1 g of dry soil then separated using solid-phase extraction cartridges (HyperSep Silica SPE columns, 200 mg/3 mL, Thermo Fisher Scientific). Methanol (2 mL) was used to elute the PLFAs, which were then subjected to mild alkaline methanolysis. The resulting PLFA methyl esters were analyzed with gas chromatography-mass spectrometry (GC-MS; 450-GC, 240-MS ion trap detector, Varian, Walnut Creek, CA). Fungal and bacteria biomass was quantified from the polar PLFA fraction based on the abundance of the fungal and bacteria biomarker. Genomic DNA was extracted from eight soil samples following a modified Miller protocol (Sagova-Mareckova et al., 2008). DNA extractions were performed in duplicate using 0.25 g of freeze-dried soil. The resulting extracts were cleaned using the Geneclean Turbo Kit (MP Biomedicals) and pooled

before PCR. The fungal ITS2 region was amplified using the primers fITS7 and ITS4 (Ihrmark et al., 2012), while the V4 region of the bacterial 16S rRNA gene was amplified using the primers 515F and 806R (Caporaso et al., 2012).

Growth and stand development

Both the C and CCF sites exhibited analogous patterns in tree growth (BAI) during the pre-harvesting period 2014–2020 (Fig 4.1). It is noteworthy that the trees at the CCF site exhibited a substantially lower BAI in comparison to those at the C site following the drought event in 2018 (on average, 560 and 614 mm² tree⁻¹ y⁻¹ at the CCF and C sites, respectively, during 2019 and 2020). A marked response of BAI to the 2021 harvest was observed at the CCF site, which exhibited a gradual increase from 2021 to 2023, at which time growth levels observed prior to the 2016 harvest were recovered (Fig. 4.1). The observed immediate response of BAI after the harvesting may be related to a period of carry-over drought stress suffered by the trees from 2018 onwards. It is also important to note that the harvesting may have partially mitigated the effect of the drought that occurred in 2021, as evidenced by the reduction in BAI observed at non-harvested C site.

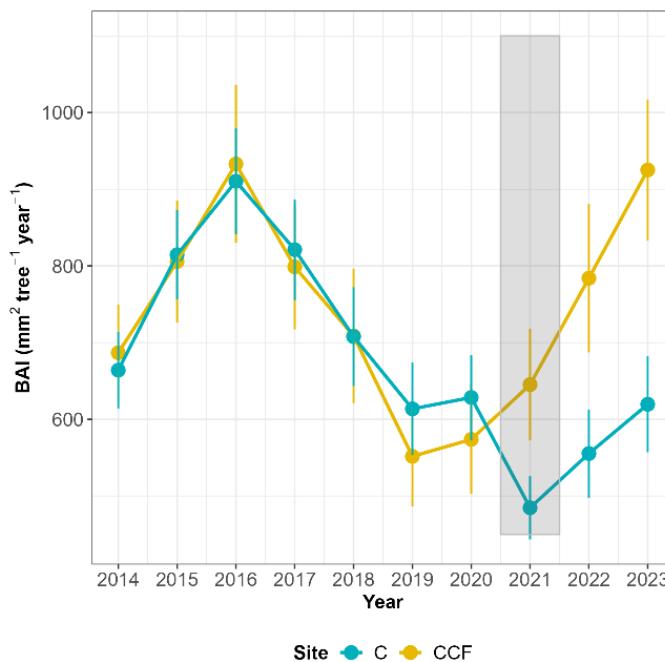


Figure 4.1. Annual variation in basal area increment (BAI, mm² tree⁻¹ y⁻¹) for the C and CCF sites during the period 2014-2023. The round symbols indicate mean annual BAI, while bars indicate standard errors of the means. Shaded area indicates the year 2021 when the selection harvesting carried out at the CCF site.

The analysis of the plot-level data indicates that the basal area (BA) in 2020 and 2024 was 30.1 ± 11.7 and 30.6 ± 11.9 m² ha⁻¹ (mean \pm SD) at the C site, respectively (Table 4.1). Consequently, a minimal BAI of 0.5 ± 1.1 m² ha⁻¹ was observed at this site during this 5-year period. In contrast, the BA values recorded at the CCF site in 2020, 2021, and 2024 were 35.4 ± 8.5 , 13.5 ± 4.2 , and 14.3 ± 4.2 m² ha⁻¹, respectively. The CCF site exhibited a BAI of 0.8 ± 1.3 during the 4-year period after selection harvesting. It is important to note that while the tree-level BAI data indicate a remarkable response after harvesting at the CCF site, the plot-level BAI was not as high due to the notable mortality that occurred in some plots located in the southern part of the site, which was caused by bark beetle attack after harvesting.

Table 4.1. Basal area (BA, $\text{m}^2 \text{ha}^{-1}$) for the C and CCF sites in 2020, 2021, and 2024. Basal area increment (BAI, $\text{m}^2 \text{ha}^{-1}$) for the periods 2020-2024 and 2021-2024 is also shown.

Site	BA 2020	BA 2021	BA 2024	BAI 2020-2024	BAI 2021-2024
C	30.1 ± 11.7	–	30.6 ± 11.9	0.5 ± 1.1	–
CCF	35.4 ± 8.5	13.5 ± 4.2	14.3 ± 4.2	–	0.8 ± 1.3

EC-based measurements at the CC site in 2022 indicate that the recovery of forest-floor vegetation was substantial, with a gross primary production (GPP) value of $406 \text{ g C m}^{-2} \text{ yr}^{-1}$, already evident in the second post-harvest year (Fig. 4.2; Tikkasalo et al., 2025). This offset more than 35% of ecosystem respiration (R_{eco}), largely attributed to soil CO_2 emissions.

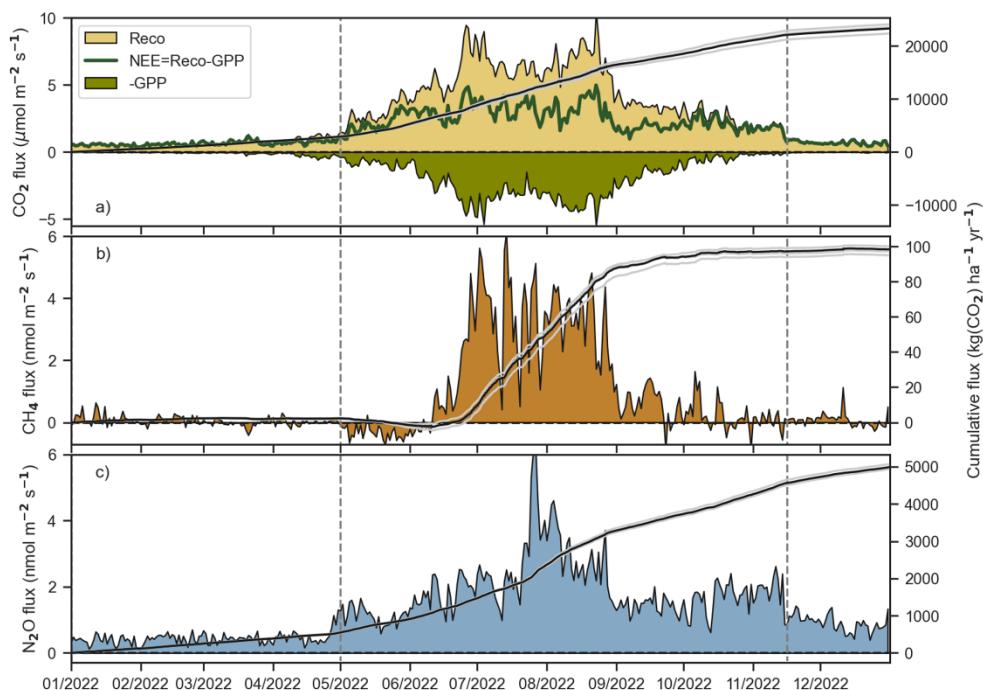


Figure 4.2. Eddy covariance-based time series of daily mean and cumulative sums of CO_2 (a), CH_4 (b) and N_2O (c) fluxes during the year 2022. Net ecosystem exchange (NEE) is partitioned into components of gross primary production (GPP) and ecosystem respiration (Reco). Vertical dash lines indicate the snow melt dates in spring and first snow in late autumn. Annual cumulative sum calculated from each flux is shown as black line.

Hydrology

Before harvesting, the water table (WT) during the growing season (May-November) exhibited slight variations among the 3 study sites (Table 4.2 and Fig. 4.3). Specifically, the WT was deeper in the order CC>C>CCF (44.8 ± 13.2 , 57.9 ± 14.7 and 65.5 ± 12.6 , respectively). After harvesting, the water table (WT) during the growing season (May-November) exhibited an immediate increase at the CC site in 2021 (Table 4.2), while no change was observed in WT at the CCF site compared to the C site (Table 4.2 and Fig. 4.3). Subsequently, the WT remained markedly higher at the CC site in comparison to the CCF and non-harvested control (Table 4.2 and Fig. 4.3). It is worthy of note that the effect of selection harvesting in the WT became discernible in 2022. Therefore, the CCF site showed slightly higher values in comparison to the C site during the years 2022 and 2023, respectively (Table 4.2). In conclusion, the results indicate a clear relationship between tree canopy cover and WT, thus reductions in canopy cover resulting higher WTs, which is likely due to a reduction in evapotranspiration.



Table 4.2. Mean (\pm standard deviation) of manually measured water table level (WT, cm), CO₂ flux (mg m⁻² h⁻¹), CH₄ flux (µg m⁻² h⁻¹) and N₂O flux (µg m⁻² h⁻¹) fluxes measured by manual chamber during growing season (May–October) before harvesting (2020) and after harvesting (2021–2023) at the C, CC, and CCF sites.

	Site	2020	2021	2022	2023
WT	C	57.9 \pm 14.7	-62.4 \pm 13.8	-65.6 \pm 12.7	-66.8 \pm 13.2
	CC	44.8 \pm 13.2	-31.6 \pm 9.9	-27.7 \pm 8.7	-32.9 \pm 7.2
	CCF	65.5 \pm 12.6	-59.3 \pm 12.6	-59.6 \pm 12.7	-60.9 \pm 11.2
CO ₂	C	592.4 \pm 149.0	468.6 \pm 181.	521.5 \pm 186.	594.9 \pm 128.5
	CC	762.7 \pm 160.3	240.6 \pm 81.8	364.6 \pm 148.8	597.4 \pm 213.1
	CCF	597.2 \pm 150.1	447.7 \pm 138.7	523.9 \pm 164.5	597.4 \pm 213.1
CH ₄	C	-102.2 \pm 58.5	-122.2 \pm 65.5	-117.3 \pm 54.7	-126.1 \pm 64.7
	CC	-143.0 \pm 26.8	-60.8 \pm 23.0	-48.6 \pm 25.1	-50.6 \pm 49.0
	CCF	-133.3 \pm 77.0	-120.4 \pm 78.1	-126.4 \pm 72.3	-149.2 \pm 86.7
N ₂ O	C	0.7 \pm 13.7	3.1 \pm 5.6	5.2 \pm 9.2	9.4 \pm 13.5
	CC	30.5 \pm 45.9	58.5 \pm 141.3	33.0 \pm 35.3	35.4 \pm 29.8
	CCF	62.6 \pm 90.1	38.7 \pm 57.2	43.2 \pm 53.5	68.7 \pm 90.3

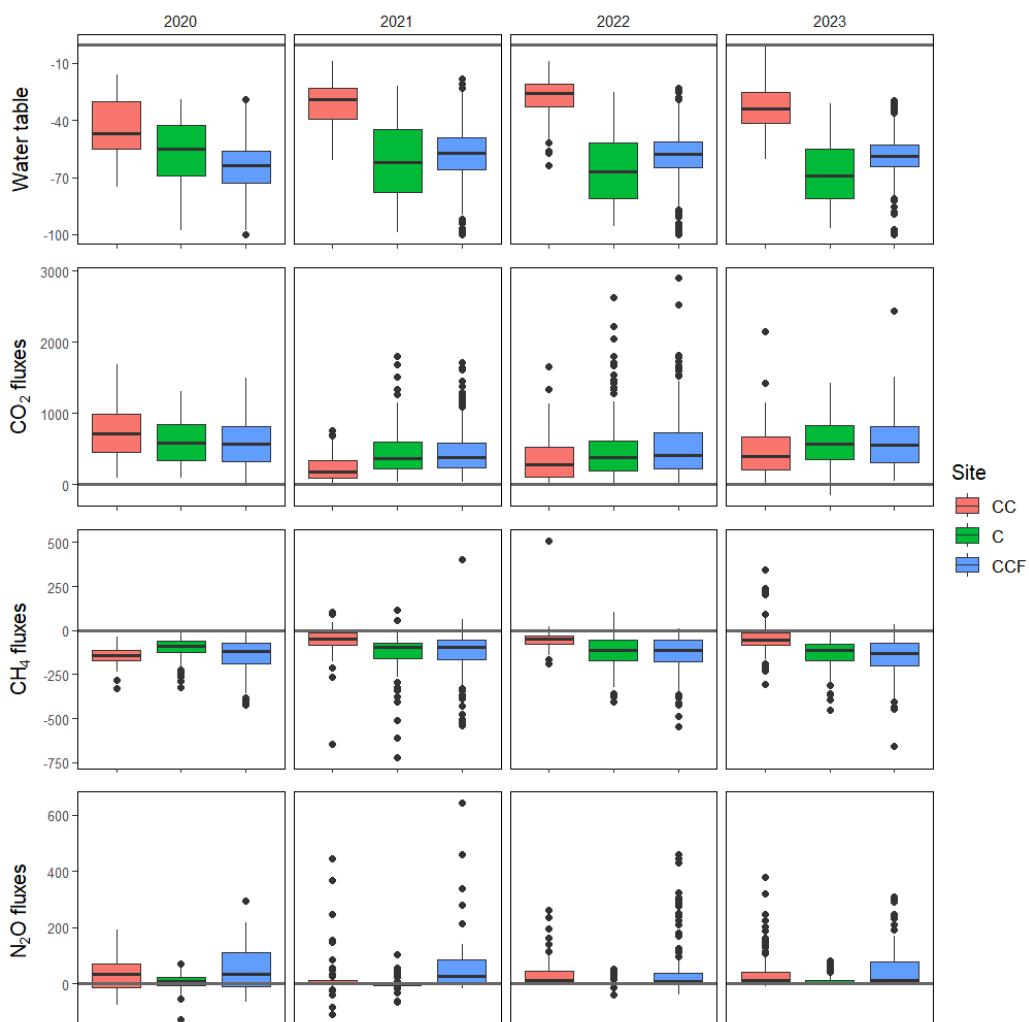


Figure 4.3. Water table level relative to the peat surface as well as CO₂, CH₄ and N₂O fluxes measured during the growing season (May–October) by manual chambers at the clearcut (CC), control (C) and continuous-cover forestry (CCF) sites before harvesting (year 2020) and after harvesting (period 2021–2023). Boxplots represent median, 25% and 75% quartile values.

Soil environmental conditions

Following the harvesting, the soil temperature (T_s , 5 cm depth, continuously monitored) was observed to be slightly higher at the CCF in comparison to the C site during the early growing season (May-June; Fig 4.4). Mean ($\pm SD$ of replicates) soil temperature during growing season from May to October in 2021–2023 was 10.4 ± 3.3 and 9.9 ± 3.8 °C for CCF and C site, respectively. The impact of clearcutting on T_s was more pronounced (mean $\pm SD$ of 11.2 ± 4.0 °C), with significant differences found throughout the entire growing season (Fig. 4.4). Both clearcutting and selection harvest treatments resulted in an increase in soil water content (SWC, 5 cm depth, continuously monitored) in comparison to the C site (Fig. 4.4). Specifically, Mean ($\pm SD$ of replicates) SWC was 64.4 ± 15.5 % for the CC site, 59.5 ± 9.1 % for the CCF site and 53.0 ± 7.7 % for the C site during May to October in 2021–2023. In conclusion, the findings suggest a clear relationship between increasing T_s and SWC with decreasing tree canopy cover, which is likely due to a reduction in tree shading and evapotranspiration processes.

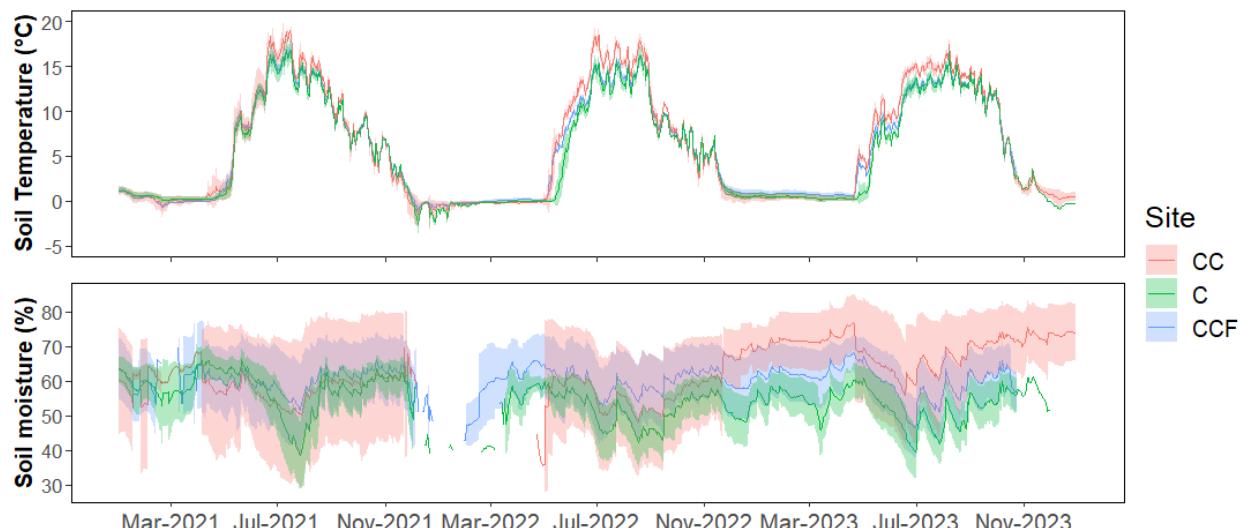


Figure 4.4. Time series of soil temperature at 5 cm depth (T_s) and soil water content at 5 cm depth (SWC) measured after harvesting (period 2021–2023) at the clearcut (CC), control (C) and continuous-cover forestry (CCF) sites. Solid lines represent the mean of replicated plots and shade areas represent the standard deviation of replicates.

Carbon dioxide (CO_2) fluxes

Soil CO_2 fluxes from the automated chambers have not yet been processed. Results of the manual chamber measurements indicated that there were no significant differences in soil CO_2 fluxes between CCF and C sites during the 3 years after harvesting (Table 4.2 and Fig. 4.3). If confirmed by the automated chambers, this result suggests that peat decomposition was not really affected by the selection harvesting treatment. In contrast, soil CO_2 fluxes in the CC site were found to be lower than those at the C and CCF sites during the 1st year after harvesting (year 2021). Thereafter, a gradual increase was noted at the CC site and the differences between sites became smaller (Table 4.2 and Fig. 4.3). The increased decomposition of residues and peat, influenced by high T_s and SWC, may be the contributing factors to the observed increase in soil CO_2 fluxes at the CC site.

Prior to the implementation of harvesting treatments, EC-based measurements conducted in 2019 indicated that the area comprising the C and CCF sites was a source of CO_2 ($360\text{ g C m}^{-2}\text{ y}^{-1}$). Both sites continued to be sources of CO_2 in 2020 (440 and $320\text{ g C m}^{-2}\text{ y}^{-1}$ for the C and CCF sites, respectively). However, due to the absence of EC-based data for the CC site in

2020, a direct comparison could not be conducted. In 2022, one year after the harvesting treatments, the C site exhibited a notable increase in its CO₂ source strength, reaching 630 g C m⁻² y⁻¹. This increase is probably related to drought events that occurred in 2021 and to a lesser extent in 2022. In contrast, the CCF site demonstrated a slight decline in its net CO₂ balance, with a value of 260 g C m⁻² y⁻¹. This decline could be attributed to the increased productivity of trees (see Fig. 4.1) and forest-floor vegetation (data not shown) after harvesting. It is also possible that the reduction in peat decomposition as the WT increased may also have been related to this effect. Furthermore, the CC site showed net CO₂ emissions that were comparable to those of the C site in 2022 (i.e., 640 g C m⁻² y⁻¹). It is noteworthy that CO₂ emissions dominated the total annual GHG balance at the CC site in 2022 (see Fig. 4.2, 23.3 t CO₂-eq ha⁻¹ y⁻¹, 82.5%; Tikkasalo et al., 2025).

Methane (CH₄) fluxes

Soil CH₄ fluxes from the automated chambers at the CCF site have not yet been processed. Results of the manual chamber measurements indicated that there were no significant differences in soil CH₄ fluxes between CCF and C sites after harvesting. Both sites acted as CH₄ sinks (Table 4.2 and Fig. 4.3). It can be posited that the slight increase in WT did not have a significant impact on the CH₄ fluxes following the selection harvesting. However, the soil CH₄ sink exhibited a notable decline in the CC site, which may have been due to a further increase in WT. This pattern was consistent over the 3-year study period after clearcutting and the CH₄ sink of the CC site exhibited a decline over time (Table 4.2 and Fig. 4.3). It is important to note that the sporadic occurrences of CH₄ emissions were observed in 2024. This indicates that clear-cutting in boreal drained peatland may result that soils become a minor source of CH₄ rather than a sink, which could potentially lead to an increase in atmospheric CH₄ levels.

EC-based measurements at the CC site in 2022 indicated that the CH₄ emissions played a negligible role in the total annual GHG balance (see Fig. 4.2, 0.1 t CO₂-eq ha⁻¹ y⁻¹, 0.4%; Tikkasalo et al., 2025)

Nitrous oxide (N₂O) fluxes

Automated chamber-based measurements (Fig. 4.5) revealed that at the C site, where the daily mean N₂O fluxes were close to be neutral (ranging between -18 and 26 µg N₂O m⁻² h⁻¹; min-max values), air and soil temperature controlled their temporal variability. In contrast, the temporal variability of the daily mean N₂O fluxes was higher at the CCF site (-24 and 109 µg N₂O m⁻² h⁻¹) and CC site (-12 and 278 µg N₂O m⁻² h⁻¹) than at the C site. The occurrence of N₂O peaks was considerably higher at the CC site during summer 2022, which might be attributable to the increased soil N availability from logging residues left on site, but SWC and WT were the dominant controlling factors at these sites.

