Organic soil earbon CO<sub>2</sub> balance in drained and undrained

# hemiboreal forests

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Abstract. Drainage of organic soils is associated with increasing soil carbon dioxide (CO2) efflux, which is typically linked to losses in soil C stock. In previous studies, drained organic forest soils have been reported as both CO2 sinks and sources depending on, e.g., soil nutrient and moisture regime. However, most of the earlier research was done in the boreal regionzone, and both the magnitude of CO2 efflux and the impact of soil moisture regime on soil C stock are likely to vary across different climatic conditions and ecosystems, depending further on vegetation. A two-year study was conducted in hemiboreal forest stands with nutrient-rich organic soil (including current and former peatlands) and a range of dominant tree species (black alder, birch, Norway spruce, Scots pine) in the Baltic states (Estonia, Latvia, Lithuania). In this study, we analyzed the  $C_{02}$  balance of organic soil in drained (19) and undrained (7) sites. To assess the  $C\underline{O_2}$  balance, soil respiration was measured along with evaluation of  $C\underline{O_2}$  influx into the soil through aboveground and belowground litter. To characterize the sites and factors influencing the CO2 fluxes, we analysed soil temperature, soil water\_table level, and physical and chemical parameters of soil and soil water. On average, no changes in soil C tocks<u>close-to-neutral soil CO<sub>2</sub> balance</u> (±0.45±0.50 t <u>CO2-</u>C ha<sup>-1</sup> year<sup>-1</sup>) <del>were-<u>was</u> observed in drained sites dominated</del> by black alder, birch, or Norway spruce, while drained Scots pine sites showed soil CO2 removals with a mean rate of ±2.77±0.36 t CO2-C ha-1 year-1. In undrained birch- and spruce-dominated sites, soil functioned as mean CO2 sink at ±1.33±0.72 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>, while the undrained black alder stands showed an uncertain CO<sub>2</sub> balance of ±1.12±2.47 t-CO2-C har year. Variationbility in the soil CO2 balances was influencedrelated to by soil macronutrient concentrations and pH: -forest types characterized characteriszed by lower nutrient availability exhibited showed greater soil CO2 sink. The variability in C balances were influenced by the nutrient-rich soil exhibiting a wide range of nutrient conditions and organic matter quality. Thus, indicating that soil macronutrient concentrations and pH can determine whether the soil functions as a C source or sink.

35 1 Introduction

36 Soil in peatlands, characterised by its high content of partially decomposed plant matter, is a major terrestrial organic carbon

(C) stock, estimated to range from 504 to 3000 Gt C (Scharlemann et al., 2014). Although northern peatlands make up only

2-4% of the global land area, they contain a substantial amount of soil C, estimates ranging from around 500 to 1,055 Gt C

(Nichols and Peteet, 2019; Yu, 2012), highlighting the significance of these lands in the global C budget. About 28% of the

pristine (undrained) peatlands globally are inherently covered by forest (Zoltai and Martikainen, 1996), and those peatland

forests in the boreal biome can accumulate C into the soil at similar rates to non-forested peatlands; the <u>higher\_faster</u> decomposition rates observed in peatland forests (Beaulne et al., 2021) can be compensated by higher litter inputs (Straková

et al., 2010). To enhance tree growth, peatland drainage for forestry has been common in the past. Drainage facilitates oxygen

access to deeper peat layers, thereby promoting tree root survival and function, but also the mineralization of organic matter

and the release of C into the atmosphere in the form of CO<sub>2</sub>. Therefore, the conservation of organic soil C stocks in managed

current and former peatlands has attracted attention in the context of climate change.

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The approximately 13 million ha of forestry-drained organic soils in Europe have been estimated to emit 17 million tons of CO<sub>2</sub> per year (European Environment Agency, 2023). Despite the temperate region-zone being characterized characterized by wide climatic gradients, currently, only a single default emission factor (EF) by the Intergovernmental Panel on Climate Change (IPCC) is available for the entire temperate elimate regionzone, to which the Baltic states correspond according to the IPCC (Hiraishi et al., 2013). The EF was developed using study results from 8 drained sites (Hiraishi et al., 2013), which were published in 5 articles (Von Arnold et al., 2005; Glenn et al., 1993; Minkkinen et al., 2007; Yamulki et al., 2013). These studies employed different CO<sub>2</sub> estimation methods, complicating the comparability of the aggregated results (Jauhiainen, 2019; Jauhiainen et al., 2023). None of the sites are in the Baltic states. Given that the soil emissions in the boreal zone are smaller than in the temperate zone (Jauhiainen et al., 2023), and Baltic states are situated in the hemiboreal vegetation zone (Ahti et al., 1968) - in transition between the temperate and boreal zones - the IPCC's default temperate EF may not be suitable for application in this region. The same issue arises on a broader geographic scale, where the use of unharmonized country-specific and default EFs creates challenges in comparing the estimated emissions both within and across different countries and climate regionszones. Discrepancies in CO2 emissions between regions can be expected due not only to climate gradient (Ojanen et al., 2010) but also to site productivity (Janssens et al., 2001). While higher ecosystem productivity is associated with increased soil respiration rates (Janssens et al., 2001) it also facilitates higher CO2 influx through litter (Krasnova et al., 2019). Therefore, EFs are most appropriately used when applied to areas with similar environmental conditions, rather than being limited by national boundaries or applied too broadly across diverse geographic regions.

In the Baltic states (Estonia, Latvia, and Lithuania)<sub>2</sub> organic soils (Eggleston et al., 2006) are current or former peatlands where a peat layer is still identifiable, or, due to high decomposition, no longer meet<sub>\(\frac{1}{2}\)</sub> the typical characteristics of peat. However, these soils by definition contain at least a 20 cm thick layer rich in organic matter (organic layer). In the region, the total area of drained organic forest soils is reported to be 0.8 million ha, with estimated emissions of 1.8 million tons of CO<sub>2</sub> per year (Konstantinavičiūtė et al., 2023; Ministry of the Environment of Republic of Estonia, 2021; Skrebele et al., 2023). Thus, countries with a relatively small total land area yet a substantial proportion of organic soil can have a considerable role in organic soil management. This underscores the importance of acquiring precise estimates of the CO<sub>2</sub> emissions from organic forest soils in this region. However, despite the Baltic States being located next to each other and thus expectedly showing similar soil CO<sub>2</sub> emissions from comparable sites and land uses, the emission estimation approach is currently not harmonized as the countries use different EF<sub>\(\frac{5}{2}\)</sub> to estimate and report emissions (Konstantinavičiūtė et al., 2023; Ministry of the Environment of Republic of Estonia, 2021; Skrebele et al., 2023). According to National Greenhouse Gas Inventories submissions of 2023, the CO<sub>2</sub> emissions of drained organic forest soil in Estonia and Lithuania were estimated using the default EF provided by IPCC for the temperate region zone (Calvo Buendia et al., 2019), while Latvia applied a country-specific EF. Due to similarities in biogeography, climate, and land-use practices, common EFs based on regionally representative, congregated data could be a better option.

A recent synthesis evaluated whether default IPCC EFs can be improved by compiling results from the most recent studies (Jauhiainen et al., 2023). Still, only modest; and insignificant changes judging by confidence intervals (CI) of IPCC EFs could be introduced for the temperate climate region-zone as a whole, due to limited data (Jauhiainen et al., 2023). Although the general driving factors of CO<sub>2</sub> emissions are known, the number and geographical representation of studies on drained soils, particularly in the temperate zone, remain too limited for stratification of EFs based on local conditions. It is widely known that soil temperature is the primary factor influencing gross CO<sub>2</sub> emissions. However, while temperature can explain variations in emissions, it does not fully account for their magnitude. The extent of CO<sub>2</sub> emissions is affected by soil properties (Basiliko et al., 2007), incorporating complex interactions between a soil's physical, chemical, and biological characteristics, including the impact of the litter quality (Berger et al., 2010). The recent synthesis confirmed that key factors influencing the magnitude of CO<sub>2</sub> emissions include soil C concentration, carbon-to-nitrogen (C:N) ratio, and bulk density, as well as stand type

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(Jauhiainen et al., 2023). Yet, the list likely remains quite incomplete, as individual studies in different site types using various methods can provide unharmonized results making it difficult to identify the relationships influencing CO<sub>2</sub> emissions. Varying and often insufficient reporting of study site conditions in previous studies (Jauhiainen, 2019) further hampers the ability to compile the results for effective synthesis and meta-analysis (Jauhiainen et al., 2023). This limitation may have hindered the identification of emission-impacting factors and the ability to quantify their relationships, underscoring the need for more localized studies to address these gaps, particularly in the hemiboreal vegetation zone which overlaps with the Cool Temperate Moist climate zone (Calvo Buendia et al., 2019) - a subregion of temperate zone as defined by the IPCCthe Cool Temperate Moist climate region (Calvo Buendia et al., 2019), which overlaps with the hemiboreal vegetation zone.

In the few studies on drained and undrained soil C orand CO2 balance conducted in the Baltic states, using both chamber and soil inventory methods, findings have been inconsistent (Butlers et al., 2022; Lazdiņš et al., 2024; Bārdule et al., 2022). Drained organic soils have been identified as both C sinks and sources, with no decisive conclusions reached regarding the factors driving such variation. Soil C loss of nutrient-rich organic soil has been identified using the soil inventory method (Lazdiņš et al., 2024) but not confirmed by the chamber method (Butlers et al., 2022; Bārdule et al., 2022) that, unlike the inventory method, targets the current situation (Jauhiainen et al. 2019). Given that nutrient-rich organic forest soils can make up to 72 % of total organic forest soils (Līcīte et al., 2019) and are associated with a higher risk of soil C loss, there is a need to enhance our understanding of the underlying drivers to improve the accuracy of CO2 balance estimates in Baltic states. Therefore, studies using harmonized harmonised data collection methods are necessary, covering a variety of nutrient-rich forest soils, recording or monitoring essential environmental conditions, and accounting for both soil CO2 efflux and influx from various litter sources.

This study aimed to quantify the soil CO<sub>2</sub> balance in hemiboreal forests with nutrient-rich organic soil and different dominant tree species. The research was carried out in 26 forest stands with organic soil in Estonia (EE), Latvia (LV), and Lithuania (LT), including both undrained and drained sites, over two years. We analysed soil CO<sub>2</sub> emissions as well as CO<sub>2</sub> inputs influx by tree fine roots, ground vegetation (below- and aboveground) and fine foliar litter. We examined factors contributing to soil CO<sub>2</sub> balance by directly or indirectly influencing soil processes, such as site type, dominant tree species, soil and soil water properties, and soil water-table level (WTL). Mean annual soil CO<sub>2</sub> balance values were estimated for potential use as emission factors EFs. For this purpose, we also assessed We also evaluated the impact of dominant tree species and whether the soil emissions CO<sub>2</sub> fluxes differed between countries. To facilitate the use of the results in future syntheses or meta-analyses, the data used for CO<sub>2</sub> balance estimations have been openly published.

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## 2 Materials and methods

## 2.1 Study sites

In total, 26 study sites (Figure 1) were established in stands dominated by black alder (*Alnus glutinosa* (L.) Gärtner), birch (*Betula pendula* Roth, *Betula pubescens* Ehrh.), Scots pine (*Pinus sylvestris* L.), or Norway spruce (*Picea abies* (L.) Karst.) of different ages (Table S1). The study sites included both drained (n=19) and undrained (n=7) organic soils (Eggleston et al., 2006), with the organic layer thickness ranging from 27 cm to over 2 meters, measured by a rod insertion. According to forest type classification, all sites were characterised as nutrient-rich based on ground vegetation and stand productivity (Bušs, 1981). Drained sites were represented by two site types: *Oxalidosa turf. mel.* (OxalidosaOxalis), which has relatively higher nutrient availability (pH, macronutrients), and *Myrtillosa turf. mel.* (MyrtillosaMyrtillus). Undrained sites were represented by the *Dryopterioso–caricosa*–site type. Soil drainage status was determined based on the presence of drainage ditches within or along the respective forest compartment (a rectangular forest area of 50 ± 25 ha used as a management unit).

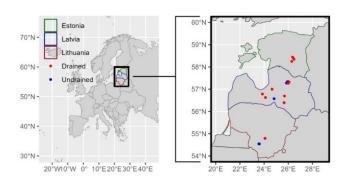


Figure 1: Locations of the study sites. Points indicate the locations of study site clusters.

Despite the greater variation in stand characteristics among the more numerous drained sites—compared to the undrained sites—which prevented direct pairwise comparisons between neighbouring drained and undrained sites, the two groups were overall comparable. Despite the more numerous drained sites showing greater variation in the stand characteristics than the undrained sites, overall, the two groups of sites were comparable (Table 1 Table 1). The mean stand age of both groups was 74 years, with a range of 26-162 years for drained and 44-96 years for undrained sites. Average basal areas in turn were 27 for drained and 30\_m² ha¹ for undrained sites, respectively. More information on stand characteristics, including mean WTL and coordinates, is provided in Table S-1. The projective cover of the most common ground vegetation species in the study sites is presented in Table S2Table S-2. All comparisons will be done at group level, since pairwise comparison, even of closely located sites that belong to the same site type, is hampered by the inherent variation in soil characteristics (see Laiho and Pearson 2016).

Both groups represent historically naturally forested lands that have undergone active forest management. While the exact time since drainage is unknown, active drainage of the forests was initiated in the mid-19th century and resumed in the mid-20th century. Drainage ditches were dug to depths of 0.8 to 1.2 m, with distances between them ranging from 100 to 400 m, and they are maintained to remain functional (Zālītis, 2012).

Table 1: Range (minimum; maximum) of tree stand characteristics in the study sites.

	Dominant tree species									
Parameter	Black	alder	Birch		Pine		Spruce			
_	Drained	Undrained	Drained	Undrained	Drained	Undrained	Drained	Undrained		
Site count	n = 2	n = 2	n = 5	n = 2	n = 5	-	n = 7	n = 3		
Age, year	30; 80	44; 74	24; 45	44; 61	60; 141	-	40; 162	81; 96		
Mean height, m	13; 20	16; 28	13; 18	9; 20	12; 21	-	10; 23	15; 20		
Mean diameter, cm	12; 21	16; 28	12; 22	8; 21	12; 22	-	10; 25	17; 21		
Basal area, $m^2 ha^{-1}$	26; 36	30; 36	15; 23	22; 23	17; 48	-	18; 36	25; 42		

At each study site, three subplots (Figure S1Figure S-1) for data collection were selected, with a minimum distance of 30 m, in an area representing typical ecosystem characteristics of the floristically defined site type, as classified under the local forest site type system (Bušs, 1981). The subplots were arranged along a transect positioned perpendicular to the closest ditch in drained areas and perpendicular to stand borders in undrained areas. In the drained sites, the first subplot was located about 20 m from the nearest drainage ditch. In the undrained sites, the first subplot was located at least 20 m from the forest border.

Empirical data was gathered from January 2021 to December 2022 in Estonia and Latvia, and from July 2021 to June 2023 in Lithuania. The sites were visited monthly in Latvia and Lithuania, and biweekly in Estonia. The meteorological conditions during the study were typical for the region (<u>Table S3Table S 3</u>).

#### 2.2 Respiration

 Measurements of forest floor respiration, which we will hereafter refer to as total respiration (Rtot), included both soil heterotrophic respiration and autotrophic dark respiration of ground vegetation (above and below-ground) as well as tree roots extending to the measurement locations. Gas samples were collected from manual closed static dark (opaque) chambers (PVC, volume 0.0655 m³) as described in the literature (Hutchinson and Livingston, 1993) for subsequent laboratory analysis. Rtot monitoring included five to six monitoring locations, divided over 3 subplots, at each site. Ring-shaped chamber collars (area 0.196 m²) were permanently installed in the soil at a depth of five cm at least one month before the first sampling to avoid the installation effect on fluxes. Collar locations reflected local diversity in vegetation and potential WTL gradient at each subplot and the encircled soil surface and vegetation were kept intact. Thus, the soil heterotrophic respiration component in Rtot includes CO<sub>2</sub> emissions caused by the decomposition of both fresh litter and soil organic matter.

Gas samples for analyses—were collected by obtaining four air samples from a closed chamber into pre-evacuated (0.3 mbar) glass vials (100 cm³). The aAir samples were taken from the chamber outlet, equipped with a valve attached in the sampling tube reaching approximately the centre of the airspace. The air within the chamber was not artificially mixed during sampling. Air sampling was done by first removing the residual air left from the sampling tube (to avoid potential impact on the concentration readout) by a syringe, and thereafter pre-evacuated glass vial was attached into the outlet. The first sample was taken immediately after attaching the chamber on the collar, and subsequent samples were taken at either 10 (Latvia) or 20 (Estonia and Lithuania) minute intervals over 30- or 60-minute monitoring periods, respectively (Butlers et al., 2022; Vigricas et al., 2024).

The gas samples were analysed using a Shimadzu GC-2015 gas chromatograph (Shimadzu USA Manufacturing, Inc., Canby, OR, USA) equipped with an electron capture detector (ECD). The uncertainty of the method used was estimated to be 20 ppm of CO<sub>2</sub> (Magnusson et al., 2017). Linear regression was applied to relate the CO<sub>2</sub> concentrations with the time elapsed since chamber closure for each measurement. Subsequently, the measurement data was screened to identify deviations from the recognized trend, considering the removal of measurements with identified errors. All measurements were discarded if the regression coefficient of determination (R<sup>2</sup>) was less than 0.9 (p<0.01), except for cases where the difference between the highest and lowest measured CO<sub>2</sub> concentration in the chamber was less than the uncertainty of the method (specifically applicable during non-vegetation periods). Consequently, a small n insignificant amount of data (<5%) was discarded.

The data that met the quality criteria were used to determine the slope coefficient of the linear regression, which was then used to calculate the instantaneous Rtot according to the ideal gas law equation (Fuss and Hueppi, 2024):

$$Rtot = \frac{M \times P \times V \times slope}{R \times T \times A \times 1000}$$
 (1)

where Rtot is the instantaneous total respiration, mg CO<sub>2</sub>-C m<sup>2</sup> h<sup>-1</sup>; M is the molar mass of CO<sub>2</sub>-C, 12.01 g mol<sup>-1</sup>; R is the universal gas constant,  $8.314 \text{ m}^3 \text{ Pa K}^{-1} \text{ mol}^{-1}$ ; P is the assumption of air pressure inside the chamber, 101.300 Pa; T is the air temperature in the chamber, K; V is the chamber volume,  $0.0655 \text{ m}^3$ ; the slope is the CO<sub>2</sub> concentration change over time, ppm h<sup>-1</sup>; and A is the collar area,  $0.19625 \text{ m}^2$ .

We also conducted measurements of heterotrophic respiration (Rhet) for comparison, as described in the Supplementary text.

#### 2.3 Environmental variables

Manual WTL measurements were carried out using nylon-mesh-coated, perforated piezometer tubes (5 cm in diameter) installed down to a 140 cm depth in all subplots. Manual soil temperature measurements were done at depths of 5, 10, 20, and 40 cm in all subplots by Comet data logger (COMET SYSTEM, s.r.o., 756 61 Roznov pod Radhostem, Czech Republic) equipped with Pt1000 temperature probes. All manual measurements were carried out at the same time as <a href="mailto:the-continuous soil temperature">the-CO2</a> flux measurements. Continuous soil temperature measurements at depths of 10 and 40 cm were carried out at 30-minute interval in the centermost subplot (Maxim Integrated DS1922L2F, iButtonLink Technology, Whitewater, WI 53190 USA).

Once per month, soil water samples were collected from perforated tubes (7.5 cm in diameter) explicitly installed for water sampling for chemical analysis. Water chemical parameters including pH, electrical conductivity (EC), and concentrations of dissolved organic carbon (DOC), total nitrogen (N), and nitrate (NO<sub>3</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>); and phosphate (PO<sub>4</sub><sup>3</sup><sup>-</sup>) ions were determined. Soil samples were taken up-down to a depth of 75 cm (0-10; 10-20; 20-30; 30-40; 40-50; 50-75cm) at two locations in each subplot during the establishment of the study sites. Two separate sample sets were collected one—for the determination of bulk density, and another for ash content and chemical parameters (pH, concentrations of total carbon (TC), nitrogen (N), and HNO<sub>3</sub> extractable phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)). The samples were collected with a volumetric 100 cm<sup>3</sup> cylinder (Cools and De Vos, 2010) at 10 cm intervals to a depth of 50 cm. Two additional samples were taken from soil depths of 50-75 and 75-100 cm with a soil auger. Soil samples collected for determination of bulk density were oven-dried (105 °C) and weighed, while soil samples for chemical analyses were prepared by air drying (≤40 °C), sieving and homogenizing (LVS ISO 11464:2006). Organic carbon (Corg) content was calculated by multiplying soil organic matter content derived from the ash content measurement result derived soil organic matter content by factor 0.5, thus assuming that organic matter is 50% Corg (Pribyl, 2010). All soil and water analyses were done in an ISO 17025-certified laboratory using ISO standard methods (Table S4Table S 4).

# 2.4 Litter input

Annual litter inputs to be used in CO<sub>2</sub> balance estimation were either measured (foliar litter) or estimated based on biomass components measured (ground vegetation, fine roots).

Foliar fine litter (fLF) was collected with conical litter traps (area 0.5 m²) set one meter above ground (Latvia, Lithuania), or with square mesh frames (0.5 x 0.5 m) placed on the ground (Estonia). In each study site, five replicate litter traps were placed in the centremost subplot of the transect. It included all fine fractions of litter, such as needles, leaves, and branches with a diameter up to 1 cm and a length up to 10 cm. Branches with larger dimensions were collected from coarse woody litter (cLF) traps (square mesh frames, 0.5 x 0.5 m) placed on the ground. The litter samples were collected from the traps once every four weeks. Due to the heterogenous nature and large dimensions of cLF, respective decomposition emissions could not be representatively included in Rtot measurements. Therefore, we considered only fLF as the litter input source, and the cLF results are presented only as indicative of the site conditions.

Ground vegetation (GV) aboveground (aGV) and belowground (bGV) biomass samples were collected at the end of the growing season (August) in 2021 in five replicates per subplot, from square sampling locations with an area of 0.0625 cm<sup>2</sup>. Aboveground biomass was separated into herbaceous (aGV) species and dwarf shrubs. bGV was collected from the top soil layer, extending down to 20 - 30 cm from the same location and area as aGV. Herbaceous ground vegetation roots (bGV) were separated from tree and shrub roots by wet sieving based on root morphological properties. We assumed that the measured herbaceous ground vegetation aboveground (aGV) and belowground (bGV) biomasses were equal to annual ground vegetation litter inputs. The contribution of shrub litter was thus not included but was assumed to be minor (see Table S-5).

Moss biomass was measured by collecting samples from  $0.01 \text{ m}^2$  square areas, with four replicates per subplot, and visually removing any dead parts. Collections were conducted concurrently with moss production sampling. Moss production (MP) samples in four replicates per subplot were collected by anchoring a square mesh  $(0.01 \text{ m}^2)$  on the moss at the end of the growing season and harvesting the moss biomass grown through the mesh by the end of the next growing season. Including moss litter in the  $CO_2$  balance estimation by aligning  $CO_2$  inputs-influx and outputs-efflux from areas with and without moss cover, without increasing uncertainty, would however have required doubling the number of spatial replicates in chamber measurements. Therefore, we did not consider moss as a litter source, which causes underestimation of litter inputs to a varying extent. The moss results are presented only as indicative of site conditions (see Table S-5).

Tree fine-root production (FRP) was estimated with the ingrowth core method (Laiho et al., 2014; Bhuiyan et al. 2017). Five replicate ingrowth cores (cylindrical mesh bags with diameter 2.5 cm, mesh size 2 mm) per subplot, filled with soil collected from the subplot, were installed in autumn or spring and removed after two growing seasons. In the laboratory, the biomass of the ingrown fine roots was determined after wet sieving, and ground vegetation roots were separated from tree roots by morphological properties. We assumed that tree fine-root biomass was essentially not changing over the study years, and thus we could assume that FRP equalled litter production. Since the ingrowth cores were removed from the soil after two growing seasons, the FRP estimate was calculated by dividing the fine-root biomass in the cores by two (Bhuiyan et al., 2017).

All litter and biomass samples were oven-dried (70 °C), weighed and milled before further analysis. Chemical analyses were performed according to ISO standard methods (<u>Table S4Table S4</u>). To estimate the annual  $CO_2$  inputs influx to be used in the  $CO_2$  balance estimation (section 1.5), the estimated annual litter inputs on a unit area were transformed to  $CO_2$  input influx by using C content values measured for each component (Table 2).

Table 2: Mean C and N content (% of dry matter) in foliar litter from trees and biomass components used for estimating other litter inputs. The values are means  $\pm$  standard deviation including both drained and undrained sites. Abbreviations: aGV and bGV – above- and belowground biomass of herbaceous vegetation, FR – tree fine roots, M – moss, fLF – foliar fine litter, cLF – coarse woody litter.

Element	aGV	bGV	FR	M	fLF	cLF
С	49.34±2.45	50.95±2.02	51.21±5.16	48.38±2.13	52.50±0.25	53.88±0.67
N	2.18±0.64	1.53±0.43	1.47±0.44	1.10+0.75	1.30±0.41	$1.04 \pm 0.20$

## 2.5 Estimation of annual soil CO2 carbon balance

We estimated the annual soil CO<sub>2</sub> balance of the sites by combining annualized CO<sub>2</sub> inputinflux (section 1.4) and outputefflux data. In exceptional cases where the CO<sub>2</sub> inputinflux from specific source was not estimated in certain countries, such as bGV and FRP in EE and FRP in some sites in LT and LV (Table S10Table S-10), we assumed the CO<sub>2</sub> inputinflux to be equivalent to the average results from sites with the same drainage status in the other countries. While we directly measured Rhet, we utilized the estimated Rhet derived from Rtot as the outputefflux value (marked from here onwards as Rhet'). Such an approach was necessary because, at all sites, our Rhet values were significantly higher (by mean 5.8±3.1 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) than Rtot, which would be logically impossible if the conditions in the measurement locations were the same. This pattern was probably mostly due to the high and variable CO<sub>2</sub> efflux from roots killed by the trenching, noted also in other studies (e.g., Hermans et al., 2022). This discrepancy could not be remedied with the root data at hand (for more details, please refer to the Supplementary text and Discussion section).

To estimate annual CO<sub>2</sub> outputefflux, at first, site-specific relationships between instantaneous Rtot fluxes and soil temperatures were established. To identify the best approach for to express the relationship, we compared the suitability of exponential regression on untransformed data and linear regression on logarithmically or Box-Cox transformed data. The performance of these models for flux data interpolation based on continuous soil temperature data was evaluated by using the root mean square error (RMSE) of prediction. It was found that the Box-Cox approach achieved the best conformity of flux

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data to a normal distribution and provided the best fit for the models. Previous studies also indicate that this method effectively addresses the typical underestimation of fluxes caused by their nonlinearity (Box and Cox, 1964; Liaw et al., 2021; Wutzler et al., 2020). Interpolation was performed by evaluating the relationship between Rtot and soil temperature (at 10-cm depth) measured at each study site and constructing site-specific linear regression equations (Table S-6) after applying Box-Cox transformation to the flux data for normalization (Box and Cox, 1964). This approach, compared to alternative methods, more successfully (lower RMSE of prediction) accounted for the exponential nature of the relationship between flux and temperature, and prevented underestimation (Liaw et al., 2021; Wutzler et al., 2020). Hourly Rtot estimates were then formed by using the hourly recorded soil temperatures (logger data) for each study site. Consecutively, site-specific annual Rtot estimate was calculated by summing the interpolated hourly emission estimates of the year.