Manual chamber-based measurements, which covered a larger spatial variability, corroborated that the N₂O fluxes during the growing season at the C site were close to neutral (median±IQR of -1.0±4.7, 0.9±6.3 and 1.6±15.0 µg N₂O m⁻² h⁻¹ in 2021, 2022 and 2023, respectively; Fig 4.3). The CCF site exhibited higher N₂O emissions (26.1±82.4, 9.1±36.3 and 10.9±75.3 µg m⁻² h⁻¹ in 2021, 2022 and 2023, respectively; Fig 4.3), caused mainly by some N₂O hotspots found within a fertile area at the CCF site. N₂O fluxes at the CC site showed high variation in 2021 immediately after harvesting (ranged from -107.2 to 445.5 µg N₂O m⁻² h⁻¹), after which N₂O fluxes appeared to stabilise in 2022 (10.8±43.22 µg N₂O m⁻² h⁻¹) and 2023 (10.9±75.3 µg N₂O m⁻² h⁻¹ Fig 4.3). Due to the N₂O measurement instrument change (Gasmet was used in 2020 and LICOR from 2021) and paucity of measurement plots at the CC site prior

to harvest in 2020, a direct comparison between the pre- and post-harvest N₂O fluxes was challenging.

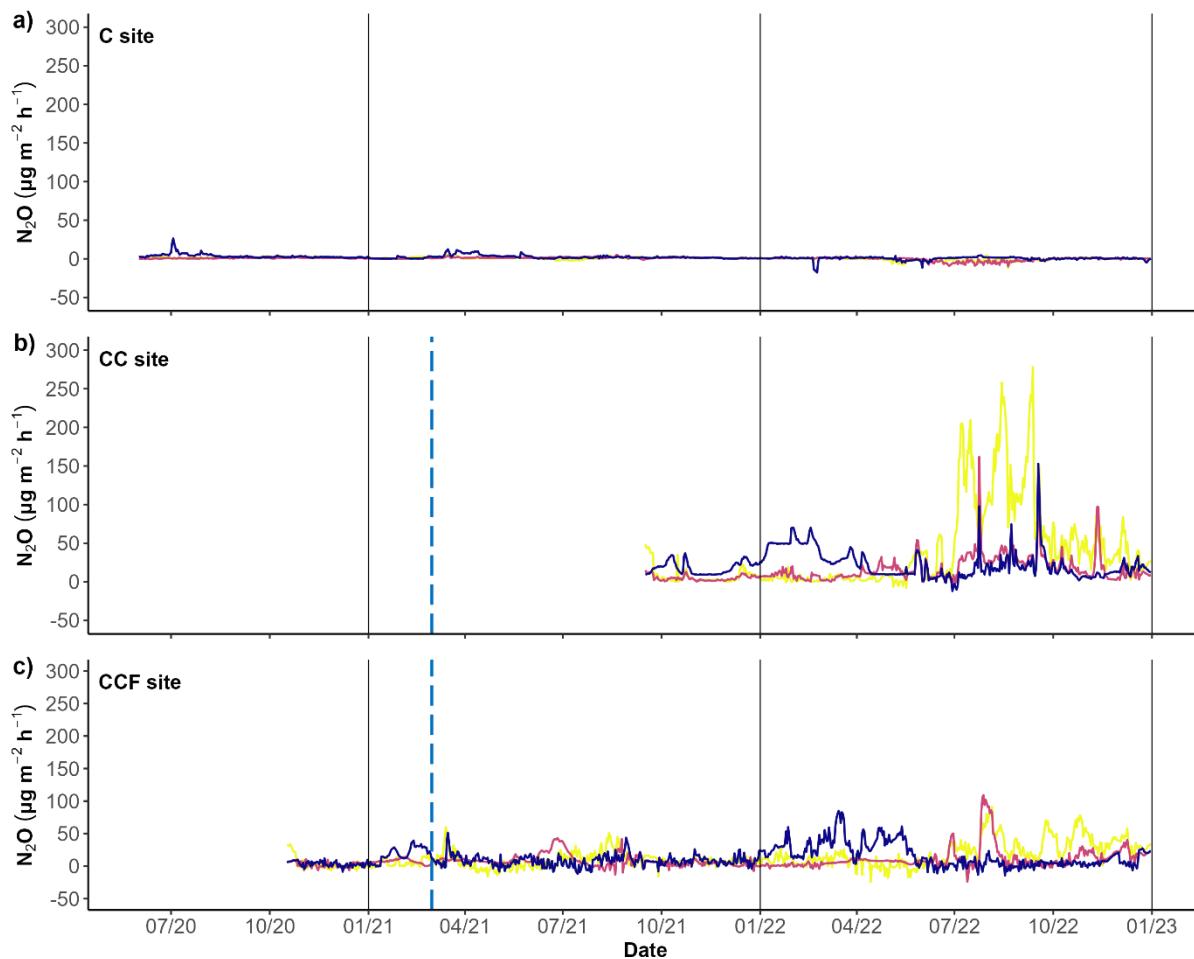


Figure 4.5. Mean daily N₂O fluxes ($\mu\text{g m}^{-2} \text{h}^{-1}$) measured by 3 automated chambers at the control (C), clearcut (CC), and continuous-cover forestry (CCF) sites during June 2020 to December 2022. The blue dashed vertical line in panels b) and c) indicates the time at which the harvesting treatments were initiated.

Using a combination of automated and manual chamber-based measurements during 2022, we found a significant spatial variation in N₂O fluxes among sites, although the presence of hot spots was higher at the CCF site. This spatial heterogeneity was found to be strongly influenced by site-level conditions such as soil fertility (i.e., K, Cu, P, N, and C:N ratio), Ts, and soil bulk density. It is also important to note that at C and CC sites, the correlation between N₂O fluxes and soil fertility (N) over 1-m depth horizon was highest just above the WT level.

Automated chamber-based measurements indicate that the annual N₂O balance was close to neutral at the C site in 2021 and 2022 (11 ± 6 and $-5 \pm 5 \text{ mg N}_2\text{O m}^{-2} \text{ y}^{-1}$, respectively; median \pm IQR). In contrast, the annual N₂O balance was higher at the CCF site in 2021 ($72 \pm 7 \text{ mg N}_2\text{O m}^{-2} \text{ y}^{-1}$) and 2022 ($145 \pm 43 \text{ mg N}_2\text{O m}^{-2} \text{ y}^{-1}$). The CC site showed the highest annual N₂O balance among the sites in 2022 ($157 \pm 150 \text{ mg N}_2\text{O m}^{-2} \text{ y}^{-1}$). However, when the analysis was extended to include the annual N₂O balances in 2022 obtained from manual chamber-based measurements, N₂O emissions increased in the order of C, CCF, and CC sites (0 ± 25 , 163 ± 533 , and $185 \pm 194 \text{ mg N}_2\text{O m}^{-2} \text{ y}^{-1}$, respectively). This pattern was like that obtained from



preliminary manual chamber-based measurements carried out prior the harvest in 2020. This result suggests that despite the effect of harvesting on increasing N₂O emissions at the study site, inherent spatial variability may have a greater influence on N₂O emissions than harvesting during the early stages of the ecosystem recovery.

EC-based measurements showed that the N₂O emissions increased by 17% the total annual GHG balance at the CC site in 2022 (see Fig. 4.2; Tikkasalo et al., 2025)

Microbial biomass and community composition

The Fungi/Bacteria (F/B) biomass ratio did not vary significantly under different forest managements in most of the years studied (Fig. 4.6-1). After harvesting (March 2021), the F/B ratio was higher in the C site than C and CC, although the difference was only significant in 2022. For the CC site, the variation of F/B ratio decreased sharply after 2021. In contrast, the C site showed relatively constant range of values over the entire study period. In 2021, Gram+/Gram- biomass ratio increased in the CCF site compared to pre-harvesting (2020), which is significantly different with CC and C site (Fig. 4.6-2). In 2022, an increase in the Gram+/Gram- biomass ratio was observed in the CCF and CC sites compared to 2021, while decreased in C site, leading to significant differences between treatments. In 2023, it decreased in the CCF and CC sites, returning to same level as C site. The harvesting treatments had significant influence on the composition of the total bacterial and fungal communities (AMF not considered). The width of the ellipses was largest for the CC site, followed by CCF, suggesting greater heterogeneity after harvesting (Fig. 4.7).

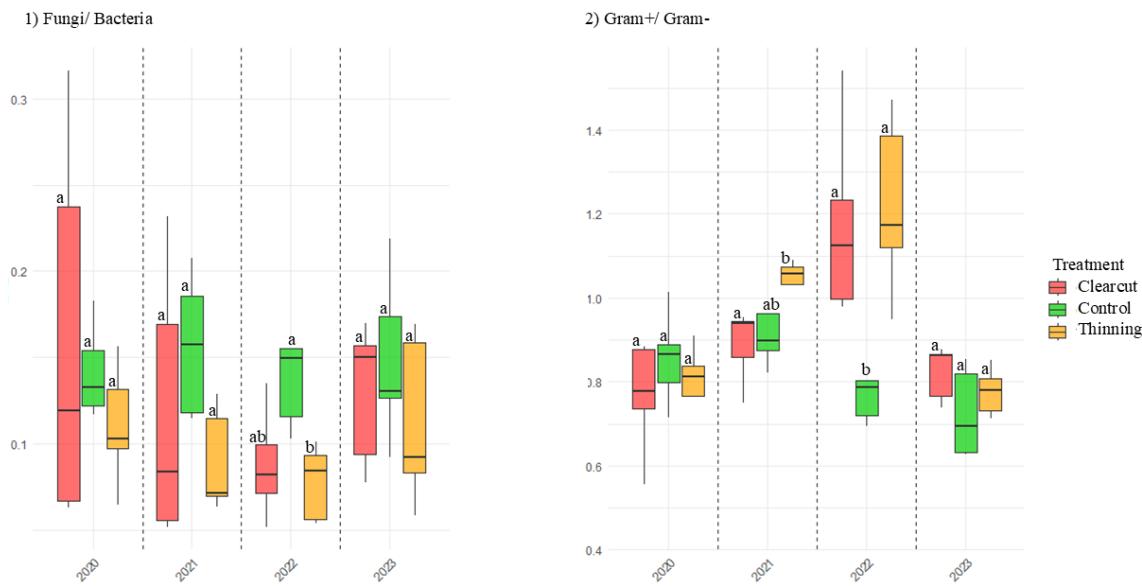


Figure 4.6. Fungi/Bacteria biomass (1) and Gram+/Gram- biomass ratio (2) in different forest management treatments from 2020 to 2023. Different letters indicate significant differences (p -value <0.05)

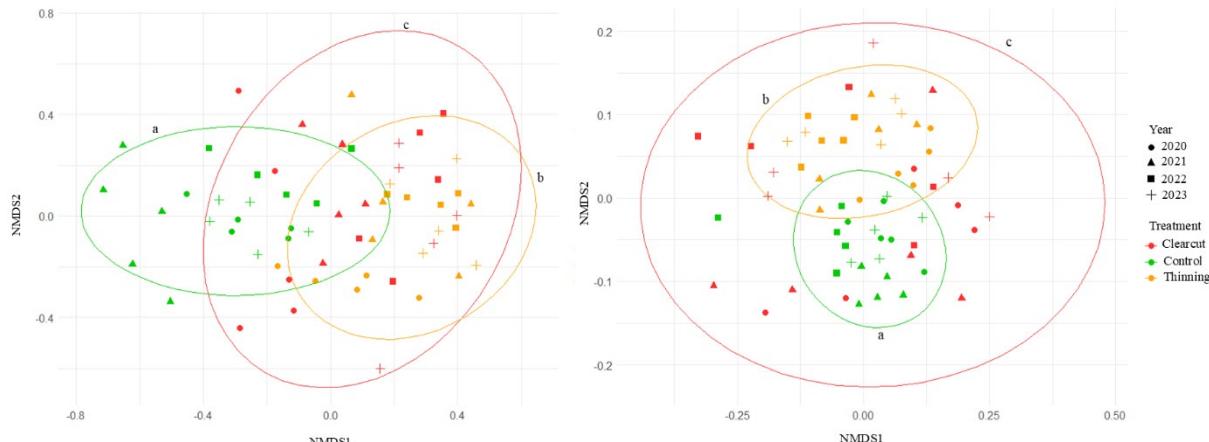


Figure 4.7. Non-metric multidimensional scaling (NMDS) plot of global fungi (except AMF; left) and bacteria (right) community composition, based on Bray-Curtis distance according to the years of sampling and forestry treatments. Letters a, b and c indicate significant differences (p -value <0.05)

Main findings and conclusions

Continuous-cover forestry (CCF) is increasingly being advocated as a climate-smart forestry (CSF) alternative to business-as-usual (BAU) rotation forestry with clear-cutting in boreal drained peatland forests, since it may improve water quality and quantity, biodiversity and reduce GHG emissions through water table management, while also proving timber provisioning. However, studies quantifying the environmental benefits of the potential impacts of a change in management regime (from BAU to CSF) on these boreal ecosystems are scarce. In this way, our work in Ränskälänkorpi study site provides a better understanding of the implementation of CSF in boreal drained peatland forests at mature stages approaching the decision point of whether to clear-cut or manage with selection harvests.

The implementation of clear-cutting resulted in the anticipated immediate and sustained elevation of the water table (WT) level near the peat surface, resulting in mean levels that were considerably higher than those observed at the control and selection harvesting sites. In contrast, the increase of the WT following selection harvesting was marginal, exhibiting higher values but remaining closer to the water table of the control site. This phenomenon could be ascribed to the markedly low levels of pre-harvest WT. This suggests that the efficacy of selection harvesting in regulating WT and associated favorable climatic impacts may be constrained to sites exhibiting intermediate WT levels.

Selection harvesting resulted in remarkably lower net ecosystem exchange than clear-cutting during the early stages following harvesting treatment (e.g., 260 versus 640 g C m⁻² y⁻¹ in 2022). This fact could be attributed to a lower decline in GPP following a more limited removal of trees, as well as an increased tree (and forest-floor vegetation) productivity, whereas all the photosynthetic biomass was harvested or destroyed in the clear-cutting site.

The control site exhibited a notable increase in its CO₂ source strength, reaching 630 g C m⁻² y⁻¹ in 2022. This increase is likely associated with the drought events that occurred in 2021 and, to a lesser extent, in 2022, as evidenced by the drastic decrease in tree growth. This outcome underscores the detrimental repercussions that this disturbance, which is anticipated to become both more intense and recurrent in the forthcoming years, may exert on the carbon balance of boreal drained peatland forests. In this regard, our findings also

demonstrated that selection harvesting could be a suitable approach to enhance the growth response of remaining trees to drought.

None of the harvesting methods appeared to be effective in preventing the loss of carbon from the soil, which is crucial for the long-term C storage of peatlands. In the clear-cutting site, this may have been due to a reduction in peat decomposition at higher WT, which may have been counteracted by an increase in the decomposition of harvesting residues favored by higher temperature. However, the raising of the WT levels did not seem to be sufficient to increase peat C storage in the selection harvesting site, so additional measures such as blocking ditches could be considered in the near future. However, the potential repercussions of this practice (i.e. increased CH₄ emissions) must be given due consideration.

The impact of selection harvesting on the soil CH₄ sink was found to be negligible, given the minor fluctuations in WT levels. Conversely, the increase in WT levels induced by clear-cutting, resulted in a reduction of soil CH₄ sink capacity and minor CH₄ emissions over time. It is important to note, however, that CH₄ emissions played a negligible role in the overall GHG balance at the clear-cutting site.

Overall, a remarkable spatial dependence of soil N₂O emissions was observed, which was induced by site fertility rather than by WT changes. Consequently, the findings indicate that, despite the impact of harvesting on increasing soil N₂O emissions, inherent spatial variability and potential hotspots may exert a greater influence on soil N₂O emissions than harvesting method during the early stages of the ecosystem recovery.

Both selection harvesting and clearcutting lead to a shift of microbial biomass and community composition. The microbial biomass showed a delay response to harvesting with significant changes observed in the 2nd year after harvest, while a fast recovery to control levels was found in the 3rd year after harvest. Furthermore, harvesting practices increased the spatial heterogeneity of fungi and microbial communities, suggesting that harvesting-induced changes in tree roots and forest-floor vegetation had a strong influence on local scale species composition. The potential impact of these changes on soil processes related to GHG emissions will be further investigated.

In view of the limited study period after harvesting treatments, preliminary results show that GHG emissions from clear-cutting can be avoided by applying CCF instead of rotation forestry. Noting that further research is required to assess whether continuous-cover forestry represents an alternative to rotation forestry from a long-term GHG perspective.

5. Dobroc, Slovakia

The role of drought and disturbance on carbon sequestration and methane oxidation in a mixed forest and Norway spruce monoculture

We evaluated tree species' long-term growth trends (2000 – 2022) and their responses to the 2022 drought, focusing on implications for carbon sequestration and storage within Norway spruce monocultures and mixed stand ecosystems. Additionally, we examine drought-induced changes in soil CO₂ and CH₄ fluxes, driven by temperature increases, altered water availability, and indirect effects of drought on tree growth.

We established the study site as part a European-wide Horizon 2020 project HoliSoils which at two adjacent forest sites (950 m a.s.l.) on deep Dystric Cambisol. At these sites we studied the effects of forest management on soil CO₂ and CH₄ fluxes: 1) The first was a mixed-species stand in a buffer zone of the Dobroc primary forest declared as a nature reserve in 1913, and 2) the second in a Norway spruce monoculture regenerated by planting 70 years ago and managed by thinnings.

Since August 2021, Carbon dioxide (CO₂) and methane (CH₄) fluxes from the soil was measured biweekly since August 2021 using the LI-COR LI-7810 CH₄/ CO₂/H₂O Trace Gas Analyzer (LI-COR Biosciences GmbH). We applied a root exclusion method to separate the heterotrophic (Rh) and autotrophic (Ra) respiration and to quantify their contribution to the total soil respiration (Rt). Tree-ring analysis and dendrometer measurements were aimed to provide insights into historical and seasonal growth dynamics, linking these processes to soil respiration and methane fluxes.

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. 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 0.25g 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 515F and 806R (Caporaso et al., 2012); both the forward and reverse primers were barcoded.

Effects of drought and disturbance on soil CO₂ and CH₄ fluxes

The 2022 drought significantly reduced total CO₂ fluxes in spruce monocultures, reaching only 25–50% of those in mixed stands, primarily due to reduced growth and root respiration. CH₄ oxidation also decreased during the drought, though differences between stands were mostly insignificant.

Following the 2023 clear-cut following a bark-beetle attack in 2022, CO₂ fluxes in the spruce stand increased significantly due to higher soil temperature and moisture (Fig. 5.1). CH₄ oxidation initially declined but returned to mixed-stand levels by 2024. Temperature best

explained the CO₂ fluxes from soil to atmosphere. The significant interaction between soil temperature and moisture indicated that soil moisture affected CO₂ fluxes, but only at higher temperatures and at much lesser intensity compared to temperature being the only predictor.

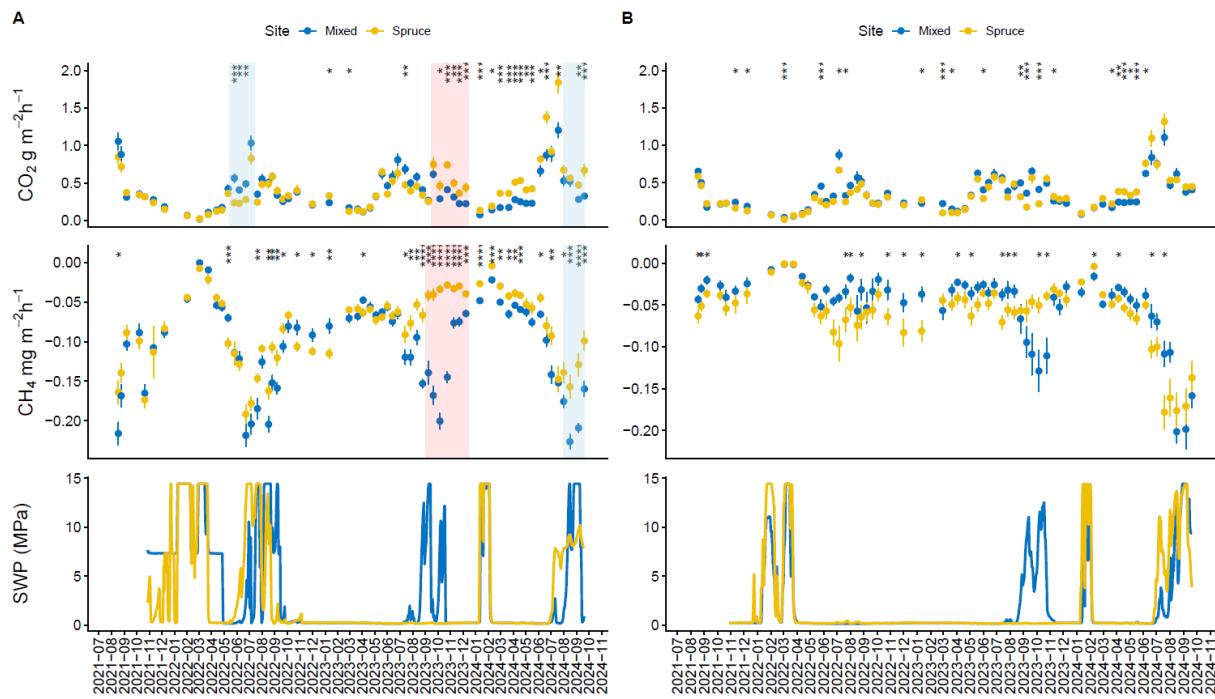


Figure 5.1. A) CO₂ and CH₄ fluxes in the study period with soil water potential (SWP) in the control plots (total soil respiration) and B) in the trenching plots (heterotrophic soil respiration). Wilcoxon test was used to test the differences between mixed and spruce stands, and the asterisks indicate the significance level (* - 0.05, ** - <0.01, *** - <0.001). Light blue rectangles indicate drought periods in 2022 and 2024, and the red rectangle the period after clear-cut in 2023.

Microbial species richness was similar across forest types, but composition varied (Fig. 5.2): ectomycorrhizal fungi dominated mixed stands, while saprotrophs prevailed in spruce stands and root-excluded plots. Bacterial communities showed no differences across treatments.