We derived site-specific annual Rhet` from estimated annual Rtot empirically. To avoid potential bias introduced by using a single fixed factor, we instead used Equation (2), accounting for the observed pattern of decreasing Rhet proportion as soil surface respiration (Rs) increases (Bond-Lamberty et al., 2004; Subke et al., 2006). The equation characterizes the relationship between Rs and Rhet, and was created using results of previous studies (Jian et al., 2021) in the boreal zone (Figure S2 Figure S-2). We assumed Rtot is equal to Rs, i.e., that aboveground autotrophic respiration has a minor role in Rtot (Hermans et al.,

2022; Munir et al., 2017), and applied the equation to annual Rtot directly.

$$Rhet' = -0.70 + 0.78 \times Rtot \tag{2}$$

Neglecting aboveground autotrophic respiration leads to a minor overestimation of Rhet.

We will use the + sign to denote positive soil CO<sub>2</sub> balance (soil CO<sub>2</sub> sink), and - sign to denote net loss of CO<sub>2</sub> from soil to

the atmosphere.

## 2.6 Statistical analysis

Statistical analyses were performed and figures prepared using the software R version 4.3.1 (packages 'MASS', 'stats', 'nlme', 'Hmisc', 'lmerTest', 'lmer4', 'vegan', 'pls', and 'caret'), using p=0.05 as the limit for statistical significance. The compliance of the data with the normal distribution was checked with the Shapiro-Wilk normality test and visually by density and quantile-quantile (Q-Q) plots. To calculate the uncertainty of the study results when combining multiple data sources, we used the root sum of the squares method to aggregate the individual uncertainties (95 % confidence interval). Therefore, for the CO2 influx, for instance, we combined the uncertainties from various influx (fLF, GV, FRP) sources. The uncertainty of Equation (2) used for the calculation of Rhet' was expressed as the root mean square error (RMSE) of the corresponding regression model. The soil CO2 balances were calculated by summing the CO2 influx and efflux for individual sites. The uncertainties of the averaged CO2 balances, categorized by drainage status, country, site type, or dominant tree species, are expressed as standard error. Figures are were prepared by using packages 'ggplot2', 'corplot', 'ggbiplot'.

Correlations between the sample groups were expressed with the Pearson correlation coefficient (r). We compared differences between two sample groups using pairwise Wilcoxon rank sum tests with continuity correction and adjusted the p-values using Bonferroni correction. The method was used to compare soil parameters and instantaneous or annualized Rtot data segregated by site type, drainage status, country or dominant tree species. In the same way, soil characteristics were compared between drained and undrained sites. Multivariate testing of flux and impacting factor relationships testing-involved assessing the significance of these factors on the relationship between soil temperature and Rtot using mixed-effects linear models. As flux impacting factors, we considered country, dominant tree species, drainage status, WTL, and a 30 cm WTL threshold distinguishing shallow and deep drainage in the IPCC guidelines. In this analysis, Box-Cox transformed flux data was fitted to linear models using the study site as a random effect. In addition, multivariate relationships were observed through Principal Component Analysis (PCA) to visualize covariation and seek observational confluences with the results of other analyses. To

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assess the contribution of influencing factors on the soil  $C\underline{O_2}$  balance, Redundancy Analysis (RDA) and Partial Least Squares Regression (PLSR) were conducted.

#### 3 Results

#### 3.1 Soil and soil water characteristics

The organic layer depth in the drained sites ranged from 27 to 212 cm (mean 81±47 cm) and in undrained sites from 100 to 230 cm (mean 167±49 cm). Soil bulk density (0-30 cm depth) in the drained sites (mean 314±215 kg m<sup>-3</sup>) was characterized by both higher variation and higher mean density (p=0.003) compared to undrained sites (mean 168±32 kg m<sup>-3</sup>) (Table S7Table-S-7). Soil drainage status had no impact on Corg content in the 0-30 cm soil layer (p=0.11, total mean 416±130 g kg<sup>-1</sup>). However, drained soils had a higher mean C:N ratio (22±7; p=0.01) than the undrained soils (17±3). A trend could be observed that undrained soils had higher nutrient concentrations and higher pH than the drained soils. Soil analysis confirmed that site types classified based on ground vegetation presence composition served as an indicator of nutrient availability (Figure 2Figure 2). In drained OxalidosaOxalis sites, mean macronutrient concentrations in the 0–30 cm soil layer was higher compared to MyrtillosaMyrtillus sites. Notably, N, Mg, Ca, and P showed statistically significant differences (p < 0.05). Additionally, when comparing drained sites, soil pH values in the OxalidosaOxalis turf. mel. sites were, on average, 1.89 units higher (p = 0.018).

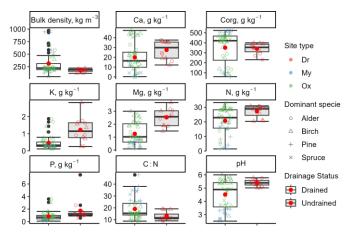


Figure 2: Variation of soil chemical and physical properties at soil depth 0-30 cm. The clear box represents the drained, and the grey-shaded box the undrained sites. Data points represent individual subplots. The bottom and top edges of the box represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles, summarizing the interquartile range (IQR). The whiskers extend to the smallest and largest values within 1.5 × IQR from the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively. Black dots mark outliers. A red dot and a solid horizontal line in the box indicate mean and median values, respectively. Corg – organic carbon; N – total nitrogen. Site types: Dr - Dryopterioso–caricosa; Ox - Oxalidosa turf. mel.; My - Myrtillosa turf. mel.

The range of mean WTL over the study period was from -23 to -112 cm (mean -60±25 cm) in the drained sites and from -7 to -17 cm (mean -13±4 cm) in the undrained sites, respectively. In the undrained sites, the WTL was mainly rather high (see interquartile range in Figure 3Figure 3) and had comparably smaller variation (mean standard deviation 16 cm) than in the drained sites (mean standard deviation 23 cm); however, in all sites except LTC108, WTLs below 30 cm were also observed

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(Figure\_4). In the undrained sites, the range of min-max WTL was from  $3\pm3$  cm to  $-63\pm27$  cm, while the WTL in drained sites had a greater absolute variation ranging from  $-14\pm19$  cm to  $-104\pm28$  cm.

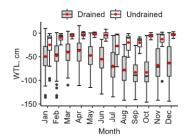


Figure 3: Yearly variation of soil water-table level (WTL) in the study sites. The edges of the box represent the 25th and 75th percentiles, encapsulating the interquartile range (IQR). The whiskers extend to the smallest and largest values within 1.5 \* IQR from the 25th and 75th percentiles, respectively. Black dots mark outliers. A red dot and a solid horizontal line indicate the average values of the date represented – mean and median, respectively.

The concentrations of all measured chemical parameters in the soil water, except for  $NH_4^+$ , were, on average, higher in the drained sites (Figure S3Figure S3). However, the results were highly variable (Table S8Table S 8).

#### 3.2 Instantaneous total respiration

In the drained sites, the mean instantaneous Rtot varied from 48 to 125 mg  $CO_2$ -C  $m^{-2}$   $h^{-1}$ , and in the undrained sites from 38 to 80 mg  $CO_2$ -C  $m^{-2}$   $h^{-1}$  (Figure 4Figure 4). In all sites combined, during the summer months (June, July, August), the interquartile range of Rtot varied from 111 to 198 mg  $CO_2$ -C  $m^{-2}$   $h^{-1}$  with a mean of  $160\pm78$  mg  $CO_2$ -C  $m^{-2}$   $h^{-1}$ . In contrast, during the winter (December, January, February), it ranged from 8 to 24 mg  $CO_2$ -C  $m^{-2}$   $h^{-1}$  with a mean of  $17\pm14$  mg  $CO_2$ -C  $m^{-2}$   $h^{-1}$ . The relative standard deviations of the instantaneous Rtot in drained ( $90\pm9\%$ ) and undrained ( $106\pm29\%$ ) sites were comparable. Although the study sites represented a broad soil WTL gradient, no significant impact of the site mean WTL on the mean instantaneous Rtot emission was observed (r=0.16, p>0.05). Furthermore, no significant correlations were found between instantaneous Rtot and soil water parameters.

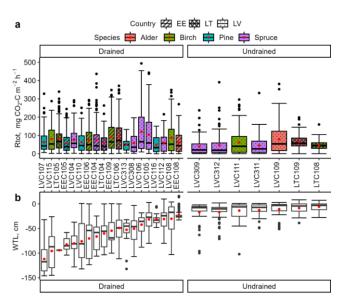


Figure 4: Variation of instantaneous total respiration (Rtot, a) and soil water-table level (WTL, b) in the study sites. The bottom and top edges of the box represent the  $25^{th}$  and  $75^{th}$  percentiles, summarizing the interquartile range (IQR). The whiskers extend to the smallest and largest values within  $1.5 \times IQR$  from the  $25^{th}$  and  $75^{th}$  percentiles, respectively. Black dots mark outliers. A red dot and a solid horizontal line in the box indicate mean and median values, respectively.

Mean Rtot in sites with the same drainage status did not differ (p>0.05) between countries (Figure S 4). The instantaneous Rtot in drained sites (mean 76±3 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>) was overall higher (p<0.05) than Rtot those fromin undrained soil sites (mean 56±5 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>) (Figure 5a). There were few apparent differences in the mean Rtot between stands of different tree species (Figure 5b). Rtot was the lowest at undrained sites dominated by spruce and the highest at drained sites dominated by birch (Figure 5Figure 5b). Rtot was significantly different (p<0.05) between coniferous forest sites with different dominant tree species and/or soil moisture regimes, wherewith Rtot ranginged from mean 42±7 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in undrained spruce forests to 59±4 and 81±6 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in drained pine and spruce forests, respectively. In deciduous stands, the moisture regime and dominant tree species had less impact on the mean flux; Rtot was higher (p<0.05) in drained birch stands (mean 84±5 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>) than in undrained sitesbirch stands (56±8 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>), while in alder stands the mean Rtot was similar regardless of the soil moisture regime (total average 67±9 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>), (Figure 5b). In drained coniferous and deciduous sites, on average, the mean Rtot was similar, but in undrained sites, emissions in deciduous forests were about 40% higher. Mean Rtot in sites with the same drainage status did not differ (p>0.05) between countries (Figure \$4, g).

\_The measured Rtot of undrained soil were smaller in both Latvia (mean 57±6 mg CO<sub>2</sub>-C m<sup>-2</sup>-h<sup>-1</sup>) and Lithuania (mean 55±6 mg CO<sub>2</sub>-C m<sup>-2</sup>-h<sup>-1</sup>) compared to Rtot from drained soil in the Baltic states ranging from mean 72±4 to 79±5 mg CO<sub>2</sub>-C m<sup>-2</sup>-h<sup>-1</sup> (Figure 5a).

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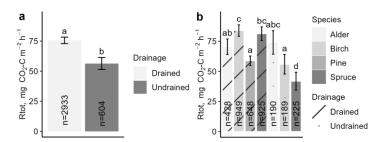


Figure 5: Mean instantaneous total respiration (Rtot) throughout the study period, categorized by drainage status and country (a) or dominant tree species and drainage status (b). Error bars indicate confidence interval. A shared letter indicates that differences are not significant.

There were few apparent differences in the mean Rtot between stands of different tree species (Figure 5b). Rtot was the lowest at undrained sites dominated by spruce and the highest at drained sites dominated by birch. Rtot was significantly different (p<0.05) between coniferous forest sites with different dominant tree species and soil moisture regimes, where Rtot ranged from mean 42±7 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in undrained spruce forests to 59±4 and 81±6 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in drained pine and spruce forests, respectively. In deciduous stands, the moisture regime and dominant tree species had less impact on the mean flux; Rtot was higher (p<0.05) in drained birch stands (mean 84±5 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>) than in undrained sites (56±8 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>), while in alder stands the mean Rtot was similar regardless of the soil moisture regime (total average 67±9 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>), (Figure 5b). In drained coniferous and deciduous sites, on average, the mean Rtot was similar, but in undrained sites, emissions in deciduous forests were about 40% higher.

The impact of WTL is reflected in the mean Rtot, which was  $87\pm3$  mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> when WTL was below 30 cm and  $57\pm3$  mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> when WTL was closer to soil surface. However, when evaluating the effect of WTL on Rtot variation in mixed-effects models predicting Rtot based on soil temperature, WTL was found to have an insignificant impact on Rtot variation. Similarly, the contribution of country and dominant tree species to Rtot prediction was marginal (Table S9). The inclusion of dominant species provided minimal model improvement ( $\Delta$ AIC = +5,  $\Delta$ logLik = 0), while country effects captured some additional variability in Rtot. However, the increase in R<sup>2</sup> due to country variables was minor (from 0.77 to 0.78), indicating limited explanatory power.

## 3.3 Annual total respiration

Soil temperature at 10 cm depth (Figure S5Figure S-5) was used in constructing Rtot prediction models and for emission interpolation needed for annualizing Rtot. The 10 cm depth was chosen because it showed the strongest correlation between instantaneous Rtot and soil temperatures measured at different depths, with a mean Pearson correlation coefficient (r) of 0.86±0.04 across the study sites. For the other soil depths (5, 20, 30, 40 cm), r ranged from 0.71±0.07 to 0.79±0.05. Linear models developed using Box-Cox transformed data provided the best Rtot prediction power. A lambda value of 0.3411 was used for all data transformations, as individual data transformations for each site resulted in comparatively less successful data normalization. With this approach, the RMSE of instantaneous Rtot predictions for individual sites decreased by an average of 16±14%, compared to linear models with logarithmically transformed data or non-linear models with untransformed data (Table S6Table S 6).

Annualized Rtot indicated similar mutual relationships among the study site dominant tree species and drainage status categories as the instantaneous Rtot. Consequently, Pooled the estimated annual emissions from neither drained sites (overall mean 6.2141±0.43-49 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) nor-and undrained sites (overall mean 4.3875±1.200.68 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>)

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differed significantly (Figure 6, a). However, when the data were further stratified by country, the differences waswere no longer statistically significant due to wider confidence intervals (Figure S4, h). between countries and were generally higher from drained soils (Figure 6a).

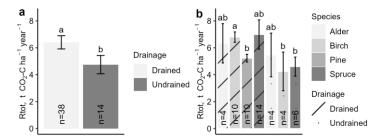


Figure 6: Annualized total respiration (Rtot) in study sites stratified by drainage status and country (a) or dominant tree species (b). Error bars indicate confidence interval. A shared letter indicates that differences are not significant.

However, wWhen categorizing data according to drainage status and dominant tree species, fewer differences were found in the annualized Rtot than in the instantaneous Rtot (Figure 6Figure 6\_b). For instance, among the drained sites, the lowest mean annual Rtot was estimated for pine forests (5.23±0.29 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>), while in spruce, birch, and alder forests, the means were similar (p>0.05) (6.71±0.31 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>). Emissions from undrained soils in alder, birch, and spruce forests were similar to each other and lower than from drained sites, ranging from 4.6±0.71 in spruce forests to 5.47±1.63 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup> in alder forests (overall mean 4.86±0.71).

The correlation between Rtot and WTL was low; however, a drainage status (drainage ditch presence) impact on Rtot is indicated by the PCA results, where undrained sites tend to have more similar characteristics while drained sites show greater diversity concerning Rtot. However, clear covariation of dominant tree species and Rtot are not recognized by PCA (Figure S6Figure S 6 and Figure S7Figure S 7). When comparing the chemical and physical properties of different soil layers with the estimated annual Rtot, as well as the measured mean Rhet, the mean measured Rhet consistently showeds a higher correlation with evaluated soil parameters (Figure S8Figure S 8). The only exception is Corg, for which no correlation was observed between Corg in different soil layers and neither Rtot or The only exception is Corg, where in all correlation combinations, itRhet-was not present (r around -0.1). Excluding Corg, the other soil chemical parameters generally hadve a low to moderate correlation (mean r=0.4) with respiration. The highest correlation is was with pH, K, Mg, and P (mean r=0.5±0.07, p<0.05), and it is was consistent across all evaluated soil layers, while the correlation with BD (mean r=-0.2, p>0.05) tends to increase with deeper soil layers reaching the highest correlation (r=-0.3) in layer 20-30 cm. In addition, a higher C:N ratio is associated with lower CO<sub>2</sub> emissions (mean r=-0.4, p<0.05).

# 3.4 Annual litter inputs

The estimated mean litter inputs at the subplot level were mostly similar between drained and undrained sites. The estimated mean litter inputs in both drained and undrained sites were mostly similar (Table S10Table S 10 and S11), typically differing by less than 20%. Only fLF and FRP tended to be considerably higher in the drained sites, FRP on average even more than twice as high. Compared to undrained sites, bGV in drained sites was about 20% higher on average, while aGV was about 20% lower on average (Figure 7Figure 7). However, regardless of the soil drainage status, the proportion of aGV in the total GV biomass was 54±18% (Table 3Table 3).

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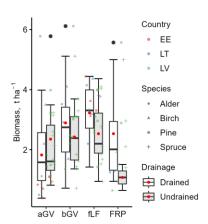


Figure 7: Variation in the biomass components used as litter input estimates. Abbreviations: aGV and bGV – above- and belowground biomass of herbaceous vegetation, fl.F – fine foliar litter (needles, leaves, fine woody litter), FRP – tree fine root production. The bottom and top edges of the box represent the 25th and 75th percentiles, summarizing the interquartile range (IQR). The whiskers extend to the smallest and largest values within 1.5 × IQR from the 25th and 75th percentiles, respectively. Black dots mark outliers. A red dot and a solid horizontal line in the box indicate mean and median values, respectively.

Table 3: Biomass (mean±CI, t dm. ha<sup>-1</sup>) components used as litter input estimates, stratified by drainage status. Abbreviations: aGV and bGV – above- and belowground biomass of herbaceous vegetation; respectively; FRP – tree fine root production; fLF – fine foliar litter (needles, leaves, fine woody litter).

Category	Drained	Undrained
aGV	1.82±0.52	2.35±1.61
bGV	$2.89 \pm 0.85$	2.42±0.84
FRP	2.53±0.77	$1.08\pm0.57$
fLF	3.22±0.44	2.53±1.06

Both bGV (r=|0.6|) and FRP (r=|0.7|) had a significant negative correlation with soil pH but a positive with the C:N ratio in soil layer 0-30 cm. Additionally, FRP had a significant negative correlation (r=|0.7|) with the contents of N, Ca, and Mg in the soil. No explanatory factors for aGV could be identified. Moderate correlation (r=0.5, p<0.05) was found between stand age and fLF.

# 3.5 Annual soil carbon balance

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The estimated Rhet' (Table S10Table S 10) proportion of Rtot varied between 54 and 71% (mean 65%). Consequently, the estimated annual gross C losses from drained soils in the form of Rhet' emissions ranged from 2.36 to 7.49 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup> (mean 4.30±1.20), while for undrained soils the range was from 1.63 to 4.68 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup> (mean 3.00±0.99). According to the RMSE of the model (Equation 2, Figure S2Figure S-2) prediction, the Rtot to Rhet' calculation introduced an uncertainty of approximately 0.32 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>. In drained and undrained sites, the total estimated CO<sub>2</sub> inputinflux ranged from 3.81 to 7.03 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup> (mean 5.20±0.91) and 2.89 to 5.98 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup> (mean 4.19±1.10), respectively (Figure 8Figure 8). The uncertainties (relative CI) in CO<sub>2</sub> efflux and influx in both drained and undrained soils were relatively uniform, with a mean uncertainty of 36±14% for the estimated individual annual CO<sub>2</sub> fluxes. The largest source of error uncertainty was CO<sub>2</sub> influx in undrained soils (49±13%), while the uncertainty of individual CO<sub>2</sub> fluxes in drained sites averaged 27±6% (Figure 8Figure 8).

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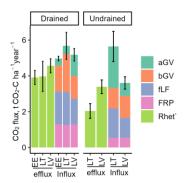


Figure 8: Components of the estimated soil CO2car-bon\_balance (sum±combined CI). Efflux is soil heterotrophic respiration (Rhet') calculated from Rtot, and influx is the estimated litter input; both are expressed as C. Thus, efflux indicates soil C losses and influx—soil C gains. Abbreviations: aGV and bGV – above- and belowground biomass of herbaceous vegetation; respectively; fLF – fine foliar litter; FRP – tree fine root production.

 The mean soil  $CO_2$  balance during the study period was  $\pm 1.06 \pm 0.45$  and  $\pm 1.27 \pm 0.73$  t  $CO_2 - C$  ha<sup>-1</sup> year<sup>-1</sup> for the drained and undrained sites, respectively (Figure 9 - a). These results indicate that long-term drainage reduced soil  $CO_2$  sequestration capacity by an average of 0.20 t  $CO_2 - C - C$  ha<sup>-1</sup> year<sup>-1</sup>.

The higher mean soil C removals estimated for both drained and undrained sites in Lithuania, compared to those in Estonia and Latvia, are primarily attributed to the greater C input from GV litter (Figure 8). However, the sites in Lithuania also stand out due to greater uncertainty in both C input and output, resulting in the soil C balance in drained sites across the countries being equivalent within the margin of error (Figure 9a). The low number of undrained sites in Lithuania (n=2) limited the ability to investigate the patterns behind the observed lower soil CO<sub>2</sub>-emissions and higher aGV litter, contributing to significantly higher soil C removals (±3.70±0.11 t C ha<sup>-1</sup> year<sup>-1</sup>) compared to sites in Latvia (±0.30±0.56 t C ha<sup>-1</sup> year<sup>-1</sup>, n=5).

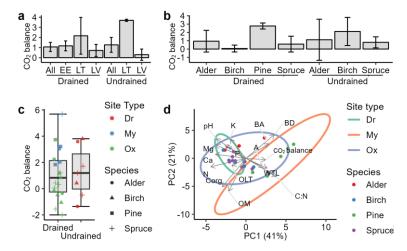


Figure 9: Soil carbon\_CO<sub>2</sub> balance (a, b, c: mean t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup>±SE) and impacting factors (d: PCA biplot). Positive values indicate soil C stock increaseCO<sub>2</sub> sink, i.e., CO<sub>2</sub> removals from the atmosphere. Abbreviations: A – stand age; BA – basal area, BD – bulk density; C:N – ratio between organic carbon and nitrogen in soil; OLT – soil organic layer thickness; WTL – water table level; pH – soil pH value; K, Ca, Mg, P, OM, Corg, N represent the content of potassium, calcium, magnesium, phosphorus, organic matter, organic

carbon and nitrogen in the top 0-30 cm layer of soil, respectively. Site types: Dr - Dryopterioso-caricosa (undrained sites); Ox - Oxalidosa turf. mel. (drained); My - Myrtillosa turf. mel. (drained).

The soil CO<sub>2</sub> balance stratified by dominant tree species (Figure 9Figure 9, b) indicates that the drained sites were skewed towards CO<sub>2</sub>C removals due to significantly higher CO<sub>2</sub>C removals in pine stands. Pine sites (n=5) showed high CO<sub>2</sub>C removals with low uncertainty (mean ±2.77±0.36 t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup>), in contrast to the other drained sites (n=19), where the mean soil CO<sub>2</sub> balance was estimated at ±0.45±0.50 t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup>, suggesting that soils in drained alder, birch and spruce sites were in near CO<sub>2</sub>C equilibrium during the study period. The soil CO<sub>2</sub>C removals identified for undrained birch and spruce stands (±1.33±0.72 t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup>, n=5) were consistent, while in alder stands, the CO<sub>2</sub> balance of ±1.12±2.47 t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup> was highly uncertain due to the number of sites being just two.

A trend of higher soil CO<sub>2</sub>C removals was observed in site types associated with relatively lower nutrient availability (Figure 9Figure 9, c). In OxalidosaOxalis sites, the mean soil CO<sub>2</sub> balance was ±0.32±0.40 t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup>, while in MyrtillosaMyrtillus sites, it was ±3.16±0.69 t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup>. Thus, drained nutrient-richer soils were approximately at CO<sub>2</sub>C equilibrium, whereas comparably nutrient-poorer soils acted as a CO<sub>2</sub> sink. The observed tendency is supported by PCA (Figure 9Figure 9, d) which indicates that higher soil CO<sub>2</sub>C removals are associated with lower nutrient concentrations and pH levels. In addition, the PCA reveals that the risk of soil CO<sub>2</sub>C loss-source is reduced in stands with higher basal area and age, as well as with a higher soil C:N ratio, which in our study likely reflects the variability in peat quality and decomposability under different vegetation types.

According to correlation analysis, soil parameters such as pH, C:N ratio, N, and P showed the strongest correlations with the soil CO<sub>2</sub> balance (Figure 10 Figure 10). Basal area was the tree stand characteristic with the strongest correlation with CO<sub>2</sub> balance, while, among the CO<sub>2</sub> flux components, bGV demonstrated the most consistent role in CO<sub>2</sub> balance. No meaningful relationship was identified between soil CO<sub>2</sub> balance and soil organic matter or C content, nor with the depth of the WTL or organic layer thickness.

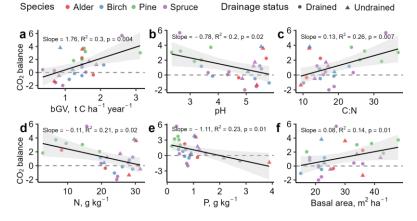


Figure 10: Relationships between soil CO<sub>2</sub> balance (t CO<sub>2</sub>-CC ha<sup>-1</sup> year<sup>-1</sup>) and impacting factors. Positive values indicate soil CO<sub>2</sub> stock increaseremovals. The figures include impacting factors showing the highest correlations found in the study dataset. Data points represent individual study sites.

Soil nutrient availability as  $CO_2$  balance affecting factor is confirmed by RDA and PLSRS models. RDA and PLSRS models (p<0.05) explained 78% and 70% of the soil  $CO_2$  balance variance, respectively. After excluding variables introducing multicollinearity, the models included WTL, organic layer thickness, pH, N, K, Ca, Mg, P, organic matter content of the soil, stand age, and basal area. The variables pH, K, and Mg showed significant contributions (p < 0.05) in the RDA model explaining the variation of soil  $CO_2$  balance. The variable N was not significant (p = 0.087) but was close to the threshold of

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significance. PLSR model indicated that pH, K, Mg and N explained 45% of the CO<sub>2</sub> balance variance. however, Although the VIP values for all potential explanatory variables were below 0.4, suggesting limited predictive power for CO<sub>2</sub> balance with the current dataset.

The higher mean soil C removals estimated for both drained and undrained sites in Lithuania, compared to those in Estonia and Latvia, are primarily attributed to the greater C input from GV litter (Figure 8). However, the sites in Lithuania also stand out due to greater uncertainty in both C input and output, resulting in the soil C balance in drained sites across the countries being equivalent within the margin of error (Figure 9a). The low number of undrained sites in Lithuania (n=2) limited the ability to investigate the patterns behind the observed lower soil CO<sub>2</sub> emissions and higher aGV litter, contributing to significantly higher soil C removals (±3.70±0.11 t C ha<sup>-1</sup> year<sup>-1</sup>) compared to sites in Latvia (±0.30±0.56 t C ha<sup>-1</sup> year<sup>-1</sup>, n=5).