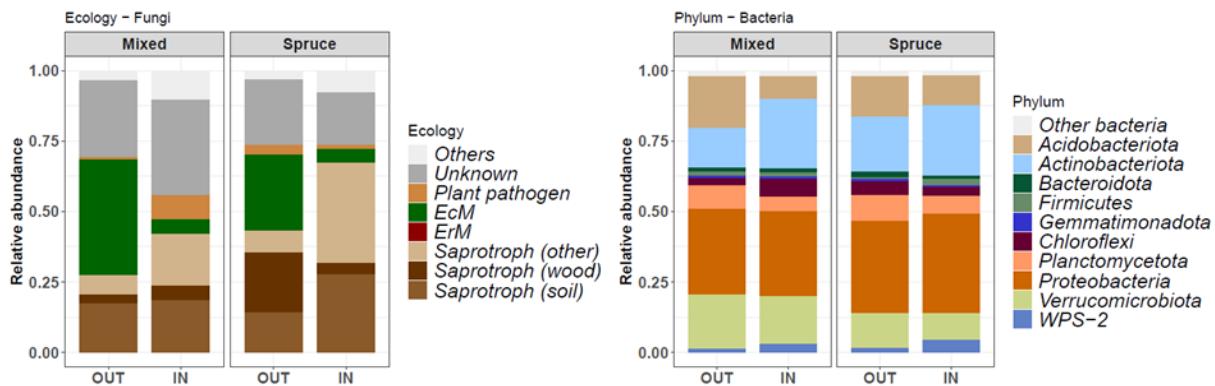


Figure 5.2. The composition of fungi and bacteria in the mixed stand and spruce monoculture inside (IN, roots excluded, heterotrophic respiration only) and outside (OUT) the trenched plots.

Despite drought-induced reductions in soil respiration in 2022, total annual C emissions (~7–8 Mg ha⁻¹ yr⁻¹) were similar between stands. Litter input in mixed stands (~3.65 Mg C ha⁻¹ yr⁻¹) accounted for half the soil heterotrophic emissions and tree biomass acted as sinks of 3



Mg C ha⁻¹ yr⁻¹, resulting in carbon sink of more than 1 Mg C per hectare for these forests. Due to the clear-cut in the mid-2023, we were not able to quantify the litter input in the spruce monoculture. Increased C emissions in 2023 at the Norway spruce monoculture resulted from clear-cutting as a response to an earlier bark-beetle infestation, and favourable decomposition conditions.

Main findings

Our study highlights significant differences between spruce monocultures and mixed stands under drought and clear-cut conditions. Spruce monocultures exhibit substantial reductions in biomass production during the periods of 2000 - 2022 and are vulnerable to drought while mixed stands of spruce, beech, and fir maintained stable growth (Fig. 5.3). Drought reduced soil CO₂ fluxes more rapidly and more strongly in monocultures, with heterotrophic respiration showed a similar but weaker response.

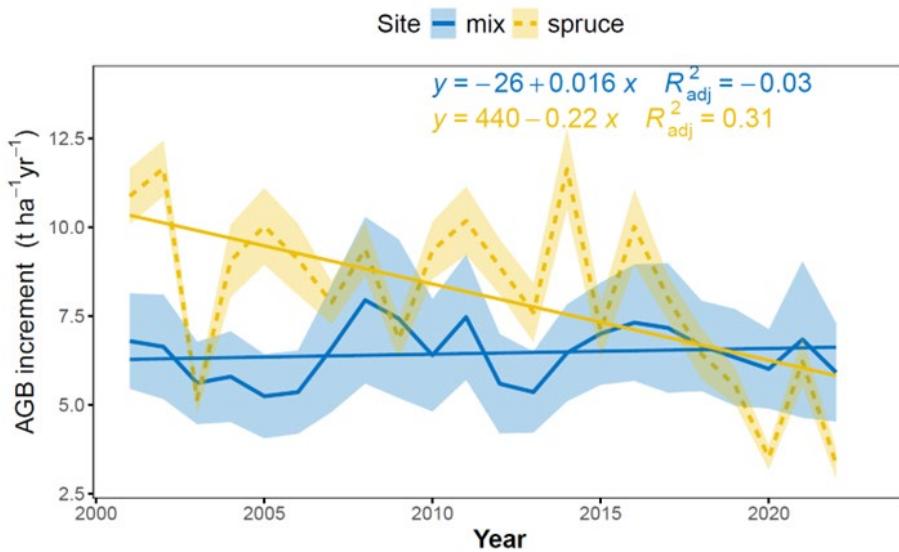


Figure 5.3. Long-term changes in above-ground biomass increment in the spruce monoculture and mixed stand

Post-clearcut, spruce stands show a 3.5-fold increase in total floor respiration and a 2.4-fold rise in heterotrophic respiration compared to mixed stands where no cutting took place (Fig. 5.4). Remarkably, soil carbon emissions during the 4-month post-clearcut period matched annual totals from 2022 and 2023. Methane oxidation peaked in Norway spruce stand during summer droughts but declined sharply after the 2023 clearcut, returning to mixed-stand levels by 2024.

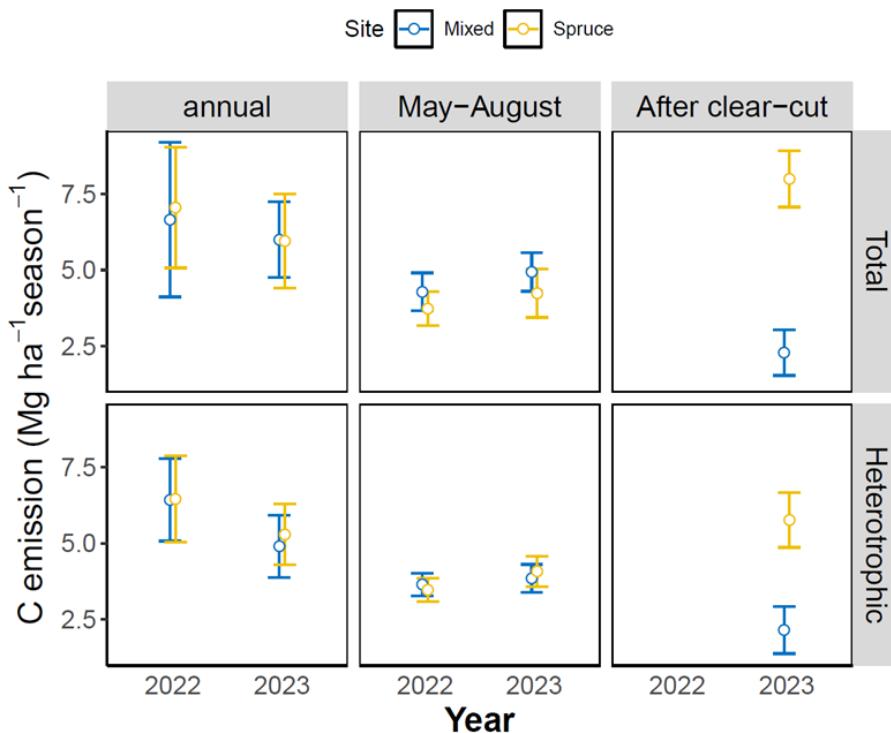


Figure 5.4. Annual, seasonal (April-September), and post-disturbance total floor and heterotrophic carbon emissions from soil based on interpolation by the mixed GAM model. The estimation of C emitted after the clear-cut sums emissions from September (when the clear-cut was realised) to December.

Recommendations for forestry practice

Our research stresses the importance and advantage of broadleaf-dominated mixed forests over spruce monocultures. Here we list the main reasons for preference of mixed forests over Norway spruce plantations:

- 1) Higher production: Mixed stands, if composed of species with different ecological niches, can have higher production because of less inter-tree competition (Bosela et al., 2015).
- 2) Higher stability of wood supply: spruce monocultures have a much higher risk of disturbances because of higher drought stress following the higher susceptibility to bark beetle outbreak – Slovakia (and central Europe) faces an unprecedented bark beetle outbreak in Norway spruce forests, and it will likely continue in the next years. Large-scale disturbance lead to carbon loss. Mixed forests growing in richer vertical structures reduce risk of large-scale disturbances and carbon loss and thus provide more sustainable wood supply (Felton et al., 2016; Field et al., 2025; Kozhoridze et al., 2024; Liu et al., 2018; Temperli et al., 2013).
- 3) Reduce the risk of biodiversity loss: To reduce the risk of large-scale disturbances in spruce monocultures, a significant decrease in rotation period is inevitable (bark beetle continues to spread fast even in younger stands of around 50 years in Slovakia). By decreasing rotation periods, however, the wood production is intensified, leading to loss of a considerable amount of biodiversity (Dobroč region was originally a broadleaf-dominated region with high biodiversity).



6. Kranzberg precipitation gradient, Germany

Precipitation gradient and drought legacy sites

We evaluated the effect of a precipitation gradient on mature Norway spruce (*Picea abies*) stands of similar structure in Bavaria (i.e. Central Europe) and compared it to mixed stands of spruce with European beech (*Fagus sylvatica*). In addition to this recording of the current state, which especially concerned soil respiration but also included data on forest inventories and microbial composition (sample processing awaiting completion), we evaluated the legacy effects of a future extreme drought scenario on mature spruce, beech, and their mixture. For the precipitation gradient, we measured soil respiration (Rs) and soil heterotrophic respiration (Rh) biweekly during the vegetation periods 2022 and 2023 and a brief period beyond. For the drought legacy scenario, monthly Rs measurements started autumn 2022 and are still ongoing. Soil moisture and temperature were continuously recorded on all sites.

Precipitation gradient and spruce vs. mixed stands

Differences in soil respiration between plots with low (385 mm) and high (640 mm) precipitation during the vegetation period were insignificant ($p>0.05$, linear mixed effect models) for pure spruce and mixed stands (Rs and Rh) when averaged across both vegetation periods. Within-year variations between gradient locations (e.g. maxima at different timepoints) and between-years' variation (dry summer 2022 resulting in two maxima for Rs but not Rh) distinguished between both, sites and years in the time-course (Fig. 6.1). However, on average only a statistically insignificant tendency ($p>0.05$, linear mixed effect model) for elevated Rs on wetter sites remains (Fig. 6.1). A matter of ongoing investigation, the soil heterotrophic (i.e. root-free) respiration for spruce was on average not significantly different from total soil respiration at the medium precipitation site which was different from the lower and higher precipitation sites (Fig. 6.1).

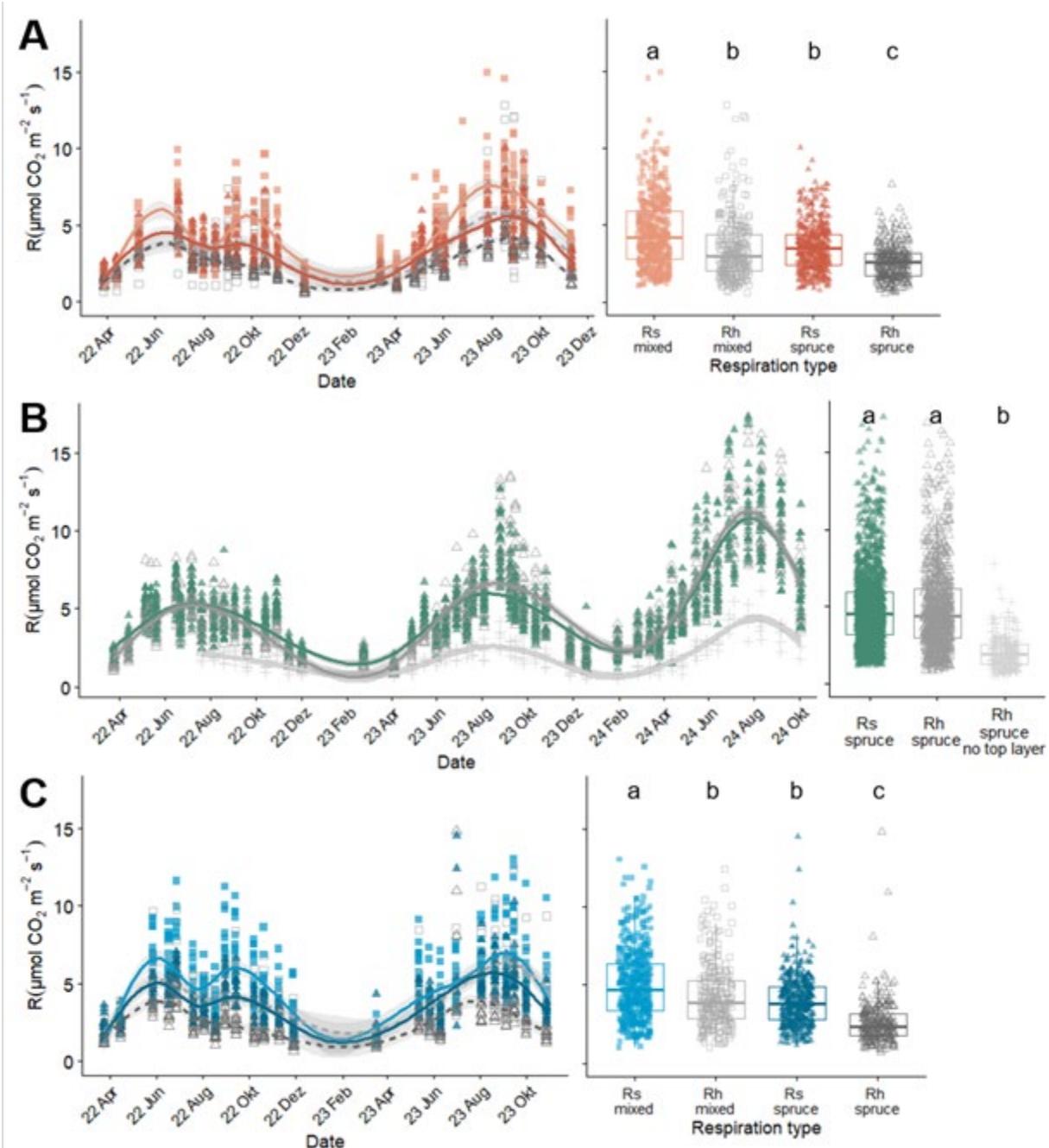


Figure 6.1. Soil respiration on the precipitation gradient. A: Kelheim (drier site). light red: Rs mixed stand, light grey: Rh mixed stand, dark red: Rs spruce, dark grey: Rh spruce. n(dates)=26. B: Kranzberg (medium precipitation site with only spruce): Green: Rs, grey: Rh, light grey: Rh without surface vegetation layer. n(dates)=58. C: Wasserburg (elevated precipitation site): light blue: Rs mixed stand, light grey: Rh mixed stand, dark blue: Rs spruce, dark grey: Rh spruce. n(dates)=25. Left panels: time course of measurements. Right panels: averages of all measurements from the left side. Filled symbols: Rs (total soil respiration), open symbols: Rh (heterotrophic respiration). Grey crosses: Rh with without surface vegetation layer (only for Kranzberg spruce). Lines: Smoothed conditional means (fitted using 'gam' $y \sim s(x, bs=cs)$) with 95% confidence intervals. Lower case letters: significantly different groups in pairwise comparison tests on linear mixed effect models (fixed effects: tree stand, respiration type; random effects: date, measurement point), n(trench)=3 (6 for Kranzberg), n(Rs measurement collars)=4, n(Rh measurement collars)=4 (3 for Kranzberg), n(Rh without vegetation layer)=1. Number of included measurements: 6741.

Averaged soil respiration on the spruce-beech mixed spruce sites was significantly higher compared with the respective pure spruce sites (Table 6.1 and Fig. 6.1). Comparing all precipitation gradient sites suggests that annual tree growth (BAI, cf. Table 6.1) could be a stronger predictor for soil respiration than tree mixing or precipitation under the range of conditions regularly observed in Central Europe; although this relationship cannot be resolved here.

We also measured soil respiration at a smaller local scale on the control plots of the drought legacy experiment, which are groups of mature trees of spruce and beech each planted in clusters. Here, elevated soil respiration is less consistently and most often seen for the beech root-zone within the plots (each plot one beech-tree cluster opposing one spruce-tree cluster), compared to similar mixed and spruce root zones (data collection still ongoing).

In conclusion, on a natural precipitation gradient in Central Europe, spruce and spruce-beech mixed stands did not show pronounced differences in soil respiration depending on the precipitation during the vegetation period. However, admixture of beech to spruce can increase soil respiration, possibly depending on the planting configuration. At the same time, it is reported that beech admixture can positively influence the growth of spruce [e.g., for these gradient sites (Rukh et al., 2020) and the related "Kroof" experimental site (Pretzsch et al., 2020), which is predicted to decline heavily due to drier future climate.

Table 6.1. Soil respiration on the gradient sites in comparison to basic stand parameters. T_v , P_v : Temperature (T) and precipitation (P) during vegetation periods (v); long term averages taken from (Pretzsch et al., 2014); Was: Wasserburg/wetter site, Kra: Kranzberg/medium precipitation site; Kel: Kelheim/drier site; BA: average basal area (2024); BAI: average (last 11 years) basal area increment; R: heterotrophic (h) and total (s) soil respiration with standard deviation (average over all measurements); Soil organic carbon content measured for Pretzsch et al. 2014, soil carbon stocks derived therefrom using the bulk density estimator from (Augusto et al., 2010); sp: spruce, be (beech).

	Was-spruce	Was-mixed	Kra-spruce	Kel-spruce	Kel-mixed
T_v [°C]	13.8	13.8	13.8	13.9	13.9
P_v [mm]	640	640	480	385	385
BA [$m^2 ha^{-1}$]	57.2	15.3 (sp) 22.8 (be)	76.5	68.4	18.7 (sp) 25.7 (be)
BAI [$m^2 ha^{-1} y^{-1}$]	1.3	0.3 (sp) 0.5 (be)	0.8	1.2	0.2 (sp) 0.5 (be)
Rh [$\mu mol CO_2 m^{-2} s^{-1}$] 2022-2023	2.9±3.9	4.3±2.0	4.3±2.2	2.6±1.1	3.4±2.0
Rs [$\mu mol CO_2 m^{-2} s^{-1}$] 2022-2023	4.1±2.2	5.0±2.3	4.3±1.8	3.6±1.5	4.5±2.3
Soil organic carbon [%]	28.6 (o) 2.7 (m)	22.4 (o) 2.7 (m)	30.1 (o) 1.2 (m)	26.4 (o) 2.7 (m)	22.3 (o) 1.2 (m)
Soil carbon stocks [$Mg C ha^{-1}$]	46.5 (o) 71.29 (m)	45.2 (o) 97.1 (m)	45.8 (o) 57.5 (m)	36.7 (o) 80.3 (m)	28.8 (o) 63.2 (m)

Drought legacy in soil respiration

The legacy effects of a future extreme drought scenario are investigated by using the plots of a former drought experiment. This so called "Kroof – Kranzberg Roof Experiment" excluded the precipitation on clusters of mature spruce and beech during five consecutive vegetation periods (until summer 2019). With HoliSoils measurements starting there in autumn 2022 and



ending in 2025 (year 3-6 post-drought), medium to long-term legacy effects of these extreme droughts are examined.

By all indications, soil respiration is significantly lowered in the long run after such an extreme drought scenario for spruce, beech and their mixed-root zone, despite the trees recovering well after the drought according to ecophysiological data, and irrespective of the year investigated (cf. Fig. 6.2). While such consecutive drought periods have been so far rare, climate change predictions point to an increased frequency of severe hot droughts in Central Europe. Therefore, these results may give an outlook on what saplings now planted will encounter in their maturity.

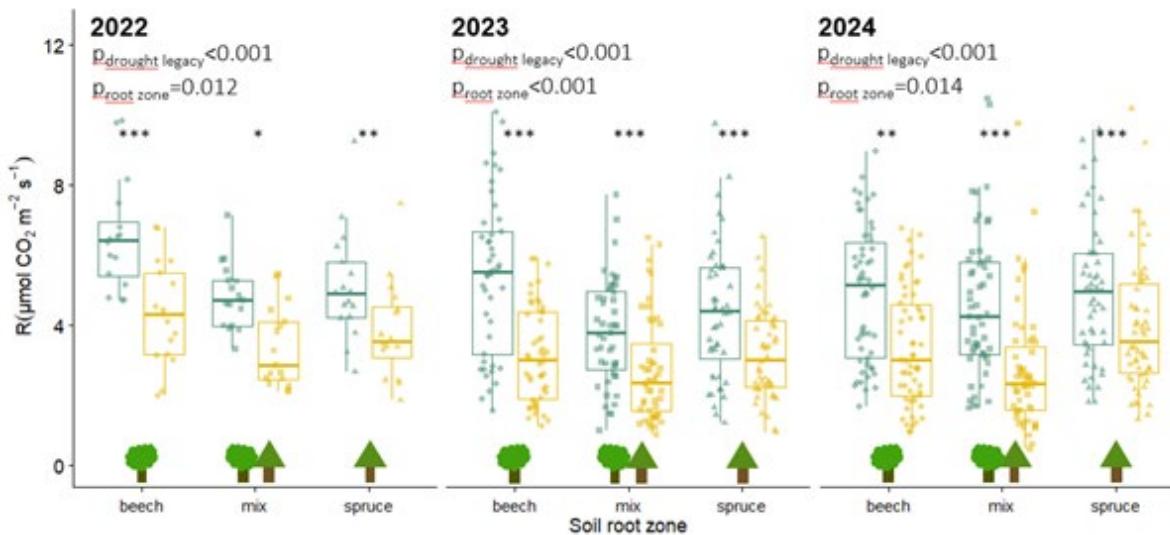


Figure 6.2. Soil respiration (Rs) in the drought legacy phase of the Kranzberg Roof Experiment. X-axis: different soil root zones within the plots (i.e. c. 150m^2 rectangles with a cluster of beech on one side and a cluster of spruce on the other; cf. (Grams et al., 2021). Green boxes/points: control plots receiving natural precipitation. Yellow boxes/points: drought legacy plots with preceding rainfall exclusion over the vegetation periods (Mar-Nov, 2014-18). P-values from linear mixed effect models; asterix: significant p-value (linear mixed effect model) in pairwise comparisons *: $p < 0.05$, **: $p < 0.01$; *** $p < 0.001$. $n(\text{plot})=8$ with 2 measurement points per root zone; $n(\text{dates})=2/6/9$ (years 2022/2023/2024). Number of included measurements: 710.