#### 4 Discussion

#### 4.1 Soil carbon CO2 balance

The soil  $CO_2$  balance of the studied drained and undrained organic forest soils fluctuated around equilibrium, demonstrating both  $CO_2$  sink and source dynamics. The reason for the uncertain  $CO_2$  balance in undrained alder stands could not be determined, as site characteristics were consistent with the patterns observed in other undrained sites showing soil  $CO_2$  removals. However, we identified soil properties as the likely reasons being for why the soil in drained pine stands showed  $CO_2$  removals, in contrast to the C-neutral soils observed at other drained sites.

Although observing a CO2 sink in drained nutrient-rich soils may seem somewhat unexpected, given that these soils have generally been estimated to on average act as a-net CO2 sources in both boreal and temperate zones (Jauhiainen et al. 2023), it is not entirely novel. Both soil CO2 sinks and sources have also been observed in earlier studies under a wide range of site conditions (e.g., Ojanen et al. 2013; Minkkinen et al., 2018; Bjarnadottir et al., 2021; Hermans et al., 2022). Many of the soil CO2 sink sites may be classified as nutrient-poor, but not all (e.g., Ojanen et al. 2013). In soil inventory studies carried out in Latvia, the soil C stocks of forestry-drained peatlands were found to be stable in all but the most nutrient-rich soil conditions, under which the C stock was reduced in the long term (Dubra et al., 2023; Lazdiņš et al., 2024). However, interpretation of soil CO<sub>2</sub> balances solely based on the nutrient status of a forest site should be approached with caution, as it is typically derived from indirect indicators such as vegetation or stand productivity, rather than a quantitative assessment of soil nutrient concentrations. Consequently, sites with variable conditions may be classified under a given nutrient status category. Similarly, drainage status does not guarantee specific WTL levels (Figure 4Figure 4). This may be the reason for varying findings on soil CO2 balances across studies that allegedly target the same soil type and drainage status, i.e. the category of drained, nutrientrich soils may be too broad, encompassing varying nutrient and moisture regimes, which prevents the expectation of similar CO2 balances, especially in different climates. In our study, this aspect was evident, as the soil CO2 balance, regardless of drainage status, appeared to be influenced by the composition and relative proportions of the dominant tree species in combination with variation in soil nutrient conditions and organic matter quality, as indicated by the C:N ratio, across the study

The soils at our study sites represented a wide range, from highly mineralized soils close to the threshold of organic soil definition (Hiraishi et al., 2013) to deep peat (Figure 2Figure 2). Similarly, the WTLs varied widely, with sites ranging from an average WTL close to the soil surface to depths exceeding one meter (Figure 4Figure 4). However, no meaningful relationship was identified between WTL variation and soil CO<sub>2</sub> emissionsefflux, nor between mean WTL and soil CO<sub>2</sub> balance. Also, soil C and organic matter contents, or the depth of the organic layer were poor predictors of soil CO<sub>2</sub> balance. Probably the importance of these factors decreases over time since initial disturbance by drainage system implementation.

Rhet has been found to decrease over time following drainage (Qiu et al., 2021), except for the initially wettest, waterlogged hollow surfaces (Munir et al., 2017). There are few long-term monitoring or chronosequence studies, but those suggest that the most intensive period of soil C loss following drainage is the first decade/decades (Hargreaves et al. 2003; Vanguelova et al., 2019). During that period, also peat subsidence following drainage is highest (Lukkala 1949), and labile substances in peat that due to lowering WTL and peat subsidence becomes exposed to oxic decomposition are largely lost, leaving more decomposition-resistant substrates behind (e.g., Jayasekara et al., 2025). Simultaneously, major changes in litter inputs and their decomposability take place (Straková et al., 2010, 2012). Considering the long period since drainage of our sites, dating back to around a century ago and potentially even to the mid-19th century (Zālītis, 2012), the initially high soil C loss due to drainage-induced increase in gross soil CO<sub>2</sub> emissions has likely been offset by enhanced biomass growth and the resulting increased litter inputs (Hommeltenberg et al., 2014). However, a comparison of soil CO<sub>2</sub> balances between drained and undrained sites still shows a negative impact of historical drainage (Table S11Table S-11). To more accurately assess the impact of drainage on soil CO<sub>2</sub> stock changesbalance and its evolution in time, long-term studies would be required.

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Soil nutrient conditions explained the observed CO2 balances better than WTL and soil C characteristics did. In our study, drained nutrient-rich soils were represented by Oxalis (Oxalidosa turf. mel.) and Myrtillosa Myrtillosa turf. mel.) site types. All our drained birch and alder sites and most of drained spruce sites belonged to Oxalis site type, but most of the pine sites belonged to the Myrtillosa Myrtillus site type. While the soil CO2 balance under the other species in drained sites was practically neutral during the study period, pine stands showed relatively high CO2 removals. In the MyrtillosaMyrtillus site type nutrient concentrations were, on average, 1.5 to 5.4 times lower compared to Oxalis sites. In addition, MyrtillosaMyrtillus sites had significantly lower pH levels and a higher C:N ratio (Figure 2Figure 2). The distinctive CO2 balance patterns between the site types suggest that, in addition to lower nutrient concentrations, increased soil acidity and differences in soil organic matter and litter input quality (decomposability) had a role in the observed CO2 removals in these sites. Previous studies have also reported a negative correlation between pH and soil C content (Zhou et al., 2019) and have linked soil acidification with increasing soil C stocks (Madsen et al., 2025; Marinos and Bernhardt, 2018). The relevance of soil chemical parameters in determining soil CQ2 balance was supported by RDA and PLSR analysis, revealing that soil pH and macronutrient concentrations were key parameters determining soil CO2 balance. Both soil pH and macronutrient concentrations at the sites may be influenced by vegetation the tree species (Reich et al., 2005; Dawud et al., 2016). Conifers, especially Scots pine, are linked with lowered soil pH (Reich et al., 2005), which may be a way to engineer the ecosystem to its own favour, as Scots pine unlike the other tree species found in our sites can also thrive in nutrient-poor peatland forests (e.g., Ohlson, 1995). Enhanced organic matter decomposition observed in deciduous stands may be attributed todriven by a priming effect (Kuzyakov et al., 2000), and whereas in coniferous stands, decomposition may be inhibited due to increased. The soil acidification in coniferous stands can both-be attributed to foliar-litter quality (Reich et al., 2005; Brock et al., 2019) caused by the litter. However, in undrained coniferous stands, soil pH was elevated clearly higher compared to drained sites, and comparablesimilar to that of undrained broadleaf sites (Figure 2). This is likely because undrained sites may receive more groundwater inputs that neutralize soil acidity than the drained sites. Our drained sites mostly had more acid soils than the undrained ones, a pattern that has previously been recorded for boreal drained peatland forests (Laiho & Laine, 1990). Apart from the reduced groundwater influence, the phenomenon has This may been explained by a reduction in soil buffering capacity resulting from the leaching and tree uptake of base elements such as Ca and Mg, as well as increased oxidation of both organic and inorganic compounds aeration following drainage, both of which can contributinge to a gradual increase in soil acidity over time (Laine et al., 20061995; Laiho & Pearson, 2016). However, in undrained coniferous stands, soil pH was comparable to that observed in deciduous stands.

Among the drained sites, soil in pine stands had the lowest mean soil CO<sub>2</sub> emissions efflux (Figure 6-Figure 6\_b). Additionally, nutrient availability correlated negatively with belowground biomass (bGV, FRP) confirming previous observations that

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greater belowground biomass is associated with reduced nutrient availability (Zhang et al., 2024). Higher CO<sub>2</sub> emissions efflux observed in Oxalis sites were consistent with the previous observations of higher organic matter decomposition rates typically observed in sites with high nutrient availability (Hiraishi et al., 2013; Shahbaz et al., 2022). However, an increased total soil CO<sub>2</sub> influx from litter was also observed in these sites during the study period, effectively offsetting soil C loss from Rhet. Another potential contributor to the stability of C stockspositive CO<sub>2</sub> balance in drained soils was soil compaction induced by drainage, as the increased BD of drained soil was found to be associated with lower Rhet emissions. The reason may be reduced soil porosity limiting gas exchange between the soil and the atmosphere (Ball, 2013; Novara et al., 2012). Thus, while drained soil may be prone to a higher decomposition rate, flux-driving processes seem to be countered by increased soil compaction.

In any case, the mean  $CO_2$  removals we estimated are uncertain and remain indicative, preventing a definitive conclusion that the soil functions as a  $CO_2$  sink. Some uncertainty in the results arises from the inherent variation of study sites eategorized categorized into different forest site types and drainage statuses; such variation is natural and cannot be considered erroneous (see, e.g., Westman & Laiho, 2000; and Ojanen et al., 2010). However, based on the observed patterns, we consider site stratification by drainage status and site type to be an appropriate approach for interpreting soil  $CO_2$  balance. This stratification captures key ecological differences that are relevant to C dynamics and supports meaningful comparisons across site conditions. The uncertainty in the results arises from the variety of included study sites. The results obtained reflect  $CO_2$  balances only for the study period, specific to the respective stands in their specific developmental stages and site conditions. They do not represent average ehanges in soil C stocks $CO_2$  balance over a longer timeframe, such as an entire forest management cycle. In similar conditions, organic soils have been found to result in C stock loss be a  $CO_2$  source following regenerative felling (Butlers et al., 2022; Korkiakoski et al., 2023). Therefore, if the impact of management practices were considered, the soil  $CO_2$  balance would likely shift towards reduced  $CO_2$  sequestration.

In addition, oOur CO<sub>2</sub> balance estimate does not include the impacts of dissolved organic carbon (DOC) leaching and methane (CH<sub>3</sub>) emissions. DOC leaching occurs from both drained and undrained organic soils; however, in drained sites, related C losses can be increased by 0.43 to 0.78 t C ha<sup>-1</sup> yr<sup>-1</sup> (Hiraishi et al., 2013). While CH<sub>4</sub> emissions from drained organic soils, including emissions from drainage ditches in temperate zone, are generally minor, averaging around 5.9 kg CH<sub>4</sub>-C ha<sup>-1</sup> year<sup>-1</sup>, C losses by CH<sub>4</sub> emissions from undrained soils are highly variable and uncertain, ranging from 0 to 856 kg CH<sub>4</sub>-C ha<sup>-1</sup> year<sup>-1</sup> (Hiraishi et al., 2013). Consequently, not accounting for the impacts of DOC leaching and CH<sub>4</sub> emissions when comparing the C balance of drained and undrained organic soils considerably increases the uncertainty, while these impacts potentially offset each other.

4.2 Soil CO<sub>2</sub> emission factors

The reported soil  $CO_2$  balance values can be directly used as EFs (Table 4). Considering the lack of sufficient evidence for differences in drained soil  $CO_2$  efflux (Table S9) or balance (Figure S4) between countries (see the Supplementary text), the use of regional soil  $CO_2$  EFs is recommended over country-specific values. For example, the mean soil  $CO_2$  balance for drained soils can be treated as EF of  $-1.06 \pm 0.45$  t  $CO_2$ -C ha<sup>-1</sup> year<sup>-1</sup>, which falls within the range of boreal forest organic soils  $CO_2$  EFs reported by previous studies (Table 4, Figure 11). The highest values observed in this study fall within the range reported in temperate-zone studies. The greater discrepancy with the temperate emission factors may be explained by the limited number of temperate studies available and their focus on a narrower range of site conditions compared to those represented in the Baltic countries.

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Table 4: Organic soil CO<sub>2</sub> emission factors (t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) of this study compared to those reported in previous studies. Values for the boreal and temperate zones are synthesised from the results of previous studies on nutrient-rich organic soils (Jauhiainen et al., 2023), while values for the hemiboreal zone reflect the EFs derived in this study. Abbreviations: NR – nutrient rich; NP – nutrient poor; Min and Max refer to the minimum and maximum values reported; Clmin and Clmax represent the lower and upper 95% confidence limits for the mean; N – number of estimates.

Climate zone	Drainage status	Mean	Min	Max	<u>CImin</u>	<u>CImin</u>	N	_
Boreal	Drained	0,71	<u>-3,70</u>	<u>7,86</u>	0,34	1,08	103	_
Hemiboreal	Drained	<u>-1,06</u>	<u>-5.69</u>	2.00	<u>-1,51</u>	<u>-0,61</u>	<u>19</u>	
Hemiboreal	Undrained	<u>-1,27</u>	<u>-3.80</u>	<u>1.35</u>	<u>-2.00</u>	<u>-0.54</u>	<u>7</u>	
Temperate	Drained	<u>1,61</u>	<u>-0,08</u>	<u>2,93</u>	<u>1,15</u>	<u>2,07</u>	<u>16</u>	

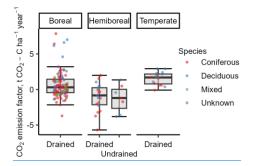


Figure 11: Organic soil CO<sub>2</sub> emission factors of this study compared to those reported in previous studies. Values for the boreal and temperate zones are synthesised from the results of previous studies on nutrient-rich organic soils (Jauhiainen et al., 2023), while values for the hemiboreal zone reflect the EFs derived in this study.

## 4.23 Total respiration

No significant differences in Rtot were observed between the countries, likely because the gradient in the mean air temperature from Estonia to Lithuania, ranging from 6.4 to 8.5 °C, was not substantial enough to introduce distinguishable differences. Nevertheless, while temperature is generally recognized recognised as a strong factor influencing soil respiration and its variation, it should not be regarded as the sole predictor of respiration. Relationships observed in one region may not directly apply to another, as differences in soil moisture (Jovani-Sancho et al., 2018) or nutrient status, as discussed earlier, can significantly alter the respiration dynamics. Similarly, no clear impact of dominant tree species on Rtot was found. This points to a minor role of dominant tree species on emissions. However, there is some evidence that emissions in undrained sites tended to be higher in deciduous stands, particularly alder stands, according to the measured instantaneous emissions. The enhanced soil CO<sub>2</sub> efflux observed in the presence of alder can probably be attributed to the symbiotic nitrogen fixation associated with these trees (Warlo et al., 2019), which increases nitrogen availability in the soil. Nitrogen availability, in turn, can stimulate decomposition processes, leading to a higher rate of CO<sub>2</sub> release. However, we did not observe increased nitrogen levels in neither the soil nor soil water of alder sites. Although statistically unconfirmed, a tendency can be noticed that in drained sites Rtot emissions tend to be higher in birch stands, but lower in pine forests. Also, previous studies indicated that deciduous stands show higher CO<sub>2</sub> emissions (Jauhiainen et al., 2023).

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While it was found that both drainage status and WTL threshold above or below 30 cm can be used as a predictor of Rtot, a meaningful correlation between WTL and Rtot was not found. Furthermore, although the absolute variation of the WTL was higher in drained sites, the relative variation in both WTL level and Rtot was indifferent to the drainage status. The observations suggest that higher WTL conditions in undrained sites, while decreasing Rtot emissions, do not guarantee higher resilience to moisture regime disturbances, i.e., more stable emissions. The main reason is that the presence of drainage ditches is not the only factor constraining WTL both spatially and temporally, and in undrained sites too, WTL frequently falls below 30 cm (Butlers et al., 2023) ensuring oxic conditions in soil layers containing labile organic matter. Furthermore, this typically happens in summer (Butlers et al., 2023) when increased temperatures further promote organic matter mineralization. The role of WTL dynamics is reflected also in PCA, showing higher dispersion of drained sites likely due to higher variation in WTL depths. This may be the reason complicating the quantification of relationships between Rtot and the affecting factors, especially in drained sites. The wide range of mean WTL measured at the drained sites also helps explain why Rtot at these sites is not necessarily significantly higher compared to undrained sites.

To achieve accurate Rtot annualization using data from periodic flux measurements, data interpolation through modelling approaches was applied. For Rtot interpolation we compared nonlinear models and linear models after logarithmic or Box-Cox transformation. Both the advantages and shortcomings of these data transformation methods and modelling approaches have been reported in previous studies. (Box and Cox, 1964; Khomik et al., 2009; Liaw et al., 2021; Moulin et al., 2014; Wutzler et al., 2020; Yueqian, 2020). Although the bias in predicted annual Rtot varied among study sites, the overall impact of different flux modelling approaches on estimated mean annual Rtot of drained and undrained sites was minimal. Specifically, the mean bias of results obtained through the implementation of the Box-Cox transformation compared to other approaches was -2±9%. Thus, while such an impact has been observed in previous studies, the skewing of results due to the annualization of respiration was not identified in our study.

## 4.3-4 Soil heterotrophic respiration

We used a Rhet value derived from Rtot - Rhet' - as the soil CO2 efflux. Rhet' was derived from Rtot empirically using the Rhet/Rs data from a large dataset (Jian et al. 2021), thereby reducing the effect of potential systematic and random errors in individual studies. Such empirical derivation of Rhet has been acknowledged to be an applicable approach and has been utilized in previous studies (Jauhiainen et al., 2019, 2023). To elaborate the recalculation model we used Rhet and Rs values from the database (Jian, J. et al., 2021) on forest soil flux in the boreal zone, as existing experience suggests that organic soil emissions in hemiboreal forests are more likely to align with boreal rather than temperate conditions (Bārdule et al., 2022; Butlers et al., 2022; Dubra et al., 2023; Heikkinen et al., 2023; Jauhiainen et al., 2023; Krasnova et al., 2019; Lazdiņš et al., 2024). The choice of using only boreal data tends towards estimating higher Rhet', compared to the use of temperate data, as illustrated in Figure S-2. This approach aimed to avoid the underestimation of soil CO2 efflux. The mean share of Rhet acquired using boreal data was 0.65±0.04 while using temperate data - 0,60±0,15, or around 10% difference. Accordingly, the Rhet proportion values we applied were higher than the typically observed range of 0.5 to 0.6 (Bond-Lamberty et al., 2004; Hanson et al., 2000), demonstrating that our approach avoided underestimating Rhet.

The role of ground vegetation autotrophic respiration in Rtot increases with its biomass (Munir et al., 2017). Therefore, the risk of underestimating Rhet by using Rhet' is further reduced because the Rhet/Rs ratio used to recalculate Rtot to Rhet' does not account for the impact of autotrophic respiration from aboveground vegetation, consequently, the approach tends to rather overestimate the Rhet. This aspect should be considered when assessing our results. When estimating the impact of historical drainage on the soil  $CO_2$  balance by comparing sites by drainage status, this bias was likely negligible, because the mean ground vegetation biomass did not significantly differ between drained and undrained sites ( $\Delta$ =0.53 t dm. ha<sup>-1</sup>).

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The applicability of the approach is supported by the comparability of the estimated Rhet' values with Rhet reported in previous studies (Bond-Lamberty and Thomson, 2010). We estimated Rhet' of drained soil to be mean 4.30±1.20 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>, which is slightly higher than both mean Rhet of forest organic soil found in the boreal zone (4.09 t C-CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) (Ojanen et al., 2010) and in a broader regional scale (3.71±0.53 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) (Jian, J. et al., 2021). When attempting to correct for the approximated overestimation of Rhet introduced by trenching (see the Supplementary text), the resulting mean Rhet would be 2.4 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>, which is considerably lower than the Rhet' values we used in the CO<sub>2</sub> balance estimates.

## 4.4-5 Carbon influx by litter inputs

In the estimation of  $CO_2$  influx, we considered data only for fLF, aGV, bGV, and FRP, excluding cLF, MP and dwarf shrubs. This approach was chosen because the  $CO_2$  emissions produced in decomposition of these litter types was directly included in the measured Rtot. For instance, cLF due to its dimensions and scarce coverage could not be objectively included in chamber measurements. Furthermore, while fLF is relatively uniform in forest areas, the coverage of mosses and dwarf shrubs is not always so, therefore it is necessary to know their area of projection to be included in the  $CO_2$  balance estimation. One solution for incorporating the "missing" litter inputs would be to use modelling approaches (Alm et al., 2023). However, we did not attempt to include those litter sources in the soil  $CO_2$  balance estimation, as doing so would have introduced additional uncertainty. We estimated that annual moss production was  $22\pm10\%$  of the average total moss biomass of  $0.50\pm0.09$  kg dry matter (dm.) m<sup>-2</sup> measured for moss patches in our sites. Thus, mosses could potentially provide annual litter inputs reaching up to  $0.98\pm0.25$  t dm. ha<sup>-1</sup> if their cover was 100%. The mean annual cLF was  $0.74\pm0.23$  t dm. ha<sup>-1</sup>. Therefore, the litter input estimates (mean  $4.70\pm1.43$  t  $CO_2$ -CC ha<sup>-1</sup> year<sup>-1</sup>) used in the calculation of soil  $CO_2$  balance likely led to overestimated soil C loss. This highlights the need to consider all litter input components in further studies, even though that may clearly increase the workload.

## 5. Conclusions

Although all soils in our study sites were classified as nutrient-rich based on forest site type taxonomy, they included a wide variety, ranging from those near the threshold of organic soil definition to soils with deep peat layers. Consequently, the soils exhibited broad variability in pH, macronutrient concentrations, and C:N ratio. That in turn contributed to the observed behaviour of the soils demonstrating both CO2 sink and source dynamics under both drained and undrained conditions. During the study period, drained soils under birch, black alder, and Norway spruce remained CO2 neutral, while in pine stands the soils were CO2 sinks, presumably due to the significantly lower nutrient availability limiting mineralization of the organic matter. The disparity in soil nutrient conditions also explains why some undrained soils, characterized by relatively high nutrient availability, acted as CO2 sources. These findings highlight the potential to improve predictions of soil CO2 balance by complementing the broad "nutrient-rich" soil classification - typically assigned using site vegetation as a proxy - with quantitative measurements of soil nutrient status.

The study provides a notable contribution through both the plot-level summary and raw data on soil CO<sub>2</sub> influx and efflux. The spatial coverage of the study sites, along with the variability in stand characteristics, soil properties, and water-table level dynamics, provides input for synthesising dynamic empirical soil CO<sub>2</sub> balance models that depend on drainage status, meteorological conditions, soil chemistry, and stand-related parameters. The reported soil CO<sub>2</sub> balance values can be directly used as EFscmission factors. For example, the mean soil C balance for drained soils can be treated as EF of 1.06±0.45 t C ha<sup>-1</sup> year<sup>-1</sup>, which falls within the 95% confidence interval range (1.46 to 0.88 t C ha<sup>-1</sup> year<sup>-1</sup>) of boreal forest EFs synthesised from previous studies. The results can be used as input for constructing emission factors or more elaborate forest organic soil CO<sub>2</sub> emission prediction methods. Additional research is necessary still needed to expand the dataset for

- 744 establishing robust quantitative relationships that can be used to reliably identify and predict whether organic soils function as
- 745 CO2 sinks, sources, or remain in neutral balance, depending on site-specific conditions and annual weather variations.

#### 746 Data availability

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747 Data used for carbon balance estimations is available at https://doi.org/10.5281/zenodo.14968843

#### Author contributions

- 749 KS, JJ, RL, AL and KA developed a harmonized harmonised methodology. ABu, DČ, TS and MKS managed and processed
  - the study data. ABu wrote the original manuscript, with significant reviewing contributions from RL. JJ, TS, ABā, IL, VS,
  - HV, IL, AH and AJ provided critical reviews and edits to the manuscript.

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  - Forest Management and Value Chain for Latvia's Growth: New Forest Services, Products and Technologies" (No.: VPP-ZM-
  - VRIIILA-2024/2-0002).

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## Supplementary text

#### 1. Soil heterotrophic respiration interpretation issues

#### 1.1. Soil heterotrophic respiration measurements

Heterotrophic soil respiration (Rhet) was measured by applying the manual closed dynamic dark chamber method (Denmead, 2008; Hutchinson and Livingston, 1993). For each measurement, a 60 x 90 cm (W x L) trenched (Ngao et al., 2007) locations was prepared at the end of the previous year's growing season to a depth of at least 40 cm, using geotextile on the sides to prevent root ingrowth and by removing alive vegetation and litter layer. In each subplot, measurements were done in 3 replicates, in total, nine measurement locations in each study site. CO<sub>2</sub> flux monitoring was made by EGM5 portable CO<sub>2</sub> gas analyser (PP Systems, Amesbury, MA, USA) and a fan-equipped chamber (area 0.07 m², volume 0.017 m³) placed in the centre of the trenched surface without using a collar. The measurement data was stored at a 1 Hz frequency over a three-minute period in each measurement. Rhet measurements were made in parallel with Rtot measurements. Between the measurement campaigns, Rhet measurement areas were covered with geotextile, which was covered with an equivalent quantity of debris and litter as nearby soil, aiming to simulate natural conditions.

Before flux calculations, the first 15 seconds of the measurement data were discarded to avoid potential error in the results due to the placing of the chamber in the soil. To estimate the slope of the linear regression equation representing  $CO_2$  concentration change in time, the same approach as for Rtot was used (1(4)).

## 1.2. Identification of Rhet overestimation

We observed an inconsistency between Rhet and Rtot, as direct comparison showed that Rhet (mean 13.0 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) exceeded Rtot by an average of 5.8±3.1 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>. The difference is evident in the observed relationship between Rhet or Rtot and temperature, indicating higher Rhet emissions at the same temperatures (Figure S.9). Rhet should not be greater than Rtot, as Rtot includes both Rhet and autotrophic respiration of plants.

The main errors in Rhet and Rtot measurements can be introduced during gas sampling and analysis (instrumental method), by site preparation (e.g., collar installation or trenching), or by site-specific factors. To identify the reason for the discrepancy between Rtot and Rhet, we undertook several steps, including investigating the comparability of the instrumental methods and analysing potential sources of error.

In some studies, it has been observed that flux can be underestimated due to nonlinearity in gas concentration increase generally caused by either the small chamber volume or by extended measurement periods (Kutzbach et al., 2007; Nakano et al., 2004; Nomura et al., 2019). Therefore, we assessed method comparability and concentration increase linearity in two steps: 1) by initial quality assurance procedure; 2) by quality control during flux calculation, we checked the linearity of each flux measurement, both visually and using R<sup>2</sup> as an indicator:

1) First, we ensured that the instrumental methods were comparable and investigated whether the discrepancy was due to Rtot underestimation. To compare the methods, we conducted a quality assurance procedure designed to eliminate the influence of site-related factors. Both instrumental methods were therefore compared under controlled conditions. In the laboratory, under constant organic soil temperature (17 °C) and moisture (50 %) conditions characteristic of natural soil conditions in a warm season, we simultaneously collected gas samples in glass vials from the Rtot measurement chamber for CO<sub>2</sub> concentration analysis with a gas chromatograph, while also measuring changes in CO<sub>2</sub> concentration using a portable gas analyser employed in the Rhet measurements. Repeated measurements (n = 6) revealed that the gas concentration changes in the chamber remained linear throughout the 30-minute measurement period. The relative standard deviation for flux measurements using a gas collection in glass vials and testing with a

gas chromatograph was 10%, while with the portable gas analyser, it was 6%. The gas fluxes obtained by the portable analyser were, on average,  $10\pm7\%$  lower. Consequently, the procedure shows that Rtot measurements could be subject to relative overestimation; however, considering the differences in measurement accuracy and precision, these differences are not significant. Hence, the comparability demonstrated alongside the observed linearity of gas concentration changes in the Rtot chamber, showed that both instrumental methods are comparable and excluded the possibility of Rtot underestimation due to longer measurement times. The nonlinearity can be induced by increasing pressure inside the chamber over time (Silva et al., 2015), consequently, this phenomenon may be more pronounced when using chambers with a small volume. For this reason, it has been advised to use small chambers to emphasize nonlinearity (Kutzbach et al., 2007). Likely, we did not identify nonlinearity as a cause for potential Rtot underestimation in our study due to the relatively large chambers used (area 0.196 m², volume 0.0655 m²). Nevertheless, it has been also advised that linearity itself should not be regarded as an indicator of measurement accuracy (Nakano et al., 2004).