7. Netherlands

Soil respiration and microclimate under varying forest management

We investigate effects of forest management on soil respiration through its dependencies on soil temperature and moisture in Scots pine (*Pinus sylvestris*) forests on poor sandy soils. The soil respiration monitoring was conducted on three out of five locations across the Netherlands; Kroondomein, Zwolse Bos and National Park Hoge Veluwe (Table 7.1).

The Scots pine forests were planted between 1968 and 1975 (Vos et al., 2023), with understorey vegetation mainly composed of *Vaccinium myrtillus*, *Deschampsia flexuosa* and mosses, however, abundance of these species vary across locations. Due to high browsing pressure from roe deer, deciduous trees (e.g. *Betula pendula*, *Sorbus aucuparia*, *Frangula alnus*, *Quercus robur*) only occur sporadically in the understory.

Table 7.1. Characteristics of soil respiration and monitoring locations in the Netherlands, including site location, soil type, and climate information such climate type, annual mean precipitation (P mean) as well as annual minimum, maximum, and mean temperatures (T min, T max, and T mean, respectively).

Site	Latitude	Longitude	Soil type	Climate type	P mean (mm)	T min (°C)	T max (°C)	T mean (°C)
Zwolse Bos	52.420669	6.034353	Albic Podzol	temperate maritime	798	2.12	18.97	10.75
Kroondomein	52.205046	5.865131	Albic Podzol	temperate maritime	791	2.12	18.97	10.75
NP Hoge Veluwe	52.089985	5.804077	Dystric Cambisol	temperate maritime	788	2.21	18.55	10.54

On each location, four forest management treatments were established representing increasing felling intensity (control, high-thinning, shelterwood and clearcut). All treatments were established in February-March 2019 as a part of the Dutch Climate-Smart-Forestry project (Sterck et al., 2022), prior to the monitoring measures carried out in the HoliSoils project (Table 7.2).

Table 7.2. Characteristics of forest management treatments (fellings) in all three locations in the Netherlands. Information includes stand and management characteristics of state and reduction of basal area (BA) before and after was conducted. CTR: control, CC: clearcut, SW: shelterwood, HT: high-thinning.

Site	BA before treatment				BA after treatment			
	CTR	CC	SW	HT	CC		SW	
	m2/ha				m2/ha	reduction %	m2/ha	reduction %
Zwolse Bos	18.0	17.0	17.2	20.8	0.0	100	4.6	61
Kroondomein	21.9	19.8	19.4	20.1	0.0	100	4.0	76
NP Hoge Veluwe	21.0	21.5	21.8	20.6	0.0	100	3.5	89

We monitored soil CO₂ fluxes (bi-weekly) soil temperature and moisture (continuously every 15 minutes) during period May 2022 – September 2024 on every treatment. For monitoring soil respiration, we used EGM-5 analyzer with soil respiration chamber (SRC-2) and TOMST TMS-4 to monitor soil microclimate. We made root exclusion trenches to separate the heterotrophic from total respiration.



Effect of felling intensity on soil microclimate

Effects on soil temperature

Our main findings suggest that in clearcut treatments the effect of felling intensity on soil temperature changes depending on the seasonal temperature variation, with colder extremes in cold seasons (December - March) and warmer extremes in warm seasons (June - September). Contrary to our expectations, soil temperature did not gradually change with increasing felling intensity across treatments (from control – high-thinning – shelterwood to clearcuts; Fig. 7.1 & 7.2). These discrepancies could be explained by weakening effect of the felling treatments over time since our monitoring started three years after the treatments were established which could allowed soil to recover from the disturbance (here as fellings) and overgrow which ground vegetation.

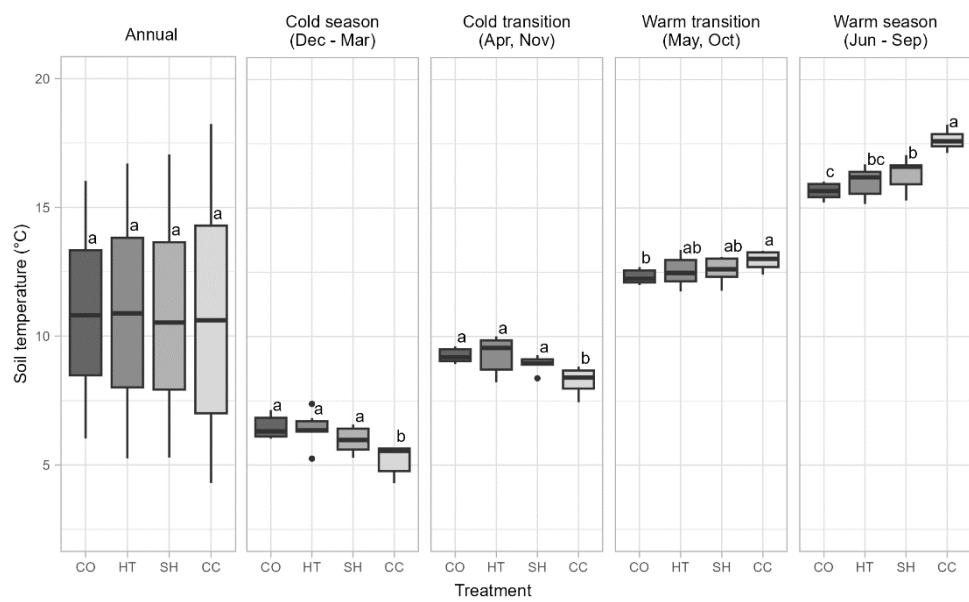


Figure 7.1. Soil temperature inside the root exclusion block. Soil temperature patterns between treatments inverse between the two colder and the two warmer seasons. Different lowercase letters express significant differences between treatments within one season at $p < 0.05$.

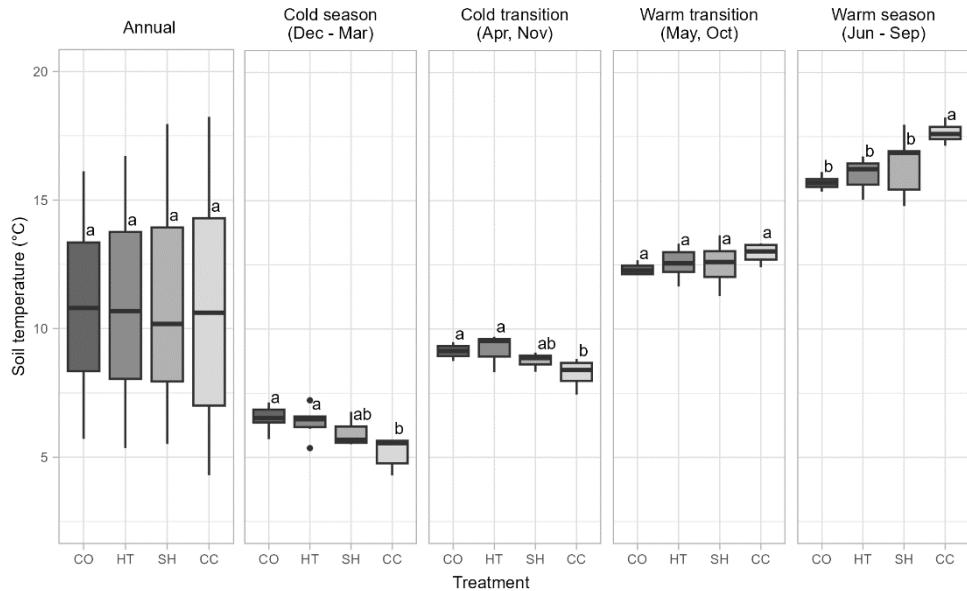


Figure 7.2. Soil temperature outside the root exclusion block. Soil temperature patterns between treatments inverse between the two colder and the two warmer seasons. Different lowercase letters express significant differences between treatments within one season at $p < 0.05$.

Effects on soil moisture

The effect on soil moisture showed an inconsistent response of the management treatment considering inside and outside of the root exclusion blocks. In contrary to our expectations, the soil moisture inside the root exclusion increased with higher felling intensities instead of remaining stable, from control to high-thinning and shelterwood, and clearcuts (Fig. 7.3). This might be a result of throughfall which we did not initially consider in our analysis. We suspect that the presence of the ground vegetation and mosses damped the evaporation which resulted in more moisture inside the exclusion block.

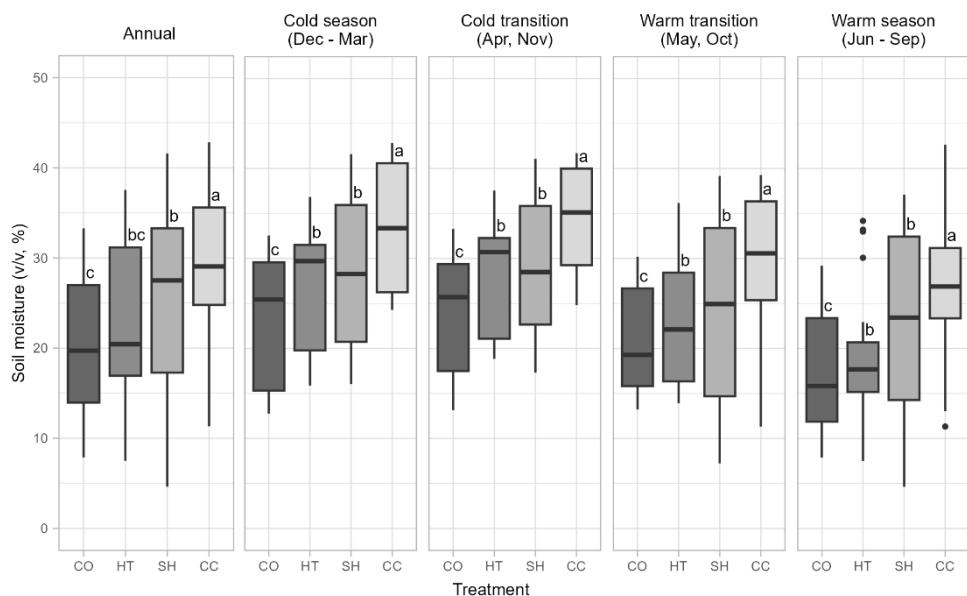


Figure 7.3. Soil moisture inside the root exclusion block. Soil moisture patterns remain stable across seasons. Different lowercase letters express significant differences between treatments within one season at $p < 0.05$.



Outside the exclusion block, the soil moisture increased with increasing felling intensity. However, in contrary to our expectations the control treatment did not have the lowest soil moisture. Instead, the measured soil moisture content was not significantly different from high-thinning and shelterwood treatments (Fig. 7.4). We think that this discrepancy can be explained by a threshold for the effect of felling on soil moisture, where hydrological processes leading to increased soil moisture only start above a certain felling intensity. For example, del Campo et al. (2022) concluded that transpiration is reduced and throughfall increased at felling intensity above 50% increasing soil moisture afterwards. Other studies also confirm this pattern by showing an increased soil moisture compared to controls at higher felling intensities but not at low to moderate intensities (Lei et al., 2018, Zhao et al., 2019). Based on our analysis, the threshold outside the root exclusion block is between the felling intensities of 83 % for shelterwood and 100 % for clearcuts.

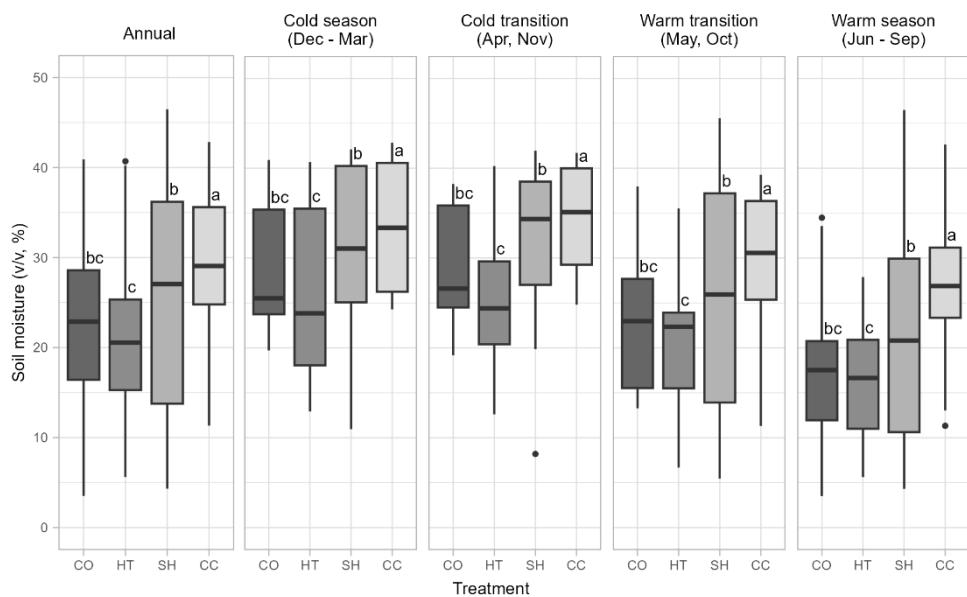


Figure 7.4. Soil moisture outside the root exclusion block. Patterns of significant differences in soil moisture remain stable across seasons, but distribution and variance change. Different lowercase letters express significant differences between treatments within one season at $p < 0.05$.

Effect of soil microclimate on soil respiration

Our results confirmed that the soil microclimatic conditions affected soil respiration. We found that the soil temperature had a strong exponential influence on soil respiration, by doubling with every 10 °C increase in soil temperature (Fig. 7.5). Besides, the effect of soil moisture showed a decrease of total soil respiration with increasing deviation from an optimum soil moisture content of 34 % (Fig. 7.5). This indicated an importance of the soil moisture component in determining total soil respiration. In addition, the effects on heterotrophic respiration were similar to those of the total respiration. The model fit and temperature sensitivity was similar for both the total and heterotrophic soil respiration. However, the expected effect of soil moisture was observed only for the total respiration. Nevertheless, we still concluded that the degree to which soil microclimate explained the variation of the two types of respirations was similar. As the total and heterotrophic soil respiration were strongly correlated, the microclimate dependence of heterotrophic respiration was consequently also shown for the total soil respiration.

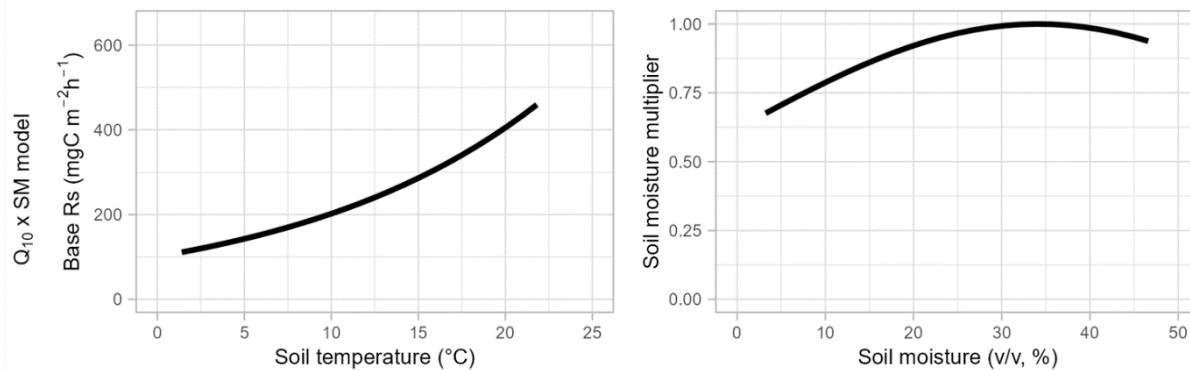
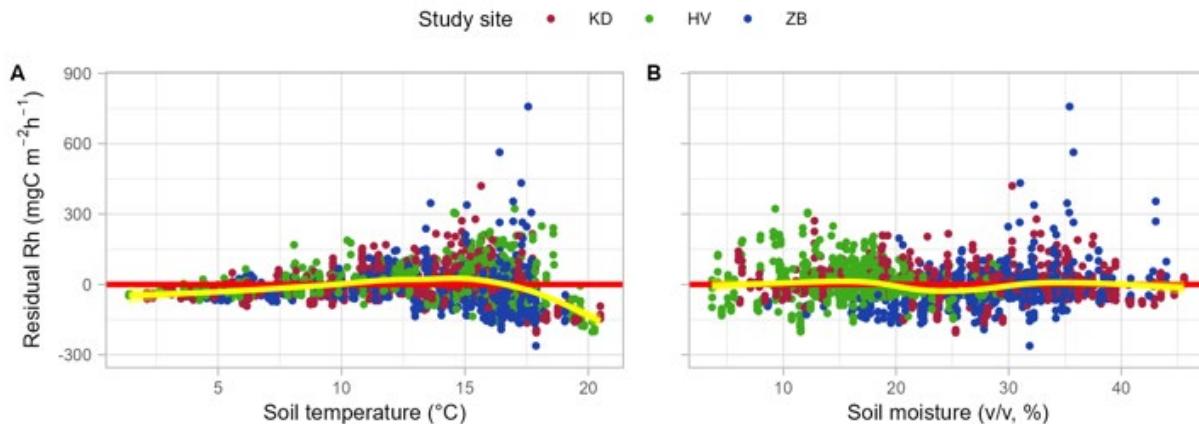


Figure 7.5. Predicted response curve of total respiration using the Q10 x SM model along the range of measured soil temperature and soil moisture. Model was fitted using all measurements from outside root exclusion blocks from all three locations. Model Q10 x SM fit ($R_{10} = 202.19^{**}$; $Q_{10} = 2.00^{**}$; $SM_{opt} = 34.12^{**}$; $A = 0.999590^{**}$, RMSE = 114.66, NRMSE = 64%). Parameter with significance $^{**}p < 0.001$.

Although our findings indicated microclimatic effect on the soil respiration, the modelling approach which we used does not lend itself to precise predictions of the soil respiration based on soil microclimate. The model residuals revealed a model bias by overpredicting the soil respiration at the lower and higher ends of the observed soil temperature spectrum (Fig. 7.6 & 7.7), which suggested that the true relationship between soil respiration and soil temperature was not exponential.



7.6. Residuals of the Q10 x SM model fitted on heterotrophic soil respiration. The red line indicates the line of perfect fit, the yellow line shows an automatically generated generalised additive model fit to better identify trends.

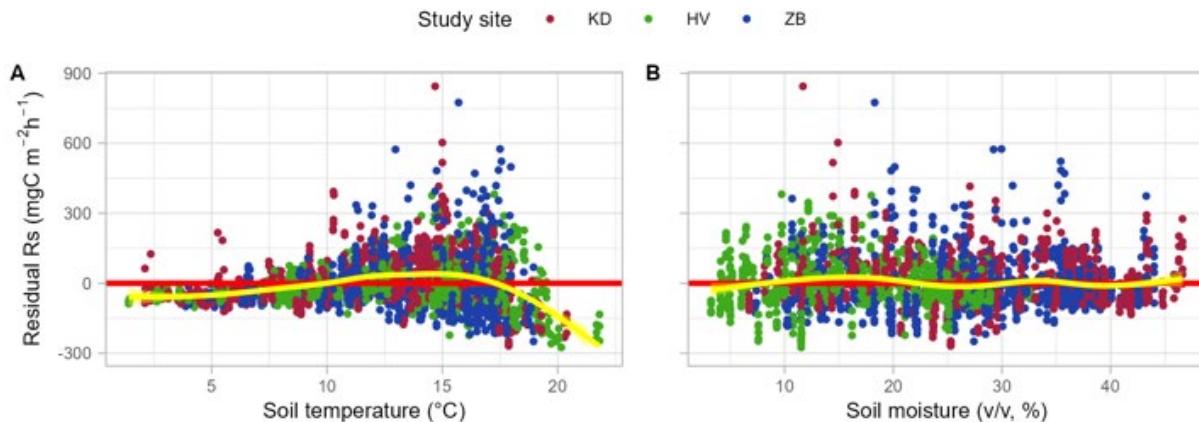


Figure 7.7. Residuals of the Q10 x SM model fitted on total soil respiration. The red line indicates the line of perfect fit, the yellow line shows an automatically generated generalised additive model fit to better identify trends.

Effect of felling intensity on soil respiration

We did not find significant effects of felling intensities on both total and heterotrophic soil respiration. However, we noticed general similar patterns of felling treatments on soil microclimate and soil respiration (Fig. 7.8 & 7.9). Considering seasonal difference, the patterns were less consistent. We observed that the total soil respiration decreased at the higher felling intensities of shelterwoods and clearcuts in the two colder seasons, showing pattern similar to that of soil temperature only in these specific conditions. In other seasons, the total soil respiration patterns did not parallel neither with soil temperature nor soil moisture changes. For the heterotrophic respiration, this was not the case even in colder seasons. Overall, we noticed that the soil microclimate and soil respiration patterns along a gradient of felling intensities did not strongly support the notion of an effect cascade from felling over microclimate on soil respiration. In our study lack of this cascading effects could be explained by (1) high variance in soil respiration affected by higher soil temperatures, (2) factors other than the felling intensity and soil microclimate which influenced the soil respiration and (3) ground cover vegetation and natural regeneration which was present on each treatment and location with varying cover and type.

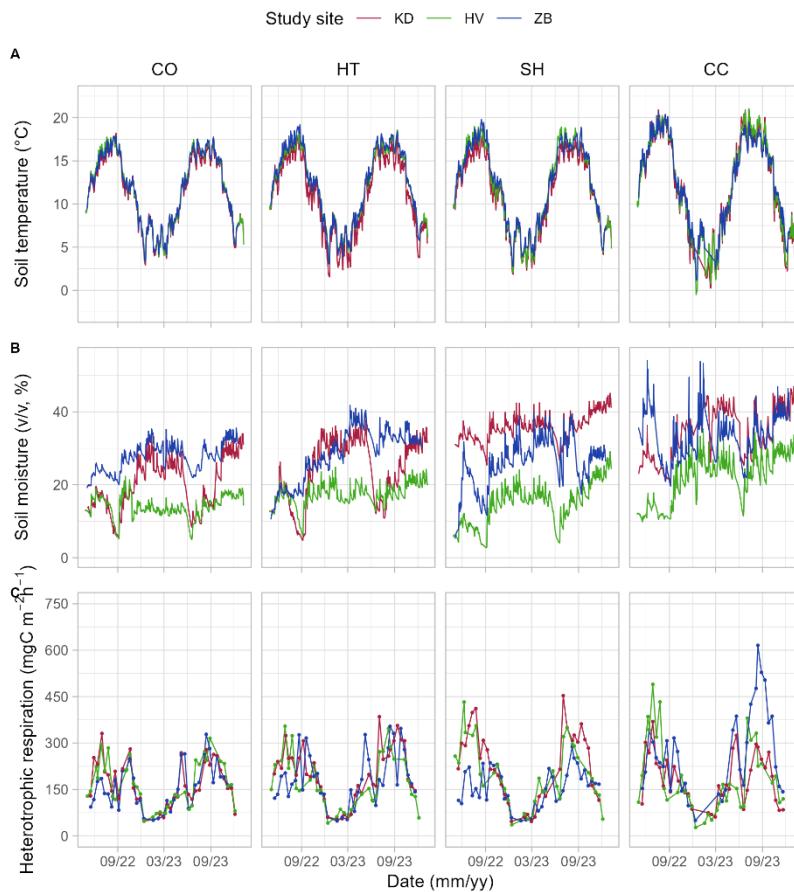


Figure 7.8. Development of soil temperature (A) and soil moisture (B) inside the root exclusion block and heterotrophic soil respiration (C) over time.