2) During flux calculation, linear regression was applied to establish a relationship between CO2 concentrations and the elapsed time since chamber closure for each measurement. The data was then screened to identify deviations from the expected trend, with erroneous measurements being removed. We used a regression coefficient of determination (R2) of 0.9 (p<0.01) as a quality threshold, except in cases where the difference between the highest and lowest measured CO<sub>2</sub> concentrations in the chamber was less than the method's uncertainty of 20 ppm. Since an insignificant amount of data was discarded during this process, it reaffirmed that nonlinearity was not a concern, both during the quality assurance procedure and throughout the entire study. However, there is some disagreement regarding the use of R2 as an indicator for identifying linearity. Recommendations exist against using R2 as an indicator (Kutzbach et al., 2007), however, these suggestions apply to continuous measurements, where the large volume of field measurement data can indeed lead to false indications of good linearity. In such cases, nonlinear regressions may be appropriate (Kutzbach et al., 2007; Nakano et al., 2004). In cases, such as ours, involving manual chamber usage with a limited number of measurements, nonlinear regression can lead to overfitting an unsuitable trend, making linear regression a safer option. For these reasons nonlinear regression is not recommended for Rtot measurements, as plant responses can be highly variable and unpredictable (Kutzbach et al., 2007). Therefore, we consider our flux estimation approach well suited for this context, as it incorporates rigorous quality control measures to ensure accuracy and reliability in the results.

To summarize, quality assurance demonstrated that nonlinearity was not a concern, while quality control validated the reliability of individual measurements. A comprehensive evaluation of these assessments led to the conclusion that Rhet measurements exceeding Rtot measurements were not because of underestimation of Rtot, but rather due to an overestimation of Rhet

# 1.3. Sources of Rhet overestimation and reduced precision

In investigating the causes of errors in Rhet measurements, we identified the main sources of accuracy errors that led to overestimation, as well as factors that led to additional precision errors, further increasing measurement uncertainty. Both types of errors were introduced by the trenching and the approach used to simulate natural conditions between measurements by covering the trenched area with geotextile, topped with a litter layer:

1) To investigate the impact of geotextile cover removal, we compared Rhet measurements taken immediately after removing the geotextile with those taken one hour later. We observed that, on average, the results were lower by a factor of  $1.6 \pm 1.1$  after one hour, with a range of variation between 0.79 and 1.8. This means that the textile reduced

the rate of CO<sub>2</sub> diffusion from soil to air, and the textile should have been removed well before the onset of the measurements.

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- 2) Soil trenching was conducted before winter, killing the roots within the trenched area. By spring, when measurements began, the cut roots decomposition was reflected in Rhet measurements. To assess the potential impact of the cut roots, we collected total belowground biomass samples from the top 40 cm of soil using a soil probe and found that the total root biomass in drained and undrained sites was, on average, 39.3±11.1 and 52.7±18.7 t ha<sup>-1</sup>, respectively. Considering that around 50% of roots can decompose over two years (Moore et al., 1999; Straková et al., 2012), the study period's underground biomass decomposition could have led to a significant artificial increase in measured Rhet of drained (11.67 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) and undrained (14.37 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) soils. Specifically, the decomposition of resulted killed roots may have raised the Rhet value by 4.90 and 6.59 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>, respectively. Although this estimation is rough, it quite well illustrates the potential overestimation generated by root decomposition, especially since the measured Rhet in the study exceeded the Rtot by an average of 5.8±3.1 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>.
  - Additional challenges in Rhet interpretation arose due to altered soil conditions caused by trenching, as indicated by the reduced correlation between Rhet and soil temperature. The reduced correlation points towards that further errors in Rhet measurements were introduced by the effects of trenching on soil temperature and, consequently, likely also on moisture levels, in spite of the use of the geotextile cover. Reduced correlation shows that the temperature readings, taken at the centre of the subplot in the untrenched area, did not accurately reflect the temperature within the trenched sections. The correlation (r) between soil temperature and Rhet ranged from a mean of  $0.28 \pm 0.12$  to  $0.51 \pm 0.12$ . This was significantly lower than the correlation found with Rtot (r=0.86), thus indicating altered soil conditions in the trenched areas. The correlation between temperature and flux should be comparable for both Rhet and Rtot since both root and microbial respiration are temperature-dependent (Davidson and Janssens, 2006). Furthermore, correlation with Rhet can be expected to be even stronger than with Rtot, as the correlation with Rtot is reduced by the variability in autotrophic respiration (Kutzbach et al., 2007). In our case, the reduced Rhet correlation seems to be generally caused by high emission outliers at elevated soil temperatures. These outliers lead to considerable overestimation of Rhet by flux interpolation models, which are constrained to predict reduced emissions at increased temperatures when soil moisture conditions do not favour microbial activity (Khomik et al., 2009; Yueqian, 2020). Soil respiration is influenced not only by soil temperature but also by water availability (Davidson and Janssens, 2006). Not accounting for moisture regime in the interpolation of flux measurement results can lead to overestimation as Rhet prediction models have to be available to predict lower emissions at even increased temperatures if soil moisture is limiting microbial activity (Jovani-Sancho et al., 2018; Liaw et al., 2021). As the soil temperature and moisture were measured in undisturbed areas of the site, our study design did not account for potential differences in environmental parameters, such as soil temperature and moisture, between trenched and untrenched areas. Therefore, we were unable to address the issue empirically, and the temperature measurements were not applicable to Rhet measurements, preventing both data correction and interpolation. Trenching-altered soil conditions (Ojanen et al., 2012) is a well-known source of Rhet measurement error (Chin et al., 2023; Comstedt et al., 2011; Díaz-Pinés et al., 2010; Epron, 2010; Ngao et al., 2007; Ryhti et al., 2021; Savage et al., 2018; Subke et al., 2006). Due to the challenges in overcoming Rhet measurement errors, root exclusion methods, including trenching, are not entirely satisfactory. As a result, the reliability of using these methods to accurately measure Rhet remains questionable. Given the questionable accuracy and precision in quantifying soil heterotrophic respiration, total soil respiration (Rtot) or soil respiration (Rs) should be considered as an alternative proxy for evaluating soil CO2 emissions. Rtot and Rs, by causing less soil disturbance, provide more reliable measurement results. Moreover, using these results for relative comparisons, which are necessary for investigating the impact of anthropogenic emissions and management practices

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on emissions, helps mitigate biases introduced by autotrophic respiration, particularly in cases where biomass is similar across the compared sites.

Considering the significant impacts formed by time from trenching prior to monitoring period and timing of geotextile removal during measurements on the overestimation of Rhet, the primary cause of overestimation could not be definitively identified. The general overestimation likely resulted from the cumulative effects of root decomposition and the CO2 flux surge following geotextile removal at the start of flux measurements. Attempts to correct these effects would introduce substantial uncertainties. Due to the combined influences of these factors, which cannot be separately isolated, and the observed high variability in overestimation, no robust empirical correction could be applied to obtain instantaneous Rhet values, that could be reliably used for Rhet interpolation. Furthermore, the ability to perform proper interpolation was reduced due to the unavailability of temperature measurements in the trenched areas, further hindering reliable flux annualization and subsequent soil CO2 balance estimation. Thus, we concluded that a more reliable soil CO2 balance result was achieved by empirically recalculating Rtot to Rhet. This approach introduced only one additional uncertainty related to the Rtot-to-Rhet conversion, estimated to be approximately 0.32 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>, based on the RMSE of the applied conversion model. In comparison, using direct Rhet measurements for soil CO2 balance estimation would introduce at least seven additional sources of uncertainty. These include root biomass in the trenched area, geotextile removal correction, turnover rates of trenched roots, ground vegetation, tree fine roots, and foliar litter. Further uncertainty would be compounded by the error introduced from using temperature measurements from the untrenched area for Rhet interpolation, as these did not accurately represent the temperature at the trenched site.

## 1.4. Summary and conclusions

Based on the quality procedures performed, we concluded that the instrumental methods were not responsible for the discrepancy between Rhet and Rtot results. Furthermore, the quality assurance procedure suggested that Rtot was more likely to be potentially slightly overestimated rather than underestimated. We found sufficient evidence of errors, including overestimation, in the Rhet results to support the conclusion that using the Rtot-to-Rhet conversion approach was a more reliable method for annual soil carbon balance estimation. Similar Rhet interpretation issues have been acknowledged by previous studies (Ngao et al., 2007; Epron, 2010; Savage et al., 2018; Chin et al., 2023).

Given the complexity and uncertainty of the Rhet overestimation found, reasonable data corrections were not possible, and thus, using the measured Rhet in CO<sub>2</sub> balance estimation would have resulted in highly unreliable outcomes. The experience emphasizes the necessity of measuring root biomass, stratified by root diameters or branching orders, and conducting a proper root decomposition study to account for the decomposition of killed roots to enable correction of the measured Rhet for CO<sub>2</sub> emissions from trenched root decomposition. It is crucial to perform simultaneous Rtot or Rs measurements in untrenched soil alongside Rhet measurements, as well as soil temperature and moisture measurements at both locations. Since root decomposition is the main concern, the trenching method may be more challenging in regions with increased biomass growth.

# 2. Country as a CO2 balance impacting factor

The mean measured Rtot of undrained soil was smaller in both Latvia (mean 57±6 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>) and Lithuania (mean 55±6 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup>) compared to Rtot from drained soil in the Baltic states ranging from mean 72±4 to 79±5 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> (Figure S4, g). Estimated annualized Rtot from neither drained sites (overall mean 6.21±0.43 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) nor undrained sites (overall mean 4.38±1.20 t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) differed significantly between countries and were generally higher from drained soils (Figure 6, h). Soil CO<sub>2</sub> balance estimates showed higher mean soil CO<sub>2</sub> removals for both drained and undrained sites in Lithuania than those in Estonia and Latvia. This was primarily attributed to greater CO<sub>2</sub> influx from ground vegetation litter (Figure 8). However, the sites in Lithuania also stand out due to greater uncertainty in both

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 $CO_2$  influx and efflux, resulting in the soil  $CO_2$  balance in drained sites across the countries being equivalent within the margin of error (Figure 9a). The low number of undrained sites in Lithuania (n=2) limited the ability to investigate the patterns behind the observed lower soil  $CO_2$  efflux and higher aGV litter, contributing to significantly higher soil  $CO_2$  removals ( $\pm 3.70 \pm 0.11$  t  $CO_2$ -C ha<sup>-1</sup> year<sup>-1</sup>) compared to sites in Latvia ( $\pm 0.30 \pm 0.56$  t  $\pm 0.5$ 

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# Supplementary tables

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**Table S-1: Study stands characteristics.** Abbreviations: species – dominant tree species; WTL – mean water table level, cm; A – age, years; D – mean tree diameter, cm; H – mean tree height, m; BA – basal area, m² ha¹. Site types: Dr - *Dryopterioso-caricosa*; Ox - *Oxalidosa turf. mel.*; My - *Myrtillosa turf.mel.* 

Site identifier	Latitude	Longitude	Species	Site type	WTL	Organic layer, cm	A	D	Н	BA	
			Drain	ned sites							
LTC106	54.79312	24.07451	Alder	Ox	-56	50	30	12	13	26	
EEC108	58.25010	26.29040		Ox	-23	35	80	21	20	36	
LVC108	57.32216	26.06411	Birch	Ox	-30	90	24	14	16	15	
LVC115	56.69388	25.68767		Ox	-96	56	33	13	16	21	
EEC106	58.43755	26.35558		Ox	-70	70	35	12	13	18	
LTC105	54.79010	24.08022		Ox	-94	50	43	22	18	23	
EEC109	58.34765	26.47599		Ox	-57	90	45	16	16	22	
EEC105	58.42870	26.37470	Pine	My	-82	90	60	22	18	17	
LVC110	56.62838	24.11370		My	-76	35	81	12	12	43	
LVC107	56.78452	23.86247		Ox	-112	27	101	22	21	48	
LVC116	57.26889	25.99285		My	-31	165	141	14	14	34	
LVC313	57.26889	25.99285		My	-53	138	141	14	13	39	
LVC104	56.99978	24.65896	Spruce	Ox	-80	50	40	22	20	33	
LVC105	56.39288	25.65370		Ox	-31	86	55	22	19	22	
LVC106	56.39495	25.65134		Ox	-42	95	55	24	21	21	
EEC104	58.43861	26.35394		Ox	-66	80	60	20	17	18	
LTC104	54.79426	24.08077		My	-63	50	70	20	17	27	
LVC308	57.34717	25.92568		Ox	-50	212	141	25	23	36	
LVC112	57.33731	26.02635		Ox	-31	68	162	10	10	21	
			Undra	ined sites							
LTC109	54.54109	23.61140	Alder	Dr	-10	150	44	16	16	30	
LVC109	56.57378	24.82944		Dr	-11	100	74	28	28	36	
LTC108	54.54396	23.56578	Birch	Dr	-7	150	44	21	20	22	
LVC111	57.29058	25.99874		Dr	-14	230	61	8	9	23	
LVC309	57.27915	25.85371	Spruce	Dr	-17	133	81	21	20	34	
LVC311	57.27887	25.85441		Dr	-13	205	88	18	17	42	
LVC312	57.31164	25.93609		Dr	-17	221	96	17	15	25	

Table S-2: Relative occurrence and mean projective cover of most common ground vegetation species in the study sites. The species are listed in descending order based on a score calculated as the sum of their cover and occurrence.