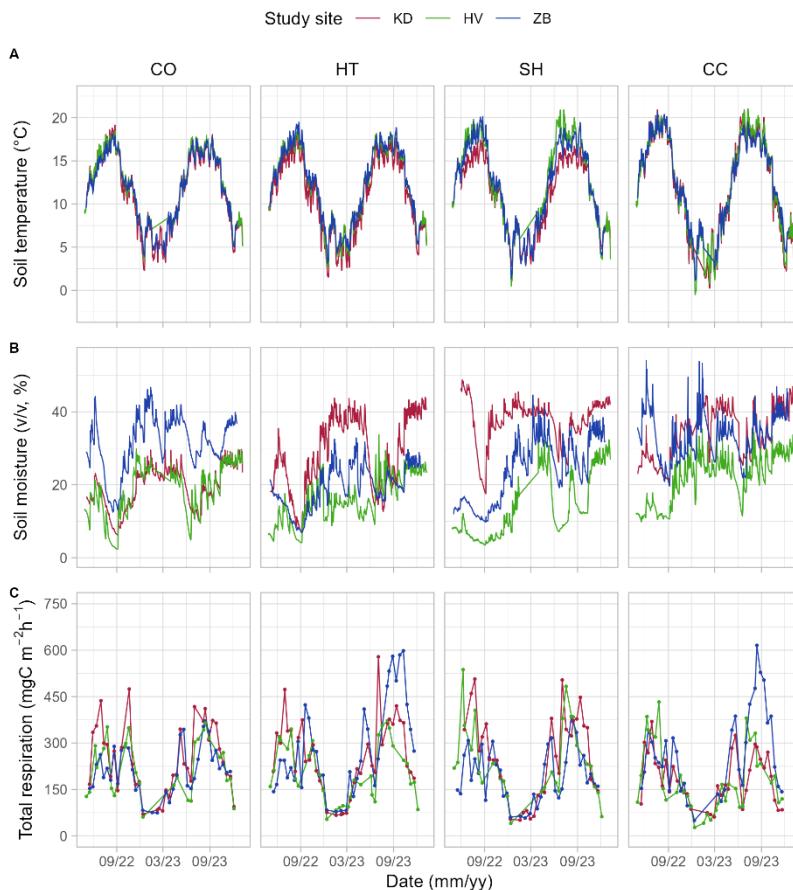


Figure 7.9. Development of soil temperature (A) and soil moisture (B) outside the root exclusion block and heterotrophic soil respiration (C) over time.

Main findings and conclusions

Although, the results did not show a clear pattern for all forest management treatments, we observed that the high felling intensities of clearcuts and, to a lesser extent shelterwoods, significantly amplified seasonal soil temperature changes and resulted in increased levels of soil moisture year-round. This observation proved that the forest canopy has a buffer function on seasonal changes in soil temperatures and supports the notion of a threshold effect between felling intensity and soil moisture. Furthermore, we found a significant positive relation between the soil temperature and soil respiration while challenging the exponential relationship between them (Curiel Yuste et al., 2004, Savage et al., 2009, Davidson et al., 2012; Drewitt et al., 2002). We also observed that the soil moisture was quadratically related to the total soil respiration, but not heterotrophic respiration. It indicates that different mechanisms determine the effects of soil moisture on heterotrophic and total soil respiration. These different mechanisms could be a result of differences in the microbial diversity and ground vegetation, which were not included in this analysis.

To our surprise, within most seasons soil respiration did not show the same patterns as the soil microclimate along the gradient of treatments with increasing felling intensities. Instead, an increase in the variance of soil respiration suggested that factors other than the soil microclimate became increasingly important in determining soil respiration when soil temperature becomes less of a limiting factor. Altogether, our results highlight that the forest



management has impacts on the soil microclimate, which changes with the seasons and that the soil respiration reacts to those changes.

Overall, our research highlighted that forests are self-regulatory and interconnected systems. In these systems, aboveground changes to canopy cover cannot be separated from belowground conditions and processes. The canopy cover plays an important role in buffering soil microclimate and soil respiration against external changes, such as seasonal temperature fluctuations (Fig. 7.1 & 7.2). It was also confirmed by Akande et al. (2023) and Zhang et al. (2018) whose findings showed an increase in soil temperatures with clearcutting and felling intensities. In the face of climate change, maintaining this self-regulatory capacity means an increased stability and resilience against extremes, which can help keep balance between carbon inputs into forest soils and carbon emissions via soil respiration (Mäkipää et al., 2023, Jandl et al., 2007). High felling intensities, such as clearcuts, erode the resilience of forest systems, make forest soils more susceptible to extreme temperature changes, and thereby threaten the balance of soil carbon dynamics. This is true even in small clearcuts of a 0.25 hectare which were a subject of our experimental site (Fig. 7.8 & 7.9). Adaptive forest management should aim to uphold the forests' capacity to self-regulate and buffer soil temperature and soil moisture, especially considering the increasing frequency of prolonged extreme weather conditions.

8. St. Mitre, France

A common management practice in Mediterranean forests is thinning, which reduces stand density. By removing some trees and thus reducing the competition between trees for resources, thinning is expected to increase the growth and productivity of the remaining trees, improves their health status and reduces the potential damages from pathogen attacks, and finally reduces the fire risk by limiting the crown fuel continuity. Similarly, in these forests, where fire is one of the most important natural disturbances shaping plant community structure and composition, the removal of the understory vegetation is also often implemented to reduce fuel accumulation and thus wildfire. However, to date, no study has investigated the combined influence of forest thinning and understory removal on microclimatic conditions, soil physical-chemical properties, the soil biodiversity and associated processes such as CO₂ emissions or litter decomposition efficiency. Our objectives were to fill this gap of knowledge by using a field experiment located in a Mediterranean forest with two forest management treatments: overstorey thinning at three intensities (i.e. no thinning, moderate or intense thinning) and understory vegetation removal (i.e. with or without understory).

Study site location and experimental treatments

The study was conducted in a naturally established 60–70-year-old *Pinus halepensis* Mill. forest (Southeast France; 43°3'N; 5°2'E, altitude: 130 m a.s.l.). The soil is calcareous, with a silty-sandy texture and an average depth of 60 cm. The understory vegetation is dominated by shrubs (*Quercus ilex* L., *Quercus coccifera* L. and *Cistus albidus* L.). The climate of the site is typically Mediterranean, with hot and dry summers and mild winters, a mean annual temperature of 16°C, a mean annual precipitation of 525 mm, and a drought period that generally lasts 4 months, centred around June and August (2008-2022, Istres weather station Météo France). The year 2022 was the warmest year in the period 2008-2022 with an annual mean temperature of 17.5°C (highest mean monthly temperature in July, 27.9°C), and one of the driest years with an annual mean precipitation of 361.6 mm and a 6-month drought period.

The experimental site is made up of twelve 25 × 25 m plots located in the pine forest. In 2007, 3 thinning intensities were implemented (Fig. 8.1): intense thinning (I) removing 2/3 of the initial basal area, moderate thinning (M) removing 1/3 of the basal area, and the control without thinning (C). In 2016, the plots of M and I treatments were divided into two parts: on one half of the surface the shrub layer was removed, while the other half remained untouched. No shrub removal was performed on the control plots (C), as the shrub layer was naturally very limited. Shrub removal was applied each year during the winter season to prevent shrub regrowth. This experimental design leads to the following five treatments: intensive thinning with or without shrubs (I+, I-), moderate thinning with or without shrubs (M+, M-) and control plots (C). In 2016, average plot characteristics including pine tree density and basal area (BA) were measured. The values recorded were the following: 1192 pine trees ha⁻¹ and BA= 37m²/ha for the control treatment (C), 632 pine tree ha⁻¹ and BA= 28m²/ha for the medium thinning treatment (M-, M+) and 236 pine tree ha⁻¹ and BA= 17m²/ha for the intense thinning treatment (I-, I+). It has to be noted that understory was more developed in the intense thinning treatment (I+) than in the medium thinning treatment (M+) due to a greater light availability: in 2016 the shrub cover and height were respectively 47% and 107 cm in the former and 33% and 93 cm in the latter (Prévosto et al., 2020).

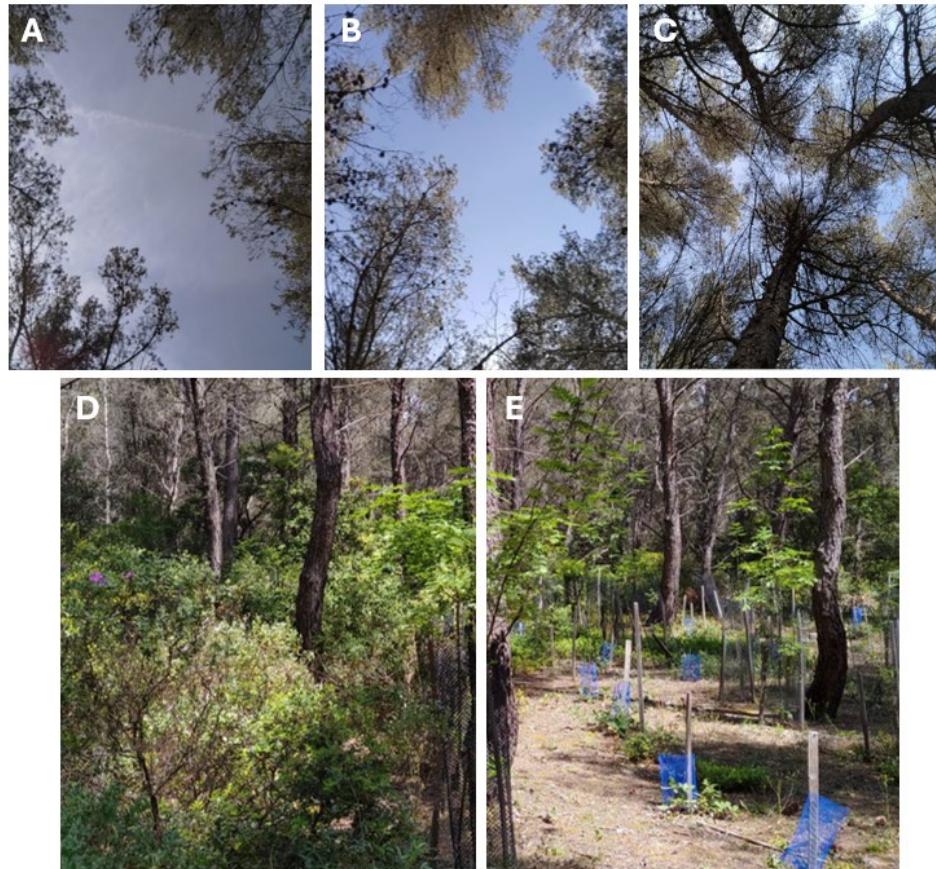


Figure 8.1. Forest thinning (top) and shrub management (bottom). A. Intense thinning, B. Moderate thinning, C. Control without thinning, D. With shrub and E. Without shrub.

Soil physical-chemical properties response to management

The physical and chemical analyses were conducted by the Teyssier laboratory (Bordeaux, France). Soil pH, total organic matter (OM), organic carbon (C), nitrogen (N), phosphorous (P, Olsen method), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), and texture (clay, silt and sand) were analyzed in each plot by a soil composite sample, made of 3 randomly-selected cores of the top 6 cm of soil, in spring 2022.

A PCA performed on the soil physico-chemical properties revealed a first axis that explained 55.3% of the variation in physico-chemical properties, mainly driven by the concentration of Mg, C and OM (Fig. 8.2). This first PCA axis highlighted a soil fertility gradient (i.e. increasing concentrations of OM, C, N, K, Mg, Ca, high CEC and low pH) and showed three distinct groups: the control plots, the plots without shrubs and the plots with shrubs, suggesting that forest management has a strong impact on soil fertility.

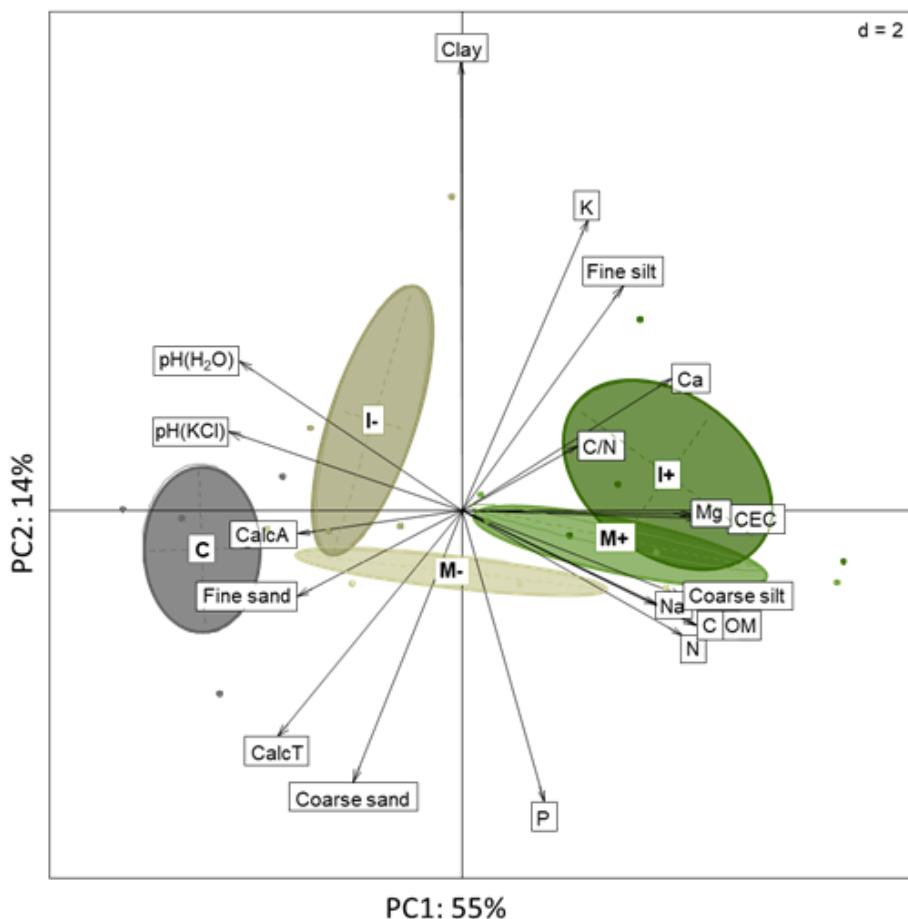


Figure 8.2. Principal component analysis (PCA) based on the soil physico-chemical properties (arrows) arranged by forest treatments (colored circles). C = control without thinning, M- = moderate thinning without shrubs, M+ = moderate thinning with shrubs, I- = intense thinning without shrubs and I+ = intense thinning with shrubs. CEC = Cation exchange capacity, pH(H₂O) = pH measured by water extraction, pH(KCl) = pH measured by KCl extraction, CalcT = total limestone, CalcA = active limestone, OM = organic matter, P = phosphorus , K = potassium, Mg = magnesium, Ca = calcium, Na = sodium, N = nitrogen, C = carbon, C/N = carbon/nitrogen ratio.

Microclimate response to management

Each plot was equipped with 2 dataloggers (TOMST®, TMS-4). These devices measure soil temperature at 6 cm depth, air temperature at 2 cm and 15 cm, and soil moisture. TMS-4 loggers record measurements every 15 min.

Soils in the intense thinning plots without shrubs had significantly higher volumetric moisture content (LMM, F= 280.0, P<0.001) and higher temperature at -6 cm depth (LMM, F= 201.7, P<0.001) than soils in plots with shrubs.

Soil organisms responses to management

Soil sampling was performed in spring (May) and fall (September) 2022, i.e. before and after the summer drought period. We sampled four groups of soil organisms: microorganisms, microfauna, mesofauna and macrofauna. We collected a total of 160 soil samples

representing a combination of 4 biotic groups × 5 forest treatments × 4 plots × 2 sampling times (before/after summer drought). Whatever the soil biota group considered, no interaction between forest treatment and sampling time was observed on their biomass or abundance, expected for the Chilopoda (predatory macrofauna).

Among the microbial biomasses, bacterial biomass was the most important (45% of the total microbial biomass), particularly that of gram-negative bacteria. Microbial biomass was not affected by forest treatment. Only bacterivore nematode abundance was influenced by forest treatment, with 193% higher abundance in the intense thinning plots with shrubs compared to the intense thinning plots without shrubs (Biryol et al., 2024).

A total of 20 500 individuals belonging to the mesofauna were counted and identified. Within the 13 mesofauna groups identified, Oribatidae represented 74% of the mesofauna abundance. Overall, the mesofauna abundance was dominated by detritivore organisms (84%, 52797 individuals/m²). The abundance of total mesofauna or mesofauna trophic groups were not affected by forest treatment. However, the abundances of Collembola, Entomobryomorpha, Polyxenidae and mesofaunal larva were higher in intense thinning plots with shrubs compared to intense thinning plots without shrubs.

A total of 2 307 individuals belonging to the macrofauna were collected and identified. Among the 17 macrofauna groups, most organisms were detritivores, including Insecta (43%), Diplopoda (16%) and Gasteropoda (14%). The abundance of macrofaunal predators and detritivores were influenced by forest treatments, with higher values found in the moderate thinning with shrubs plots compared to the control plots and the plots without shrubs (from 92% to 376%). Chilopoda abundance response to forest treatment was dependent on the season considered: In May, Chilopoda abundance was higher in the intense thinned plots with shrubs compared to control plots and the plots without shrubs, whereas in September, their abundance was not affected by forest management.

Litter decomposition and soil respiration responses to management

A litterbag experiment was used to assess litter decomposition response to forest management. Litterbags (20 × 20 cm, 5 mm mesh size) filled with 10 g of senescent needles of *Pinus halepensis* alone or in mixture with companion species (*Cistus albidus* and *Quercus coccifera*) were placed on forest soil in August 2022. Litterbags were collected after 6, 12 and 24 months of decomposition. The data of this experiment are currently analysed.

Soil respiration was recorded monthly in each plot using a portable gas analyzer (IRGA, EGM-5, PP Systems) connected to a closed soil respiration chamber (SRC-1, PP Systems, Massachusetts). Prior to measurements, the chamber was placed on a PVC tube (10 cm diameter) buried (5 cm deep) into the soil to prevent CO₂ leakage. Concomitantly, soil temperature and humidity were respectively recorded at 7 cm depth using a soil temperature probe (STP-1, PP Systems) and a portable time-domain reflectometry (TDR) device (Delta-T Devices, ML2 Theta Probes) connected to the respirometer. Soil respiration data have been uploaded to the Luke database and will be analysed in the future.



Synthesis of the preliminary main findings

The development of a dense understory vegetation with the reduction of pine cover in the intense thinned plots led to an increase in soil fertility (i.e. increased OM and nutrient contents due to higher quality litter input compared to pine litter), which could increase the availability of habitats and resources for soil organisms. Indeed, the presence of the litter from other tree species (e.g. *Quercus coccifera* L., *Cistus albidus* L.), could increase the structural complexity of habitats available for the soil biodiversity, and provide them a larger quantity and diversity of resources than a litter composed only of pine needles. Thus, plots with understory vegetation showed a higher abundance of bacterivore nematodes and macrofauna compared to plot without understory vegetation, demonstrating the critical role of understory vegetation for soil organisms.

These results indicate that the management of shrub vegetation, rather than that of the tree layer, influences soil fauna. Indeed, the suppression of understory vegetation, compared with plots where it is maintained, reduces the abundance of soil macrofauna, whether the forest thinning is moderate or intense. However, regardless of the type of forest management applied, no reduction in the abundance of soil organisms was observed compared with control plots. This indicates that, over the long term (17 years after thinning), forest management has no negative impact on soil biodiversity compared with unmanaged forests. Yet, when forest thinning is implemented, maintaining the shrub layer helps prevent a negative effect on soil fauna. These finding provide an overview of the effect of forest management practices commonly employed to preserve soil biodiversity.

In a context of climate change, where the frequency and intensity of summer droughts are increasing in the Mediterranean region, it is important to propose management methods that increase soil water availability and buffer rising temperatures. Thus, promoting the conservation of understory vegetation i) improves microclimatic conditions, ii) facilitates forest regeneration, iii) favours communities of soil organisms, but also iv) increases competition with the tree layer for nutrients and water, v) increases the vulnerability of forests to fire and, finally, vi) reduces forest productivity. Effective forest management must balance these trade-offs to promote the sustainability of forest ecosystems and the services they provide.



9. Solsona, Spain

Assessment of the impacts on soil C stocks and dynamics of different management strategies to increase fire resistance

The combination of increased temperatures resulting from climate change led to a notably increase in the frequency and intensity of drought events combined with the decrease in forest management practices over the last decades this elevated the risk of wildfires in the Mediterranean basin, particularly in southern areas. Within the Holisoils project, we analysed the effect on soil C stocks and dynamics of different management options to enhance the resistance and resilience of Mediterranean forests to drought and mitigate the severity and propagation of potential wildfires. The effects of these management strategies on forest resistance to drought have already been reported (Vilà-Villardell et al., 2024). In summary, the results showed that tree growth and drought resistance increased in thinned stands, particularly when slash burning was also applied.

The study was conducted in three sites located in sub-Mediterranean black pine (*Pinus nigra* subsp. *salzmannii* (Dunal) Franco) forests, at the southern foothills of eastern Pre-Pyrenees: Llobera, Secanella, and Madrona (Fig. 9.1). At each site, two thinning intensities were applied: light thinning (Lth) and heavy thinning (Hth), which corresponded with a 10% and 40% of reduction in basal area, respectively. For each thinning intensity, two debris management strategies were applied: prescribed burning (PB) and non-burned (NB), leaving the debris on the forest floor. Therefore, at each site, the experimental design comprised five treatments: HthNB, HthPB, LthNB, LthPB, and an untreated control (Cont). Management practices were implemented between 2015 and 2017.

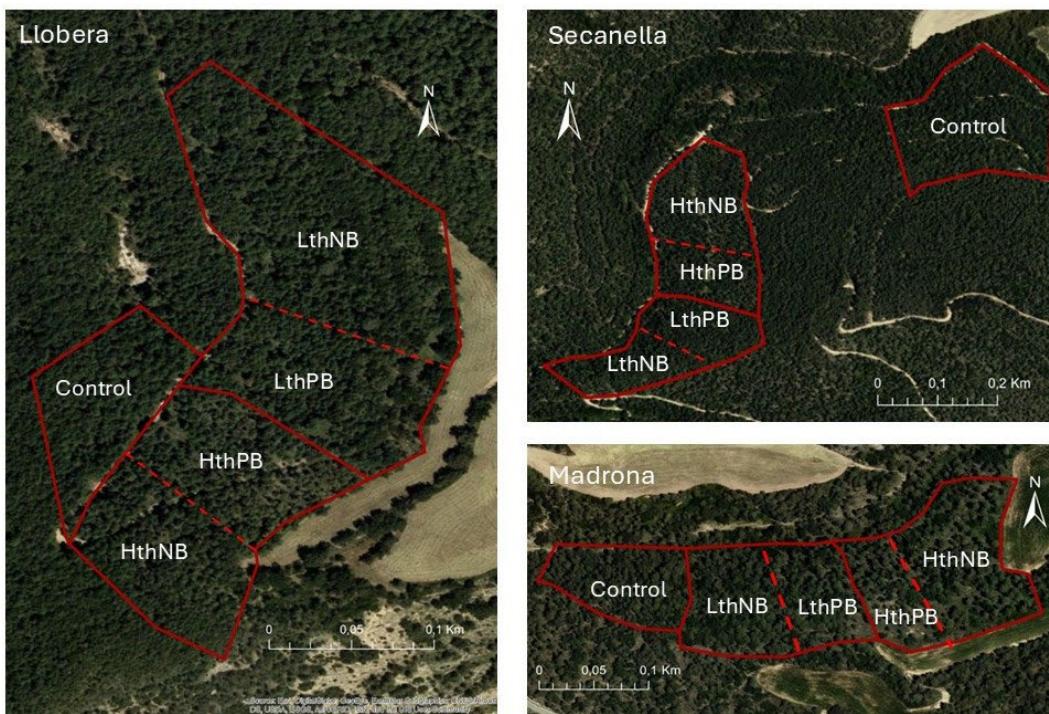


Figure 9.1. Thinning and slash burning treatments in the three experimental sites: Llobera (left), Secanella (right, top) and Madrona (right, bottom). Lth: light thinning; Hth: heavy thinning; PB: prescribed debris burning; NB: not-burned.

Impact of forest management on soil C stocks

Soil organic carbon stocks were estimated at three sites five years after the implementation of the forest management treatments (Fig. 9.2). At each site, we quantified C stocks in the forest floor and the top mineral soil (0–30 cm depth). Prescribed burning partially consumed the forest floor organic matter. However, the results indicate that the impact of this practice on the forest floor C is still relevant 5 years after its application. Indeed, organic C stocks in the H horizon of burned stands represented approximately 48.7% of those present in unburned stands. By contrast, C stocks in the mineral soil were found to be unaffected by burning (Tudela Haberland et al., 2025).

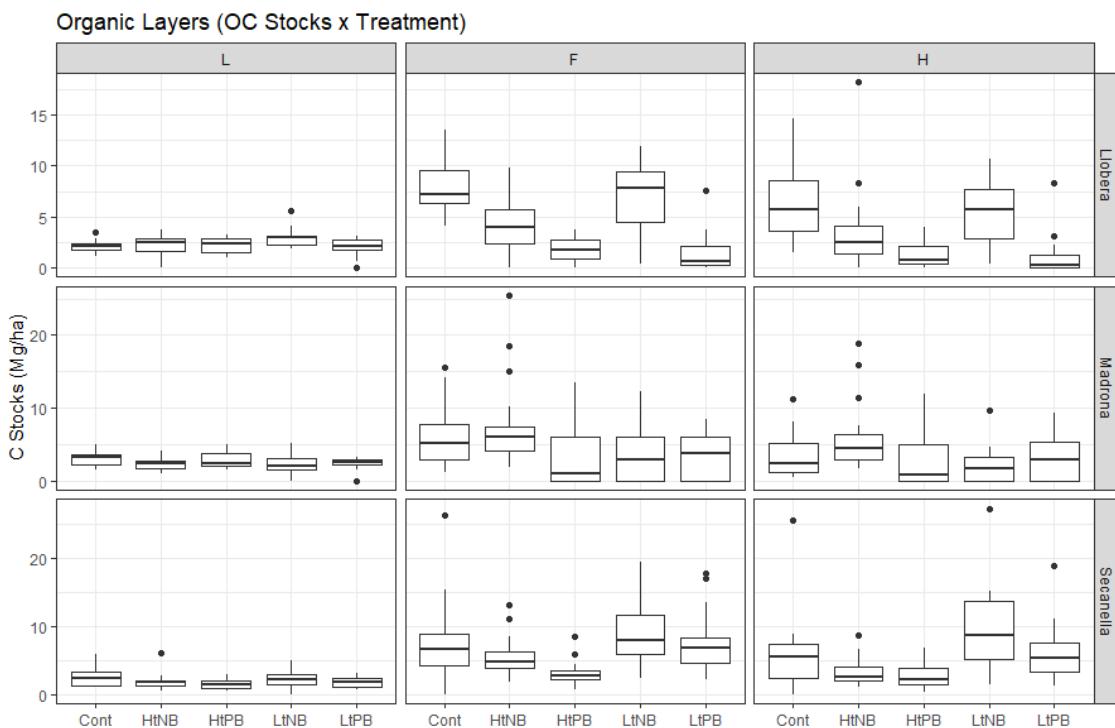


Figure 9.2. Organic carbon stock (Mg C/ha) of each organic horizon (L, F and H horizons) five years after treatments, per treatment at each site. Cont: untreated control; HtNB: heavy-thinned, no burning; HtPB: heavy-thinned, prescribed burning, LtNB: light-thinned, no burning; LtPB: light-thinned, prescribed burning.

Prescribed burning effect on soil microbial activity and functions in forest soils

The effects of fire on soils are mostly dependent on the intensity of fire. Prescribed fires are usually low-intensity fires, which are expected to exert a limited effect on soil properties and facilitate a faster recovery than that observed in high-intensity wildfires. The use of soil from Llobera in a laboratory experiment involving soil heating and incubation revealed changes in the functional diversity of soil communities (Fig. 9.3). However, these changes were observed to recover more rapidly after low (200°C) than after high (450°C) temperature heating (Garcia-Pausas et al., 2022). In addition, we incubated surface mineral soil (0–5 cm depth) from Llobera burned stands (HtPB) in the laboratory and compared it with the soil from unburned stands (HtNB) to assess the basal soil respiration, as a measure of organic matter stability, and the community-level physiological profiles using the Microresp system, to assess the soil functional diversity. At each managed stand, microsites with high and low amounts of organic

forest floor were selected for investigation of the role of organic horizons. Preliminary results indicate that five years after the implementation of the treatments, the soil organic matter from the burned stands exhibited higher stability (i.e., lower basal soil respiration) than that from the unburned stands. However, we did not find significant effects on soil functional diversity.

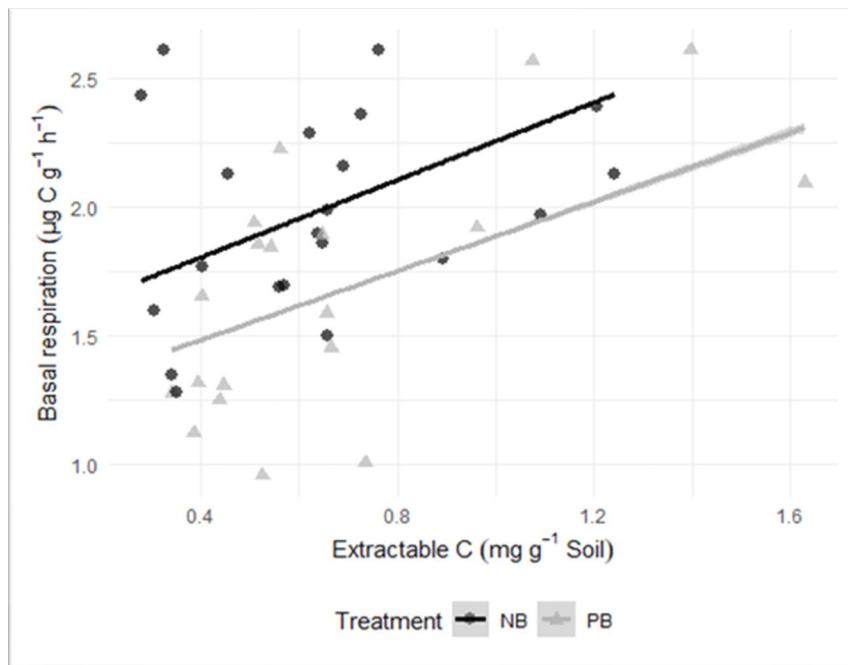


Figure 9.3. Relationships between basal respiration ($\text{C mg h}^{-1} \text{ soil g}^{-1}$) and extractable C in the mineral topsoil (0–5 cm) five years after prescribed burning (PB) or no burning (NB) debris treatments. Lines indicate the output of the model that includes extractable C and treatment.

Forest management impact on C and N distribution between soil physical fractions

Long-term stabilisation of soil organic matter mostly relies on its interaction with the mineral matrix. To study the effects of forest management on the stabilisation of organic matter, the topsoil (0–30 cm) from the three study sites (Llobera, Madrona and Secanella) was sampled and subjected to a physical fractionation by particle size (wet sieving). The distribution of organic C and N between fractions was then analysed.

Preliminary results show a difference in behaviour between the Llobera plots and the others (Fig. 9.4). In both Madrona and Secanella, thinning consistently results in an increase of C in both particulate organic matter (POM) and the organo-mineral complex (OMC). This effect is reversed by prescribed burning, which results in a decrease in the amounts of organic carbon in POM and OMC. Llobera plots exhibit a different behaviour: thinning increases the amounts of OMC but not POM. In addition, the effect of further prescribed fire is inconsistent: it increases in soil surface (0–5 cm) but decreases in depth (5–15 cm). For coarse organic fragments (COF: > 200 µm), the effect of both thinning and prescribed fire is inconsistent: it depends on site and of depth. The distribution of N between fractions is currently being analysed.

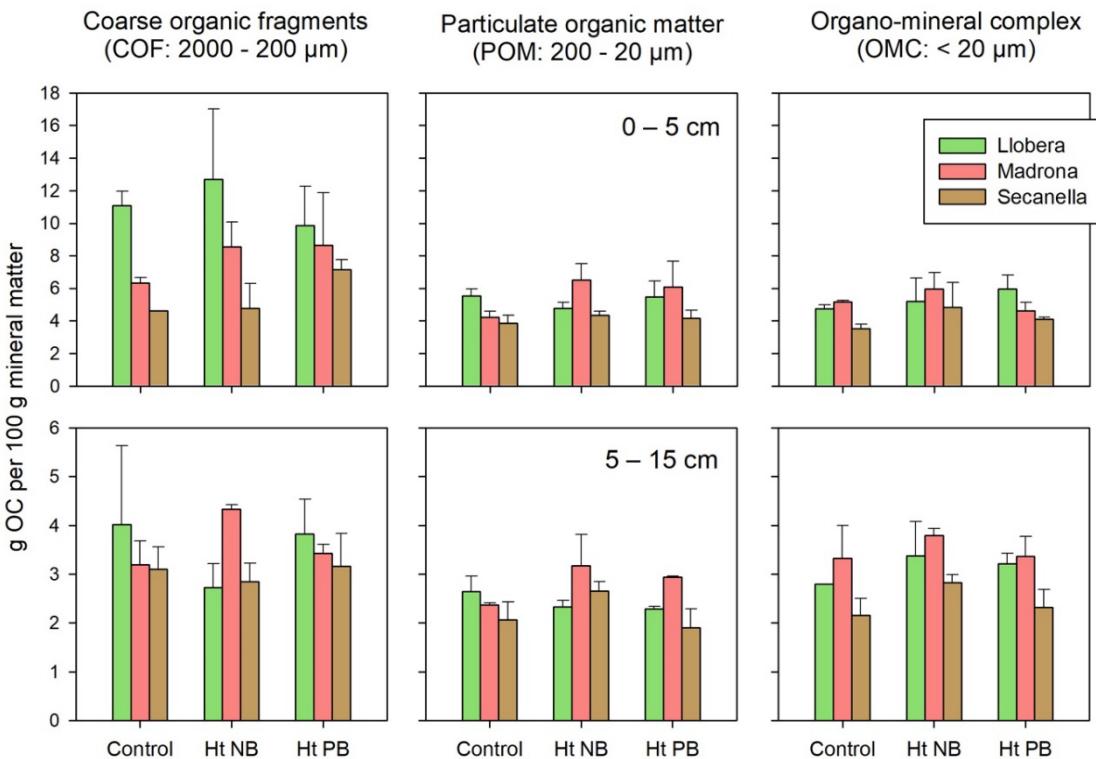


Figure 9.4. Effect of the three tested treatments in the soil organic matter: Control (no treatment), HtNB (heavy thinning, no burning), and HtPB (heavy thinning, prescribed burning). Behaviour of organic carbon in the three main fractions of soil organic matter: coarse organic fragments (2000-200 µm), particulate organic matter (200-20 µm) and organo-mineral complex (< 20 µm). Upper plots correspond to 0-5 cm soil depth and the bottom plots to 5-15 cm depth. Values are the averages of 2 measurements, while the vertical bars indicate the standard deviations.

Effect of forest management on soil respiration

In two sites (Llobera and Secanella) and in each treatment, three root-exclusion trenching experimental areas of approximately 2 × 1 m were established in autumn 2021. Total soil respiration was measured inside and outside the trenched areas using an EGM-5 CO₂ analyser connected to a soil respiration chamber (SRC-2). Measurements were conducted from spring 2022 until the end of 2023, with some additional measurements in spring and summer of 2024. A total of 28 soil respiration samplings have been performed at each site. Additionally, soil temperature and moisture, as well as air temperature, were monitored inside and outside one trenching area of each treatment using TMS-4 (TOMST) dataloggers and sensors.

Main findings and conclusions

Forest thinning removes a fraction of aboveground biomass and therefore reduces the surface organic matter inputs into the soil, at least during the first years after the thinning. For this reason, the organic horizons are the most sensitive C pool to forest management. However, leaving the thinning residues spread on the forest floor can compensate it in the short term. Also, as the reduction of competition for resources enhanced tree productivity (Vilà-Villardell et al, 2024), lower surface C inputs were possibly partially compensated in the mid-term. Our work in the framework of HoliSoils project showed that, 5 years after thinning, the C stocks in



the organic horizons was similar to those in non-thinned stands, indicating that thinning did not cause a great C loss.

Slash burning is a practice recommended in strategic areas to reduce wildfire hazards after thinning and understory clearing. This practice has trade-offs: while reducing debris by burning increases the resistance to eventual fires or to severe droughts due to the lower fuel load and lower competition for water (Vilà-Villardell et al., 2024, 2023), the effect of fire consumption of surface organic horizons is still evident five years after burning. Slash burning produces charcoal or partially charred materials that could be more resistant to decomposition than non-charred organic matter. Basal respiration measurements indicated that organic matter in uppermost mineral soils from burned stands were more stable, despite not having effects on the microbial community-level physiological profiles. This result suggests the presence of recalcitrant compounds, presumably coming from the charred material produced with fire. On the other hand, it seems that burned stands tend to contain a lower proportion of coarse fractions of organic matter, contributing to its higher stability. The ongoing analysis of field measurements of soil respiration is expected to provide further clues on the importance of this on soil CO₂ efflux.

Overall, the retention of harvest residues on the forest floor had little impact on the soil C pools 5 years after the thinning, while there is a loss of C from organic layers when the residues are burned; however, the increase in the stability of organic matter in the mineral soil may partially compensate C loss. Long-term monitoring of managed stands could help to understand whether the increased tree productivity and soil organic matter stability compensate the initial C loss of burned stands.

10. Karstula, Finland

Role of nitrogen fertilisation in boreal forest ecosystems

Nitrogen is usually limiting tree growth in boreal forest. Application of nitrogen increases N availability in forest soil resulting in a relative short and intensive pulse of additional tree growth. In practical forestry nitrogen fertilisations is often conducted a decade before final felling to ensure profitability of the investment due to the short payback time. Nitrogen fertilisation may change understorey vegetation composition, soil acidity, microbial communities, and soil fauna.

Study site

The study was conducted in a 62-year-old Scots pine (*Pinus sylvestris*) stand located in Karstula, central Finland ($62^{\circ}54'43.343''$ N; $24^{\circ}34'16.021''$ E) (Fig. 10.1). The understory vegetation primarily consisted of ericoid species such as *Vaccinium vitis-idaea*, *Vaccinium myrtillus*, *Empetrum nigrum*, and *Calluna vulgaris*, with low presence of herbs and grasses. The soil at the site is characterized as sandy loam. Temperature measurements at a soil depth of 5 cm indicated an average of 0.8°C during winter (November–April) and 10.3°C in summer (May–October), resulting in an annual mean temperature of 5.9°C. The well-drained sandy soil exhibited an average annual volumetric water content of 0.28 m³/m³.

Initiated in 1959 by the Finnish Forest Research Institute (now part of the Natural Resources Institute Finland), a factorial fertilization experiment was established comprising eight 25 × 25 m plots, each treatment replicated across three blocks (n = 24). Plots were spaced at least 10 m apart. Treatments were randomly assigned within each block and included: control (CTRL, unfertilized), Ca, N, P, Ca + N, Ca + P, N + P, and Ca + N + P additions. Fertilizer types and application rates were adjusted over time following best practice guidelines.

Between 1960 and 2020, nitrogen treatments were administered seven times, totalling 1102 kg ha⁻¹ of nitrogen over six decades. The initial application in spring 1960 involved 82 kg ha⁻¹ of ammonium sulfate (26% N). In 1970, 120 kg ha⁻¹ of urea (46% N) was applied. Subsequent treatments in 1980 and 1990 utilized calcium nitrate (27% N, 6% Ca, 3% Mg) at 180 kg ha⁻¹. The final three applications in 2000, 2010, and 2020 employed saltpeter (26% N, 3% Ca, 1% Mg, 0.02% B) at 180 kg ha⁻¹ per application.

Calcium was applied twice: 2000 kg ha⁻¹ of dolomite powder in summer 1959 and 4000 kg ha⁻¹ in spring 1980. Phosphorus fertilization occurred three times: initially with 29 kg ha⁻¹ of fine phosphate (36% Ca, 14% P) in summer 1959, followed by 40 kg ha⁻¹ of superphosphate (16% Ca, 20% P) in spring 1980 and spring 2020.

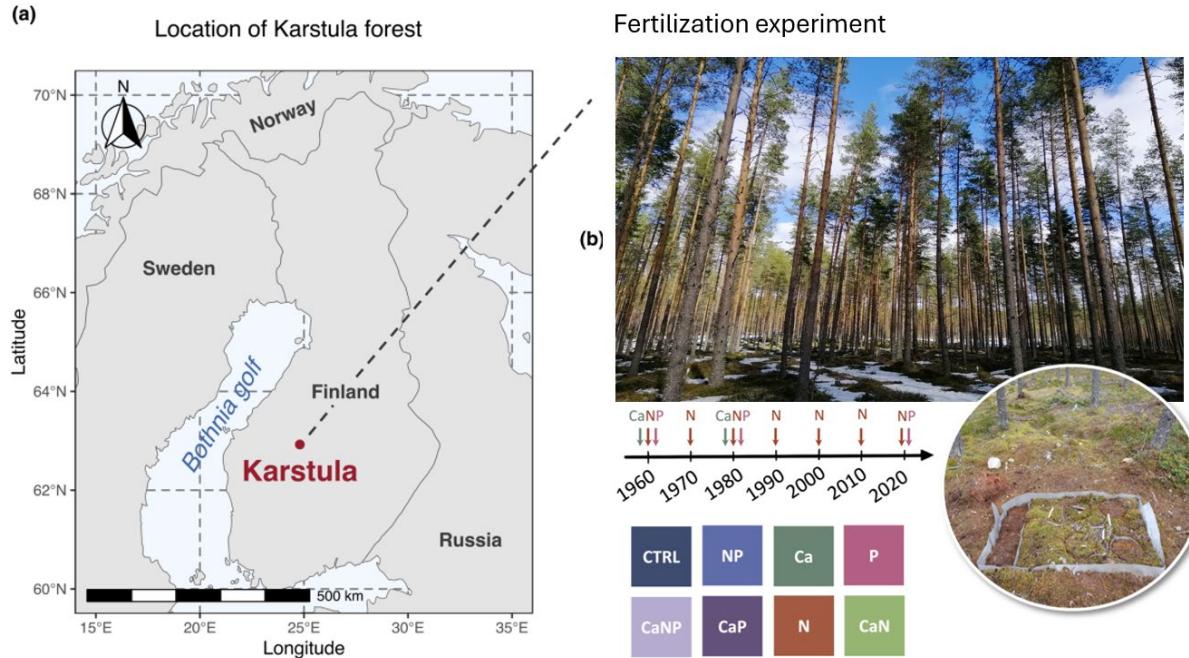


Figure 10.1. a) Karstula forest study site location in Finland; b) schematic illustrating factorial fertilization experiment along with photographs of the forest stand and forest floor plots for soil sampling and measuring soil respiration.

GHG exchange between soils and atmosphere

We evaluated the effects of long-term N addition (once per decade since 1960 until 2020) on soil heterotrophic respiration (R_h) and its dependence on soil temperature and moisture in an originally N limited boreal Scots pine (*Pinus sylvestris*) forest (Fig. 10.1). Tree growth was continuously measured every 5 -10 years since 1960. The R_h , soil temperature and soil moisture were measured biweekly during the vegetative seasons of 2021-2023. Soil organic C stock was measured in 2023. We fitted R_h rates to soil temperature and moisture separately for the control and N fertilization treatment using parametric non-linear regression models and non-parametric models. Both methods agreed on the form of the relations between R_h , temperature and moisture.

Soil organic carbon (SOC) increased under N fertilization, reaching 5.6 kg C m^{-2} in N+ compared to 4.9 kg C m^{-2} in CTR by 2023, which was consistent with increased biomass and litterfall in N+. This indicated enhanced SOC retention alongside aboveground carbon storage due to reduced microbial respiration. However, main findings reveal that N-enriched soils exhibited reduced sensitivity of microbial respiration to soil moisture, which may contribute to enhanced soil carbon sequestration (Fig. 10.2). These soils also show increased temperature sensitivity, particularly at higher temperatures (Fig. 10.2), suggesting potential vulnerabilities to warming. The shifts in litter quality, specifically C and N ratios, modifying soil respiration rates especially under N fertilization and drought conditions (Nair et al., 2024), likely contribute to divergent R_h responses to temperature and moisture (Robinson et al., 2020). This dual response underscores the need for soil C models to incorporate nitrogen effects on microbial responses to moisture and temperature sensitivities for C projections in boreal ecosystems.

Annually, N-fertilized plots respired 11.2% of SOC, compared to 12.6% in controls, suggesting N fertilization promoted SOC retention (Fig. 10.3) and was in line with previous studies (Janssens et al., 2010). Consequently, N fertilization reduced net CO₂ emissions by 262.5 g CO₂ m⁻² year⁻¹, while combined effects on CH₄ and N₂O fluxes and the production energy of N fertilizer contributed a minor CO₂-equivalent increase of 15.8 g CO₂-eq m⁻² year⁻¹. Consistently with biomass and litterfall, soil organic carbon (SOC) increased under N fertilization, reaching 5.6 kg C m⁻² in N+ compared to 4.9 kg C m⁻² in CTR by 2023, indicating enhanced SOC retention alongside aboveground carbon storage due to reduced microbial respiration (Janssens et al., 2010). However, increased soil carbon stocks, by reducing soil respiration with minor negative impact on CH₄, and N₂O, and known increases in tree C sink, may be also slightly impacted by the N impact on shortwave surface albedo (Ollinger et al., 2008).

Thus despite the associated emissions with fertilizers productions which amounted to 9 g CO₂-eq m⁻² year⁻¹ in our study (Osorio-Tejada et al., 2022), the 246.7 CO₂-eq m⁻² year⁻¹ emission reduction related to N fertilization in boreal forest, thus needs to be considered as a key factor contributing to GHG mitigation. More detailed information is available in published preprint paper (Ricky et al., 2024; Tupek et al., 2024).

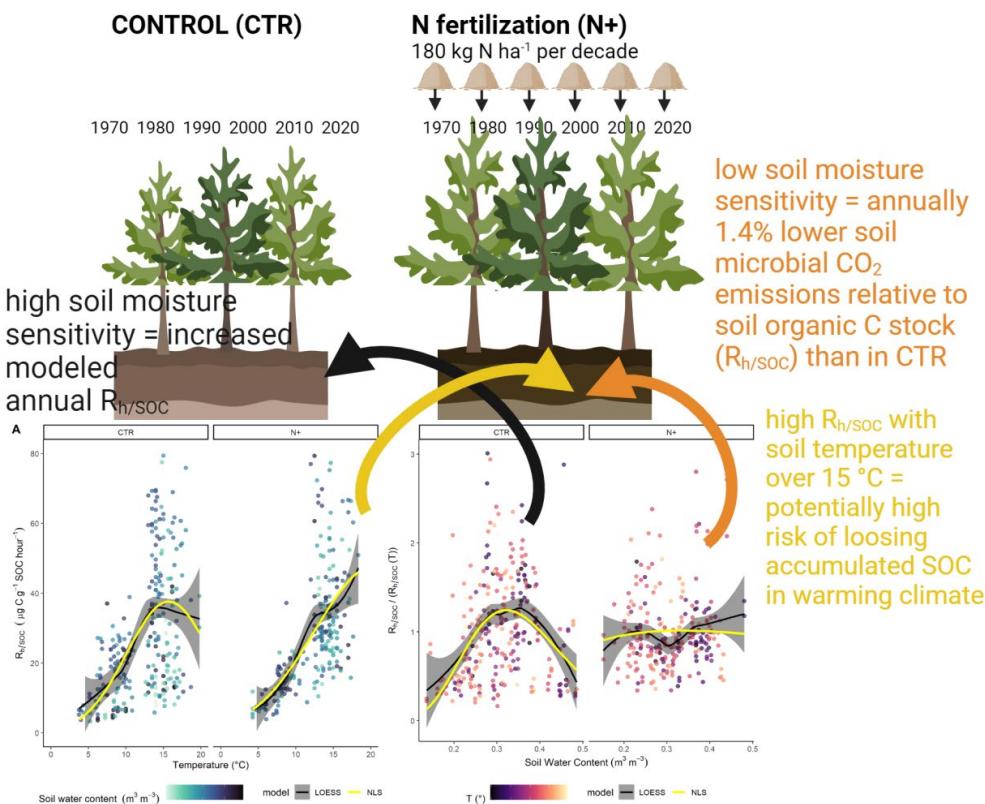


Figure 10.2. Illustration of the study design and results for decomposition kinetics for Karstula fertilisation treatment.

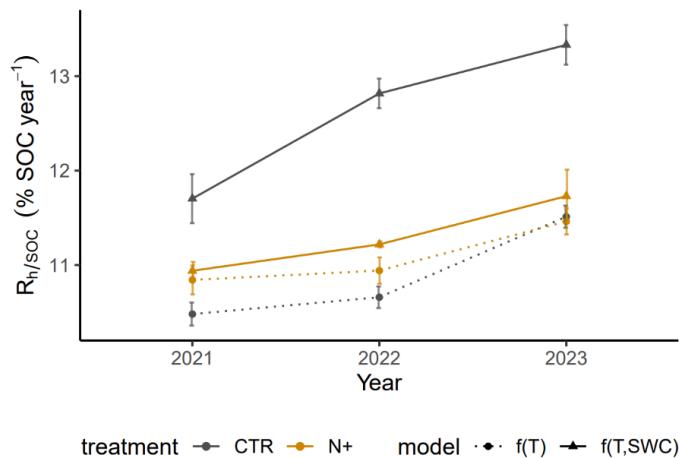


Figure 10.3. The annual sum of heterotrophic respiration per unit SOC ($R_{h/SOC}$; i.e., the annual SOC turnover expressed as % SOC year⁻¹) simulated with nonlinear least square (NLS) functional dependencies between short-term measured $R_{h/SOC}$ and soil temperature $f(T)$ (Eq.1, Tab. 1), or combined soil temperature and moisture functions $f(T, SWC)$.

Soil microbial processes and fertilization

Fertilization impacted soil microbial communities, but its effects differed between bacteria and fungi. For bacteria, fertilization did not change overall biomass but significantly shifted community composition. Nitrogen addition had the most substantial influence, increasing the proportion of gram-positive bacteria relative to gram-negative bacteria. This shift was characterized by a decrease in the relative abundance of Proteobacteria and Acidobacteria, coupled with an increase of Actinobacteria. The more acidic and humid conditions in nitrogen-treated soils contributed to these patterns, while calcium-treated soils led to a more diverse bacterial community with greater evenness.

In contrast to bacteria, fertilization affected both the biomass and composition of fungal communities. Nitrogen addition increased fungal biomass and altered the balance between saprotrophic and ectomycorrhizal fungi. Specifically, the proportion of ectomycorrhizal fungi was lower than that of saprotrophs in NP and CaN plots, proportional in Ca and P plots, and higher in N, unfertilized, CaP, and CaNP plots. Additionally, *Penicillium sp.* showed a 2.3-fold increase in relative abundance in NP plots compared to unfertilized plots. Among the fertilization treatments, calcium had the greatest impact on fungal community composition, followed by vegetation cover, such as lichen and litter. Fertilization also enhanced fungal richness, particularly in CaP and NP plots, driven by the increased richness of saprotrophs in NP plots and ectomycorrhizal fungi in CaP plots.

These shifts in microbial community composition directly influenced soil microbial processes. Nitrogen addition with phosphorus favoured copiotrophic microorganisms like saprotrophic fungi, *Penicillium sp.*, and Actinobacteria, which possess a wide range of hydrolytic enzymes involved in organic matter decomposition. This led to increased biopolymer degradation, resulting in limited soil carbon accumulation. Conversely, the combination of nitrogen, calcium, and phosphorus increased the proportion of lignin-oxidizing ectomycorrhizal fungi and enhanced manganese peroxidase activity. These fungi retain oxidative capacities that enable them to mine nitrogen from recalcitrant organic matter, releasing previously



sequestered carbon into simpler organic molecules. These molecules are then mineralized into CO₂ by soil microorganisms, increasing soil respiration and further limiting long-term carbon storage. Importantly, significant carbon sequestration was observed only in plots fertilized with nitrogen without phosphorus—in CaN (z test, $z = -2.15, p = 0.03$) and N ($z = -2.32, p = 0.02$) treatments.

In boreal forests, the alleviation of nitrogen-limitation stimulates tree growth, leading to increased carbon input into the soil through rhizodeposition and aboveground detritus. Since microorganisms require more phosphorus than trees per unit of carbon for biomass production, microbial activity is more sensitive to phosphorus limitation. As a result, nitrogen addition promotes tree growth and increases carbon input into the soil, but phosphorus limitation on microbial decomposition ultimately leads to long-term carbon sequestration in boreal forest soils. This finding underscores the importance of the entire nutrient balance for long-term carbon storage. More detailed information is available in recently published paper Richy et al. (2024).

Impact of N addition to soil properties

N fertilization has a significant effect on soil processes and plant and microbial communities and their metabolism. We observed higher content of C and N under N fertilization treatment and higher concentration of stable C pool in the soil organic layer. Also, microbial biomass, necromass and activity of extracellular enzymes increased under N addition, but not greenhouse gas production. These changes were mainly observed in organic layer of the soil, but not in mineral layer. Mechanistically, N addition elevated soil C stock via increased litter input and higher transfer of C to soil microorganisms resulting in enhanced formation of fungal biomass and further necromass, which was stabilized in the soil (Fig. 10.4). Though our study supports that employing targeted management strategies to increase microbial necromass in mineral-associated organic matter C could be considered an effective means to increase C stabilization in soil to help mitigate climate change, future studies should consider also potential side-effects of N fertilization, as changes in functioning of tree metabolism, effect on forest resilience and long-term effect on changes in microbial community structure and their activities.

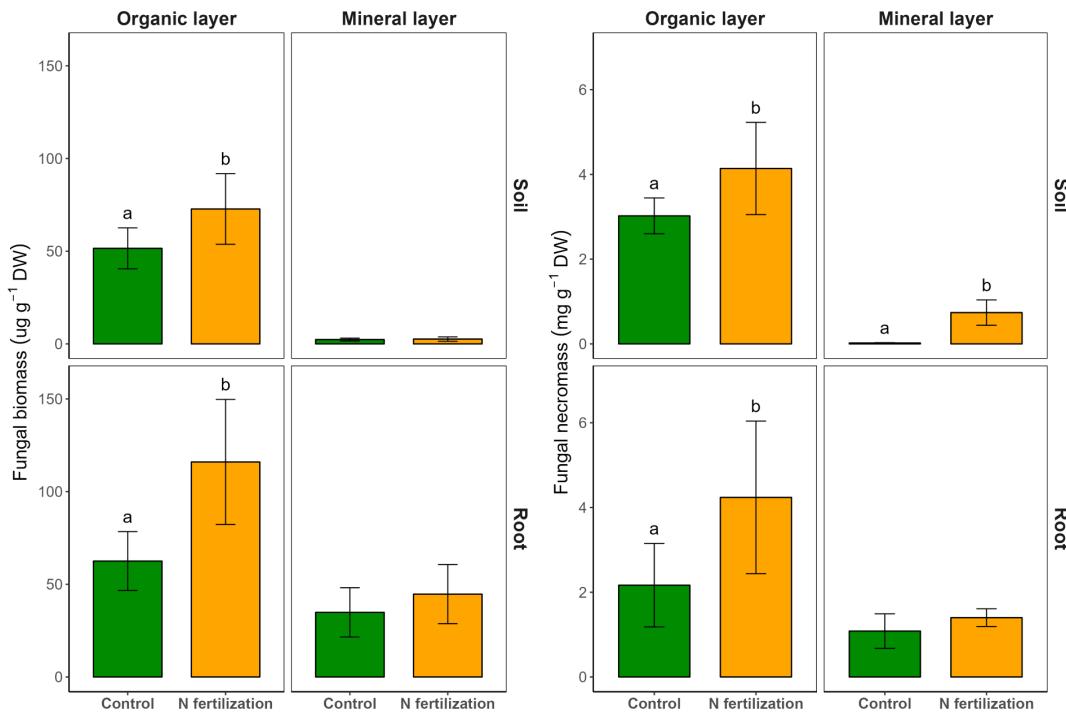


Figure 10.4. Fungal biomass and -necromass in the organic- and mineral soil layers of soil- and root samples Karstula control and nitrogen fertilization experiment.

Other studies also observed increases of SOC after N fertilization (Tang et al., 2023), however, others reported no increase of SOC (Baek et al., 2023) or even negative effect of N fertilization on SOC (Neff et al., 2002). Differences in the response of SOC size to N fertilization may emerge from various doses of N applied, frequency of N application and N status of the ecosystem. Moreover, also differences in plant and microbial communities may drive the effect of SOC accumulation under N fertilization. It was proposed that the effect of N fertilization on C stabilization depends on the N status of the ecosystem, thus on its ability to retain N (Lucander et al., 2021). Low dose of N input can stimulate plant growth (Nohrstedt, 2001), but high N load results likely cause soil acidification (De Vries et al., 2014; Lucas et al., 2011). In line with that, N-saturated ecosystems may leach additional N resulting in eutrophication and acidification of aquatic systems (Lucander et al., 2021). Response of the ecosystem to N addition depends also on site-specific differences, including various plant and microbial community structures and even microclimatic conditions.

Nitrogen fertilization as a climate smart forest management practice

It has been shown earlier that nitrogen fertilization in boreal forests typically increases tree biomass accumulation substantially (Hedwall et al., 2014). But if a site is fertile and nitrogen availability is not limiting tree growth, added nitrogen may not contribute by increasing tree growth, see e.g. Mäkinen et al. (2025). The impact of fertilization to tree growth is limited in time, but typically trees can grow faster for 2-7 years after fertilization and during that time they accumulate more foliage biomass to support their increased growth.

For soils we found that addition to increase tree biomass, nitrogen fertilization increases substantially carbon stock of the organic layer of the Scots pine dominated forests (Ricky et al., 2024), agreeing with Saarsalmi et al. (2007). Nitrogen fertilization seems a promising method as a climate smart forestry practice in boreal region when potential nutrient loading



to waterways is avoided by appropriate management plans. A prerequisite for nitrogen fertilization in forest areas is that there are suitable fertilizers available to be applied with forests and that the price of those fertilizers is reasonable relative to benefits. And moreover, it is important that the climate impact of nitrogen production is less than climate benefits of nitrogen fertilization.

11. Kačerginė, Lithuania

The study site is located in central region of Lithuania in Kačerginė forest ($54^{\circ}55'N$, $23^{\circ}43'E$) of the Dubrava regional unit (Lithuanian State Forest Enterprise). In 2002, a wood ash and nitrogen fertilization experiment was set up for a first-generation Scots pine (*Pinus sylvestris* L.) stand (Wood-En-Man experiment; (Ozolinčius et al., 2007)). The trees were planted in 1964, on a sandy limnoglacial plain overlying old fluvioglacial sands. The soil is classified as a Haplic Arenosol (Baxter, 2007). The stand represents a typical Scots pine forests of *Pinetum vacciniosum* site, which is most common in Lithuania. The ground vegetation layer in the stand is dominated by different species of moss (*Pleurozium schreberi* (Brid.) Mitt., *Dicranum polysetum* Sw., *Dicranum scoparium* Hedw. and *Hylocomium splendens* (Hedw.) Schimp.) and grasses (*Festuca ovina* L. and *Calluna vulgaris* (L.) Hull) (Table 11.1).

Table 11.1. Characteristics of Scots pine (*Pinus sylvestris* L.) stand in Kačerginė forest.

Plot	Age, years	Species composition	Total stem volume, $m^3 ha^{-1}$	Stocking level
Scots pine stand before fertilization treatment (in 2002)	35	90% <i>Pinus sylvestris</i> L. 10% <i>Betula pendula</i> Roth	332	0.8
Scots pine stand within 2002 proceeded treatments (in 2024)	57	100% <i>Pinus sylvestris</i> L.	269	0.9
Clear-cut of Scots pine stand within 2002 proceeded N treatment (in 2024)	5	90% <i>Betula pendula</i> Roth 10% <i>Pinus sylvestris</i> L.	6	1.0

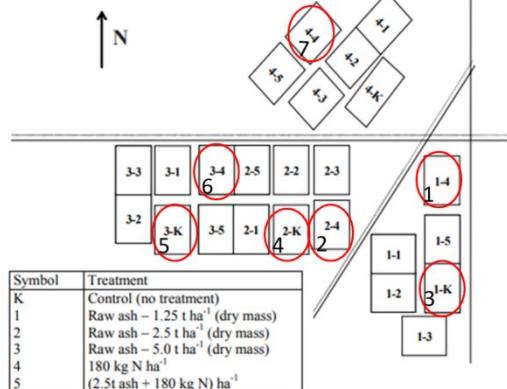
For the evaluation of the remaining nitrogen fertilization impact, out of the existing 23 research plots from 2002 ($15 \times 20 m^2$), 7 were selected in 2022 (Fig. 11.1): (1) 3 plots received $180 kg ha^{-1}$ of N (N fertilizer ammonium nitrate; (N)); (2) 3 plots were not treated and used as the control (C); and (3) in 2018 1 plot was clear-cut (CC).

Role of nitrogen fertilization and clearcutting on carbon fluxes

We evaluated the effect of N fertilization on forest floor and microbial soil respiration in a Scots pine forest in connection to soil chemistry (C, N, and P concentrations) and microbial diversity indices. The study site was fertilized with wood and ash in 2002, and 5 plots were established reflecting the amount of N and ash used. Design of experiment is outlined in Fig. 11.1. In addition, we used trenching technique (cutting tree roots) to separate autotrophic respiration (R_a) from total forest floor respiration (R_{tot}) and to determine forest soil microbial respiration (R_h). The CO_2 fluxes, soil temperature and moisture were measured biweekly during the vegetative season in 2023 and 2024.



Wood ash and nitrogen applied in 25-27 June 2002



○ Plots with trenching's installed in 2022;
Measurements started in April 2022/2023.
7 Clear-cut area (clear-cutting in 2018)

Figure 11.1. Photograph of the tree stand and forest floor with plots for measuring respiration after trenching (left), and an outline of spatial location of plots with varying doses of ash and N fertilizer (right).

Preliminary results indicate minor respiration differences between N fertilization and the control plots (Fig. 11.2). No significant difference was observed in mean R_h values between control and N fertilization (paired t-test, $n = 288$, $p = 0.2$), but R_h in the clearcut was significantly lower than R_h values of the both control and N fertilized plots (paired t-test, n-clearcut = 119, n-control and n-N-fertilized = 288, both p values < 0.0001). Lower observed R_h of clearcut area compared to forest stand area was presumably due to lower remaining soil C stock in the clearcut area 5 years after harvesting, as well as affected by site preparation (ploughing) and slow initial tree stand growth of the regrowing pine seedlings.

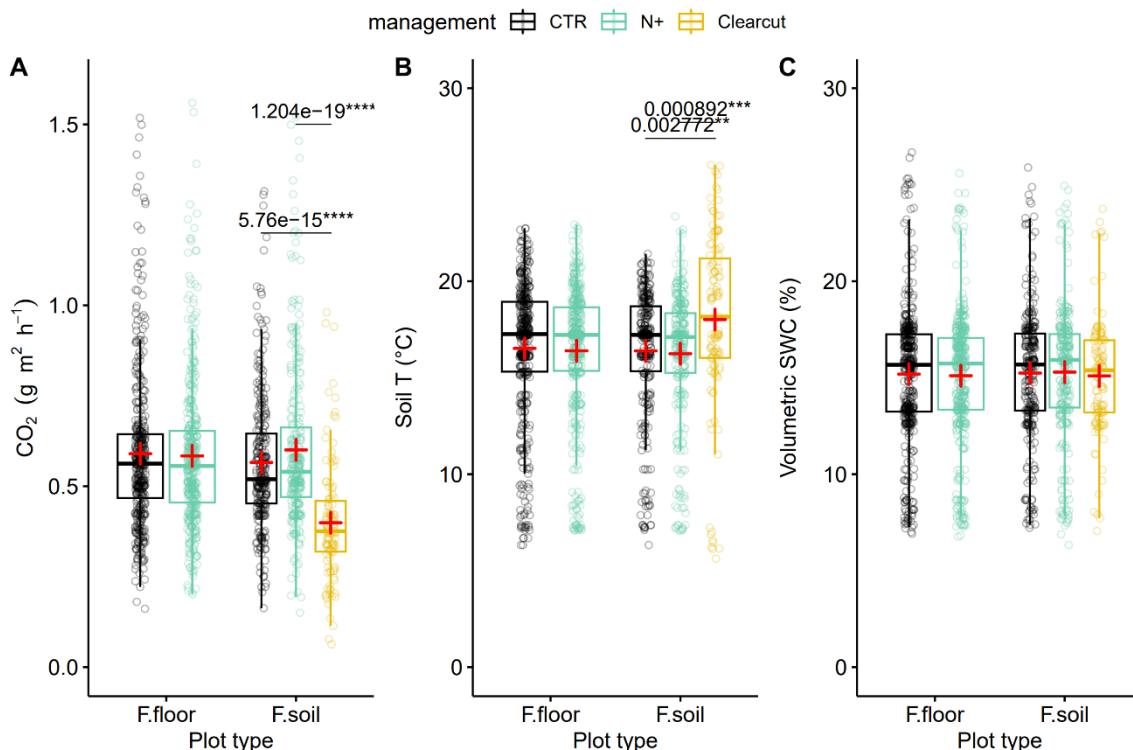


Figure 11.2. Forest floor respiration and forest soil microbial respiration (A), soil temperature (B) and soil volumetric water content (C) after N fertilization (N+) in comparison to control (CTR) at the Scots pine forest site Kačerginé.

Effect of microclimate on carbon fluxes

The site is relatively dry. During the growing seasons of 2023 and 2024, the median observed volumetric soil moisture was 0.16 and the median soil temperature 17.2 °C. Preliminary analysis indicates a strong relationship between observed R_h and soil moisture at the control and N fertilized plots in 2024, indicating that soil moisture and not soil temperature was limiting respiration during the growing season, but no such relationship could be found in 2023. In 2023, both temperature and moisture could not explain the observed CO₂ fluxes very well, which needs to be further investigated. For the clearcut plots, less data points were available, but in 2024 both soil temperature and soil moisture positively correlated with observed CO₂ fluxes. Like the control and N fertilized plots, no clear relationship could be seen in 2023. The effects of microclimate on the observed CO₂ fluxes (R_{tot} and R_h) in light of different management will be further analysed in the coming months.

Soil microbial diversity and N fertilisation

Preliminary results show that while there were some differences in the community composition of fungi, bacteria, mycorrhizal fungi and saprotrophic fungi between fertilized and unfertilized plots and between trenched and non-trenched plots, these differences were significant only for saprotrophic fungi (Fig. 11.3). The soil sampled inside and outside of the trenches hosted distinct communities of saprotrophs, suggesting that trenching caused differences in substrate availability. However, neither the nitrogen fertilization treatment nor the trenching had any significant effect on microbial diversity.

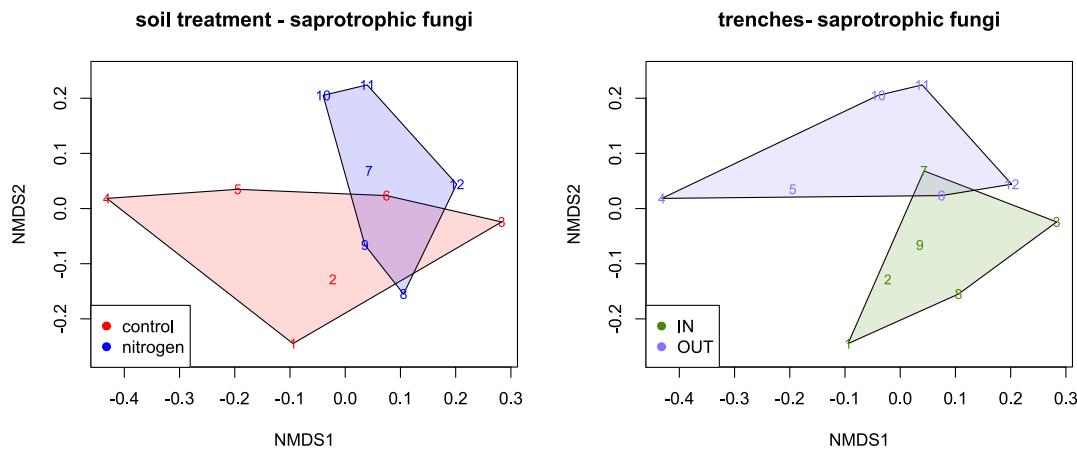


Figure 11.3. Non-metric multidimensional scaling (NMDS) plots of saprotrophic fungi communities. The plot visualizes dissimilarities in community composition between samples based on a Bray-Curtis dissimilarity matrix: points (i.e. the microbial community found in each soil sample) further apart are less similar.

The data collected also indicated that the relationship between microbial diversity and CO₂ fluxes differs significantly between fungi and bacteria, with bacterial diversity showing a positive correlation with the GHG fluxes measured in the field, and fungal diversity showing no significant correlation (Fig. 11.4).

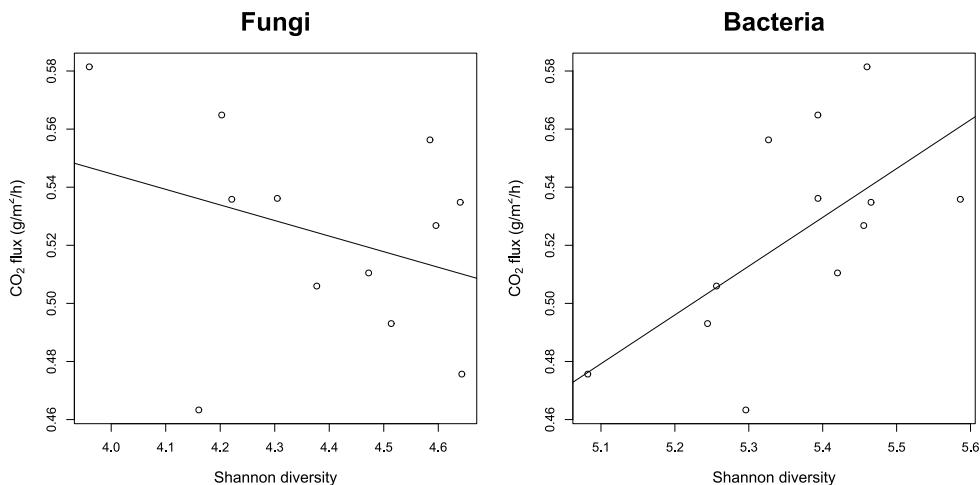


Figure 11.4. Correlation between CO₂ fluxes (g/m²/h) and Shannon diversity index for Fungi (left, non-significant) and Bacteria (right, significant correlation, (p = 0.03, cor = 0.63).

Conclusions

The nitrogen fertilization did not impact significantly microbial respiration (Rh), while clearcutting resulted in significantly lower Rh, likely due to reduced soil carbon stocks. Microclimatic conditions, particularly soil moisture, influenced respiration rates in 2023 but not in 2024, suggesting interannual variability in environmental controls. Soil microbial diversity was generally unaffected by nitrogen fertilization or trenching, with only saprotrophic fungi showing significant differences between treatments. Bacterial diversity correlated



positively with greenhouse gas fluxes, whereas fungal diversity showed no clear relationship. These findings highlight the complexity of nitrogen fertilization effects on carbon fluxes and microbial dynamics, emphasizing the need for further investigation into long-term soil responses.

12. Tacuarembó, Uruguay

Impact of afforestation with *Eucalyptus species* on the soil properties of sandy soils in northern Uruguay

Over the past few decades, Uruguay has undergone a significant transformation with the adoption of an intensive forestation policy. This policy has resulted in the afforestation of more than 1 million hectares of land previously used for grazing purposes, mainly consisting of pastures (Cartografía Nacional Forestal 2021). This practice has the potential to enhance aboveground carbon sequestration (Hirigoyen et al., 2021; Silveira et al., 2020), although further research is required to elucidate the effects of *Eucalyptus* plantations on soil properties in comparison to grassland-dominated areas.

Within the HoliSoils project, two experimental areas were established in the Tacuarembó region of northern Uruguay in August 2022. The experiments aim to assess the impact of *Eucalyptus* afforestation in sandy soil in comparison to native pasture on soil carbon sequestration and respiration processes (area 1) as well as soil microbial activity and health indicators (area 2). Furthermore, the reversibility of changes and delayed effects induced by the afforestation were also assessed in a regenerated pasture at the area 2. Data collection continued until December 2024, thereby facilitating the analysis of both short- and long-term changes induced by afforestation.

Experimental area and design used to analyze changes in soil organic carbon and respiration

The experimental area 1, designated as 'Rincon de Batovi', comprises two distinct vegetation types: native pasture and a 30-year-old plantation of *Eucalyptus grandis* and *Eucalyptus dunnii* (1400 trees/ha), planted in 2017 as a third rotation. It is important to note that the plantation was previously used as pastureland. The design consists of three levels, repeated four times: native pasture (CN), forestation inside the tree rows (Fin), and forestation between the tree rows (Fbt).

Soil respiration has been measured biweekly since January 2023 using a LI-COR LI-870 CO₂/H₂O analyzer with a Smart Chamber. The instrument records CO₂ concentrations to calculate the soil total respiration (Rs). To obtain the partitioning of Rs into its heterotrophic (Rh) and autotrophic (Ra) components, a trenching experiment allowing for root exclusion was replicated four times at each level. Concurrently, soil temperature and moisture are monitored to evaluate their influence on CO₂ fluxes. Furthermore, soil samples (0–10 cm depth) were collected at six-month intervals, starting in March 2023. These samples were analyzed for various physical, chemical, and biological soil properties. Soil organic carbon (SOC) was determined using infrared CO₂ emission measurement after combustion, adjusted to the Wright and Bailey method (Wright and Bailey, 2001). Exchangeable calcium (Ca), sodium (Na), magnesium (Mg), and potassium (K) forms were extracted using ammonium acetate at pH 7, with K and Na measured by atomic emission and Ca and Mg determined by atomic absorption or emission spectroscopy using ICP-OES (Jackson, 1964). Available phosphorus (P) was quantified using the Bray-1 method (Bray and Kurtz, 1945), involving a 1:10 soil-to-extractant ratio and subsequent determination of the reduced molybdatephosphate complex by absorbance at 882 nm. Soil pH was measured using a 1:2.5 soil-to-water ratio and agitation (Beretta et al., 2014). Bulk density was determined by weighing the dry soil taken from a known volume cylinder according to Blake et al. (1986). Microbial activity was also assessed through in vitro respiration assays and analysis of the labile carbon fraction.



Experimental area and design used to analyze changes in soil microbial activity and health indicators

The experimental area 2 is comprised of three sites, designated as 'Rincón de Batoví', 'Los Higuerones', and 'La Abuelita'. The sites are characterized by a variety of land-use stages, including native pasture, *Eucalyptus* afforestation of varying ages, and regenerating pastures post-afforestation. Soil samples (0–10 and 10–30 cm depth) were collected in the spring and autumn of 2023 and 2024 and analysed for labile carbon, in vitro respiration, and citrate-extractable protein levels according to Moebius-Clune et al. (2016). The microbial community was assessed using a combination of techniques, including Phospholipid Fatty Acid (PLFA) extraction, and molecular tools such as 16S rRNA gene sequencing for bacterial and archaeal communities and ITS (Internal Transcribed Spacer) sequencing for fungal communities. Furthermore, soil organic carbon (SOC) content was measured to monitor changes in carbon storage. Different soil analyses were also carried out for several physical and chemical properties (i.e., pH, Ca, Na, Mg, K, and P). Additionally, the enzymatic activities associated with the carbon cycle have yet to be determined, which will provide further insights into the functional shifts in soil processes resulting from afforestation.

Impact of afforestation-associated changes on soil organic carbon and respiration

Preliminary results from the first soil measurement indicate a slight decrease in soil carbon content within the surface soils of the afforestation in comparison to the native pasture. The reduction in soil carbon content is particularly noticeable in areas located directly beneath the trees (i.e., inside the tree rows), whereas soils located between the tree rows exhibit comparatively minor alterations (Table 12.1). Total soil respiration rates have consistently been lower within the *Eucalyptus* plantation. These variations appear to be influenced by differences in root density and microclimatic conditions.

Although SOC stocks show distinct differences in spatial variation within the afforestation sites –such as between areas inside tree rows and those between rows where decomposing residues from previous plantations are present– these patterns are not consistently observed across all locations. This suggests that additional factors may also be influencing SOC dynamics. It is noteworthy that in situ respiration rates were not significantly affected by spatial location within the afforestation sites (i.e., inside versus between tree rows). However, in vitro respiration measurements revealed more pronounced differences between these locations. This discrepancy suggests that environmental conditions measured in situ may mask underlying biological differences that become apparent under controlled laboratory conditions.



Table 12.1. Soil properties measured at the 'Rincón de Batovi' site to a depth of 10 cm across different plot modalities in spring 2023. For native pasture, regenerating pasture, *Eucalyptus* plantation and separately for *Eucalyptus* between tree and inside tree rows. Values indicate means, while standard deviations are shown in brackets.

	Organic C	C Stock	Labil C	N	Protein Index	P av (Bray I)	pH	Ca
	%	MgC/ha	ppm	%	mg/g dry soil	µgP/g		meq/10 0 g
Native Pasture	1.51 (0.32)	21.2 (4.2)	498 (142)	0.11 (0.03)	5.61 (1.94)	5.5 (3.1)	5.2 (0.3)	1.1 (0.2)
Regenerating Pasture	1.18 (0.29)	16.7 (4.1)	450 (146)	0.08 (0.03)	4.72 (1.54)	4.1 (1.8)	5.6 (0.3)	1.6 (0.4)
Eucalyptus plantation	1.17 (0.19)	17.8 (2.9)	439 (109)	0.06 (0.03)	6.17 (2.58)	5.1 (1.4)	5.3 (0.3)	2.6 (0.5)
E. plantation between rows	1.39 (0.36)	20.9 (5.3)	436 (176)	0.06 (0.03)	9.5 (4.40)	8.3 (5.6)	5.5 (0.5)	4.0 (1.8)
E. plantation inside rows	0.97 (0.23)	15.1 (3.6)	268 (159)	0.04 (0.03)	5.07 (1.85)	5.4 (2.6)	5.1 (0.2)	1.2 (0.3)

Impact of afforestation-associated changes on soil properties

Afforestation has resulted in notable changes on soil properties, particularly regarding nutrient cycling and soil composition. Important changes were observed in total nitrogen content, available phosphorus (P-Bray), labile carbon, citrate-extractable protein, and calcium content (Table 12.1). The soils inside the tree rows exhibit distinct patterns of nutrient availability and microbial activity when compared to soils between the tree rows, reflecting the influence of tree roots on the nutrient cycling. The accumulation of organic matter and root exudates beneath the trees has resulted in alterations to microbial processes, while the decomposition of decaying tree stumps from the previous rotation, which were left in place between the tree rows, has likely contributed to the observed differences in soil nutrient dynamics.

Impact of previous afforestation on soil properties of regenerating pasture

Preliminary results on the long-term impact of *Eucalyptus* afforestation on soil properties suggest that the effects on topsoil are still present, even after afforested areas have been converted back to native pasture. Total bacterial populations in the soil were reduced by approximately 50% in comparison to the levels observed in the native pasture. This reduction in microbial activity was sustained even after 15 years of pasture regeneration, with bacterial counts remaining below the levels recorded prior to afforestation. This suggests that the legacy of afforestation may have long-lasting effects on soil microbial communities and soil health.

Furthermore, a notable increase in soil pH has been observed in the *Eucalyptus* afforestation, which correlates with the release of calcium cations from decomposing *Eucalyptus* stems and roots. The release of calcium has served as a natural buffer, resulting in an elevated soil pH in afforested plots relative to native pastures. This pH increase may have implications for nutrient availability and microbial community dynamics, particularly in terms of phosphorus solubility and microbial processes related to nutrient cycling.



Main findings and conclusions

The expansion of fast-growing tree woody species into grasslands is a global phenomenon, particularly evident in Uruguay with the extensive *Eucalyptus* afforestation. For tree biomass quantity it is known that well growing *Eucalyptus* plantations have stem volumes up to 400 m³ per ha at age of 16 years, which makes ca. 150 Mg C per ha as a carbon stock. Average carbon stock of woody biomass in these systems can vary around 50-80 Mg C per ha. While tree biomass carbon stocks are relatively easy to quantify, less is known about changes in soil properties due to afforestation.

Afforestation of grasslands could exert a detrimental effect on ecosystem services, including soil organic carbon storage, pH, bulk density, nutrient availability, and microbial community structure, due to changes in the aboveground plant biomass as well as the quantity, quality, and distribution of C inputs. Also, management practices that include use of pesticides may alter soil properties and soil functioning. To assess the potential impact of this land change use, it is necessary to improve current understanding of the effects of intensively managed *Eucalyptus* plantations of former grasslands on soil properties.

Native grassland afforestation caused a slight decrease in topsoil carbon sequestration. It was estimated that there was also a decrease in soil respiration rates after *Eucalyptus* plantation, which may suggest that this land use change can contribute to reducing soil greenhouse gas (GHG) emissions.

The present study also facilitated an assessment of the alterations in different soil properties during the re-conversion process from plantation to grassland. The results suggest that the *Eucalyptus* afforestation may have long-lasting effects on soil microbial communities and soil health in areas designated for pasture regeneration, suggesting that the recovery of original soil properties is a relatively slow process following this change in land use.

Our results may have practical application in further short-rotation afforestation and re-conversion strategies for Uruguayan plantations in sandy soils.

Preliminary results obtained from this study provide valuable insights into the effects of *Eucalyptus* afforestation and re-conversion to grassland on soil properties. However, further comprehensive analysis is still needed to achieve a complete understanding of the broader implications of these practices, particularly in relation to their long-term effects on soil carbon dynamics, nutrient availability, pH adjustments, and microbial community recovery.



13. Summary findings

In this Deliverable, a comparison across different test sites was made of current (business-as-usual, BAU) forest management practices that have been modified to respond current knowledge requirements that relate to climate-smart forestry (CSF) practices. Specifically, we examined the effects of implementing the proposed and tested sustainable CSF practices on various soil factors. The results are summarized in Table 13.1.

In summary, the key findings are as follows:

- **Peatland forest hydrology management:**
 - **Harvesting methods:** Avoiding clear-cutting through selection harvesting, which promotes the raising of the water table level, reduces soil CO₂ and CH₄ emissions in the short term after harvesting in our study site located in a boreal drained peatland forest in Finland. Harvesting can also increase soil N₂O emissions during the early stages of stand recovery, but spatial variability in nutrient availability appears to play a greater role in their control. In addition, soil fungal and bacteria biomass decreased, community composition and spatial variability are significantly altered by harvesting.
- **Tree stand management:**
 - **Mixed species:** Dobroc study site located in a temperate forest in Slovakia, which was affected by a spruce bark beetle outbreak, shows that in comparison to mixed stands where no harvesting took place, post-clearcut monoculture Norway spruce stands exhibit a significant increase in soil CO₂ fluxes and minimal and transient changes in soil CH₄ fluxes. In addition, at two sites representing the higher and lower ends of a precipitation gradient in Bavaria, soil respiration in mixed stands was significantly higher than in pure spruce stands, despite stand level tree growth of the spruce stands being higher in both cases. Further comparison suggest that soil respiration here may be linked more strongly to annual tree growth than to the tree species.
 - **Thinning:** An increase in thinning intensity exerts a positive effect on understory vegetation in the St. Mitre study site located in a Mediterranean Aleppo pine forest in France, leading to increases in soil organic carbon and nutrient contents, with cascading positive effects on soil fauna. In addition, the study site located in a sub-Mediterranean Black pine forest in Spain demonstrates that thinning as well as the retention of harvest residues on the forest floor exert a minimal influence on soil carbon stocks. Besides, the study sites in Scots pine forest in the Netherlands show that clearcuts, and to a lesser extent shelterwood systems, amplify the seasonal soil temperature changes and increase levels of soil moisture year-round. Whereas the soil respiration exhibited no significant response to the different felling intensities.
- **Afforestation:**
 - The afforestation of native grassland with Eucalyptus in the study site in Uruguay causes a modest decline in soil CO₂ fluxes and topsoil carbon sequestration. Furthermore, *Eucalyptus* afforestation may have long-lasting effects on soil microbial communities and soil health in areas designated for pasture regeneration, suggesting that the recovery of original soil properties is a relatively slow process following this change in land use.
- **Disturbance management:**
 - **Drought:** The Dobroc study site located in a temperate forest in Slovakia reveals that mixed forest stands may be more resistant and resilient to drought events



than Norway spruce monocultures, and as such may be able to modulate the effects of drought on the soil CO₂ fluxes. However, very severe experimental droughts caused a long-lasting and significant reduction in soil respiration in both, pure and mixed root zones of a mature spruce-beech stand at Kranzberg, Germany.

- **Prescribed fire:** Prescribed fire in a sub-Mediterranean Black pine forest site in Spain induces loss of C from topsoil organic layers, but the increase in the stability of organic matter in the mineral soil may partially compensate C loss.
- **Nutrient management:**
 - **N fertilization:** Our long-term N fertilization experiments in Scots pine forest sites in Finland and Lithuania reveal that N addition alters soil properties and microbial communities, while simultaneously increasing soil carbon storage. Furthermore, the addition of N suppresses soil respiration in both sites. Focusing on the study site in Finland, N fertilization also led to a shift in microbial composition, favoring gram-positive bacteria and saprotrophic fungi, while concurrently decreasing microbial respiration sensitivity to soil moisture. Additionally, results show that the application of N results in the accumulation of soil organic carbon, with the highest levels being observed in treatments that included N alone. This phenomenon can be attributed to the restriction of microbial decomposition caused by P limitation, thereby supporting long-term carbon storage.

The results of this study were mostly consistent with earlier findings reported in the review article by Mäkipää et al. (2023) and European wide guidance for climate-smart forestry can be developed. However, we showed that the effects of stand management, tree species mixtures and afforestation on soil C stock and GHG fluxes varied across test sites, where also soil properties and stand characteristics also varied. Consequently, the development of climate-smart forest management guidance will vary according to region and site-specific conditions, preventing the provision of general European management recommendations.

Table 13.1. Effects of Climate Smart Forestry (CSF) practices on soil factors at each test site. The effects are indicated using specific symbols: '+' denotes increase, '-' denotes decrease, '=' denotes no effect, and '*' denotes no data. Blue and red symbol denote positive and negative effects, respectively. Green-shaded cells highlight results that align with the findings of earlier review paper on effects of forest management on soil carbon stocks and GHG fluxes (Mäkipää et al., 2023), whereas violet-shaded cells indicate contradictory.

Management practice		Test site		Soil factors							
Main	Site specific	Code	Country	CO ₂	CH ₄	N ₂ O	C stock	Litter	Root biomass	Microbiology	Fauna
Peatland forest hydrology management	Harvesting methods	A	FI	-	+	+	*	*	*	-	*
	Mixed species	D	SK	=	=	*	+/-	*	+	+	*
	Mixed species	E	DE	+	*	*	=	*	*	=	*
	Thinning	F	NL	=	*	*	*	*	*	*	*
	Thinning	G	FR	*	*	*	+	*	*	=	+
	Thinning	H	ES	*	*	*	=	=	*	*	*
	Afforestation	L	UY	-	*	*	-	*	*	-	*
Disturbance management	Drought	E	DE	-	*	*	+/-	-	-	-	*
	Drought	D	SK	-	-	*	-	*	*	*	*
	Prescribed fire	H	ES	*	*	*	-	-	*	*	*
Nutrient management	N fertilization	J	FI	-	+	+	+	+	*	+	*
	N fertilization	K	LT	=	*	*	*	*	*	+	*

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