Drain	ed		Undrained			
Species	Cover	Occurrence	Species	Cover	Occurrence	
		S	hrub layer			
Picea abies	35	20	Picea abies	19	19	
Frangula alnus	21	16	Salix sp.	13	29	
Fraxinus excelsior	28	7	Alnus glutinosa	11	24	
Betula pendula	14	20	Sorbus aucuparia	11	14	
Salix sp.	23	9	Populus tremula	10	10	
Sorbus aucuparia	12	18	Betula pendula	5	5	
Viburnum opulus	20	9	-	-	-	
Prunus padus	18	7	-	-	-	
Populus tremula	20	2	-	-	-	
		Her	paceous layer			
Oxalis acetosella	20	54	Epilobium hirsutum	39	23	
Rubus idaeus	17	50	Epilobium parviflorum	18	39	
Carex echinata	53	2	Galium palustre	12	44	
Vaccinium myrtillus	18	36	Cirsium oleraceum	29	14	
Urtica dioica	17	28	Deschampsia cespitosa	21	22	
Stellaria nemorum	18	26	Filipendula ulmaria	20	23	
Dryopteris carthusiana	8	29	Carex cinerea	24	16	
Mycelis muralis	3	30	Rubus idaeus	15	24	
Poa angustifolia	28	3	Athyrium filix-femina	16	22	
Carex remota	25	5	Carex sp.	12	26	
Geranium robertianum	6	22	Oxalis acetosella	3	32	
Mercurialis perennis	26	22	Salix sp.	10	23	
			•			
Vaccinium vitis-idaea	10	15	Chamaenerion angustifolium	30	2	
Cirsium oleraceum	20	5	Galium uliginosum	30	1	
Carex cespitosa	18	5	Dryopteris carthusiana	5	26	
Trientalis europaea	2	21	Chrysosplenium alternifolium	4	26	
Calamagrostis arundinacea	17	6	Scirpus sylvaticus	25	4	
Galeopsis tetrahit	4	19	Luzula pilosa	9	20	
Phragmites australis	19	3	Carex vesicaria	25	1	
Betula pendula	9	12	Urtica dioica	7	18	
Viburnum opulus	20	1	Stellaria nemorum	8	17	
		Moss	and lichen layer			
Hylocomium splendens	34	45	Hylocomium splendens	14	30	
Plagiomnium cuspidatum	16	38	Eurhynchium hians	17	26	
Pleurozium schreberi	18	35	Climacium dendroides	10	26	
Polytrichum commune	40	1	Rhytidiadelphus triquetrus	10	25	
Eurhynchium angustirete	13	24	Plagiomnium cuspidatum	4	23	
Plagiomnium affine	5	27	Cirriphyllum piliferum	7	19	
Rhytidiadelphus triquetrus	10	16	Plagiomnium affine	3	22	
Dicranum polysetum	3	24	Pleurozium schreberi	5	19	
Brachythecium rutabulum	20	2	Dicranum scoparium	4	14	
Dicranum scoparium	2	20	Polytrichum juniperinum	9	8	
Cirriphyllum piliferum	14	7	Eurhynchium striatum	10	4	
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 Table S-3: Meteorological conditions during the study period (Estonian Environment Agency. Climate normals, 2024; Latvian Environment, Geology and Meteorology Centre. Climate normals, 2024; Lithuanian Hydrometeorological Service. Climate normals, 2024)

D .	37 111	Estonia		Lat	tvia	Lithuania	
Parameter	Variable	1st year	2 <sup>nd</sup> year	1st year	2 <sup>nd</sup> year	1st year	2 <sup>nd</sup> year
Annual air	Mean	6.4	6.9	7.0	7.3	7.8	8.5
temperature. °C	Range	-22.427.2	-14.9, 25.6	-31.0, 33.7	-23.2, 33.7	-12.0, 27.2	-11.3, 25.0
	Climate normal <sup>(1)</sup>	6	.4	6	.8	7.	4
Annual precipitation. mm	Sum	597.0	472.9	676.3	685.8	639.8	533.6
	Climate normal <sup>(1)</sup>	6	62	68	86	69	5

<sup>(1) 30-</sup>year averages for climate variables

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Table S-4: Laboratory standard methods used for sample analysis.

Parameter	Unit	Method principle	Standard method
1	Analysis o	of soil and biomass samples	
Bulk density	kg m <sup>-3</sup>	Gravimetry	LVS ISO 11272:2017
pH	unit	Potentiometry	LVS ISO 10390:2021
Total C	g kg <sup>-1</sup>	Elementary analysis (dry combustion)	LVS ISO 10694:2006
Total N	g kg <sup>-1</sup>	Elementary analysis (dry combustion)	LVS ISO 13878:1998
Ash content	g kg <sup>-1</sup>	Gravimetry	LVS EN ISO 10693:2014
Concentrated HNO3 extractable potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P)	g kg-l	ICP-OES	LVS EN ISO 11885:2009
	Anal	ysis of water samples	
рН	unit	Potentiometry	LVS EN ISO 10523:2012
DOC	mg L <sup>-1</sup>	Catalytical combustion with infrared detection	LVS EN 1484:2000
Total N	mg L <sup>-1</sup>	Catalytical combustion with chemiluminescence detection	LVS EN 1484:2000
NO <sub>3</sub> -, PO <sub>4</sub> <sup>3</sup> -	mg L <sup>-1</sup>	Ion chromatography	LVS EN ISO 10304 – 1:200
$\mathrm{NH_{4}^{+}}$	mg L <sup>-1</sup>	Photometry	LVS ISO 7150-1:1984
K, Ca, Mg	mg L-1	Flame atomic absorption spectrometry	LVS EN ISO 7980:2000

Table S-5: Biomass (t dm.  $ha^{-1}$ ) measurement results (mean±SD) stratified by drainage status and country. Abbreviations: aGV and bGV – aboveground and belowground biomass of herbaceous ground vegetation, respectively, S – shrubs, FR- fine roots, FRP – fine root production, M – moss, MP – moss production, fLF – foliar fine litter, cLF – coarse woody litter, RB – total root biomass in depth 0-40 cm. NE – not estimated. \*Data used for soil  $C\Omega$ : balance estimation, \*\* assuming 100% moss cover.

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Cotoonia		Drained		Undr	ained
Category	EE	LT	LV	LT	LV
aGV*	$0.79\pm0.33$	1.34±2.98	2.38±0.63	4.64±14.53	1.44±0.63
bGV*	NE	4.24±2.01	2.52±0.96	2.3±7.74	$2.47{\pm}1.26$
S	$0.36 \pm 0.36$	2.04±2.42	NE	4.27±24.24	NE
FR	NE	NE	NE	NE	NE
FRP*	NE	$2.51\pm5.35$	$2.54{\pm}1.02$	NE	$1.08\pm0.76$
M**	4.8±2.49	NE	NE	NE	NE
MP**	$0.92 \pm 0.48$	NE	$0.87 \pm 0.32$	NE	$1.01\pm0.31$
fLF*	$3.66 \pm 0.64$	3.7±1.2	2.9±0.69	$3.28 \pm 13.77$	$2.23{\pm}1.23$
cLF	$1.39\pm0.65$	$0.33 \pm 0.62$	$0.54 \pm 0.26$	1.35±6.61	$0.55 \pm 0.68$
RB	NE	NE	39.3±11.1	NE	52.7±18.7

Table S-6: Characteristics of total soil respiration (Rtot) prediction models used for interpolation of Box-Cox transformed hourly emissions. Abbreviations: R10 - Rtot when soil temperature is 10 °C at 10 cm depth, RMSE – root mean square error of the model prediction. RMSE improvement and R10 increase are relative differences of corresponding model characteristics compared to linear models fitted using log10 transformed data. Model describes:  $\frac{Rtot^{lambda-1}}{lambda} = a*T+b. \text{ where:}$ 

 $Rtot-soil\ instantaneous\ total\ respiration\ (mg\ CO_2\text{-}C\ m^{-2}\ h^{-1}), lambda-lambda\ value\ used\ for\ Rtot\ data\ transformation,\ a-coefficient\ a\ of\ linear\ model,\ b-coefficient\ b\ of\ a\ linear\ model,\ T-soil\ temperature\ at\ 10\ cm\ depth\ (^{\circ}C).$ 

Site identifier	Coefficient a	Coefficient b	$\mathbb{R}^2$	R10	RMSE	RMSE improvement	R10 increase
LVC104	0.4903	5.6964	0.71	89	35	7%	10%
LVC105	0.74315	4.44323	0.84	116	59	8%	19%
LVC106	0.7318	5.0111	0.85	127	59	14%	20%
LVC107	0.53295	4.32402	0.70	72	36	6%	10%
LVC108	0.70835	3.45753	0.78	88	45	20%	26%
LVC109	0.68144	3.27991	0.80	80	43	34%	24%
LVC110	0.60544	3.41507	0.72	69	31	11%	15%
LVC111	0.70313	2.31005	0.78	67	31	7%	28%
LVC112	0.6929	3.341	0.84	83	24	43%	12%
LVC115	0.61712	4.19065	0.75	85	38	25%	17%
LVC116	0.6038	3.5911	0.77	72	21	31%	12%
LVC308	0.65977	3.86443	0.82	87	23	38%	14%
LVC309	0.5781	2.4058	0.74	50	24	40%	30%
LVC311	0.5456	3.1613	0.63	56	31	6%	29%
LVC312	0.6539	2.1481	0.73	57	33	-11%	35%
LVC313	0.52151	4.277	0.80	69	17	25%	6%
EEC108	0.59162	3.17106	0.82	63	25	27%	19%
EEC106	0.5715	4.2928	0.82	78	37	32%	20%
EEC105	0.44359	4.60199	0.75	62	32	20%	15%
EEC104	0.55427	4.47956	0.72	78	53	14%	20%
EEC109	0.499	5.732	0.63	92	52	6%	26%
LTC104	0.35744	4.25538	0.46	46	38	5%	17%
LTC105	0.4589	5.07975	0.52	72	54	6%	15%
LTC106	0.5431	4.8996	0.54	84	68	7%	22%
LTC108	0.28306	4.04885	0.39	35	26	3%	28%
LTC109	0.36444	4.24811	0.43	46	44	-4%	36%

 $\begin{tabular}{ll} \textbf{Table S-7: Summary of soil characteristics in 0-30 cm depth in study sites (mean \pm SD). BD - bulk density, Corg - organic carbon, N-Nitrogen, C:N-C:N ratio, P-phosphorous, K-potassium, Ca-calcium, Mg-magnesium. } \end{tabular}$ 

D (	TT '4		Drained			Undrained	
Parameter	Unit	0-10	0-20	0-30	0-10	0-20	0-30
BD	kg m <sup>-3</sup>	221±86	270±135	314±214	173±38	174±32	168±32
pH	units	$3.9{\pm}1.1$	4.1±1.1	4.2±1.1	5.3±0.3	5.3±0.3	5.3±0.3
Corg	$\rm g~kg^{-1}$	412±91	416±121	406±153	411±64	432±39	443±39
N	$\rm g~kg^{-1}$	23±6	22±8	20±9	27±4	27±4	27±4
C:N	ratio	19±6	21±6	22±7	15±3	16±3	17±3
P	$\rm g \ kg^{-1}$	$0.9\pm0.4$	$0.8 \pm 0.5$	$0.8\pm0.6$	2±1.7	1.8±1.2	1.6±1.1
K	$\rm g \ kg^{-1}$	$0.7 \pm 0.4$	$0.5\pm0.3$	$0.4\pm0.3$	1.6±1.1	1.3±0.8	1.1±0.6
Ca	$\rm g~kg^{-1}$	15±13	17±14	17±14	28±7	28±7	28±8
Mg	$g\ kg^{-1}$	1.2±0.7	1.2±0.8	1.2±0.8	2.6±0.8	2.5±0.8	2.4±0.7

Table S-8: Summary of soil water characteristics in the study sites (mean  $\pm SD$  ).

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D	Unit		Drained		Undrained		
Parameter	Unit	EE	LT	LV	LT	LV	
pН	unit	6.9±0.6	6.5±0.6	6.1±1.2	7±0.5	7±0.5	
DOC	$mg\;L^{-1}$	$69.4 \pm 23.1$	93.5±64.5	95.4±57.4	103.3±23.7	41.7±30	
N	$mg\;L^{-1}$	13.2±11.5	12.7±15.4	5.4±4.7	5.5±1.7	$2.1 \pm 1.4$	
$NH_4{^+}$	$mg\;L^{-1}$	$0.6\pm0.9$	0.5±0.7	$0.4\pm0.4$	0.7±0.9	$0.6\pm0.7$	
$\mathrm{NO_{3}^{-}}$	$mg\;L^{-1}$	11.5±12.9	10.7±17.4	2.5±4.3	1.3±1.6	0.5±0.5	
$PO_4^{3-}$	$mg\;L^{-1}$	$0.1 \pm 0.1$	$0.1\pm0.2$	0.1±0.2	0.1±0.1	0.1±0.1	
K	$mg\;L^{-1}$	$0.4\pm0.3$	2.8±3.5	$0.6\pm0.4$	1.9±0.7	$0.6 \pm 0.3$	
Ca	$mg\;L^{-1}$	$63.6\pm27.8$	77.7±51	27.5±16.9	71.1±15.5	29±9.2	
Mg	$mg\;L^{-1}$	7.5±4.9	14.6±8.2	5.6±4.6	12.9±2.2	6±1.4	

Table S-9: Characteristics of linear mixed-effects models predicting total forest floor respiration (Rtot) incorporating a random effect for study site. AIC - Akaike information criterion, BIC - Bayesian information criterion, logLik - log-likelihood value, R<sup>2</sup> marginal - variance explained by fixed effects, R<sup>2</sup> conditional - variance explained by fixed and random effects, \*p<0.1, \*\*p<0.05, \*\*\*p<0.01.

Variable	Coefficient ± standard error				
(Intercept)	3,8±0,12***	3,43±0,16***	3,2±0,36***	3,42±0,34***	3,61±1,25***
T	0,35±0***	0,35±0***	0,35±0***	0,35±0***	0,35±0***
Drainage: undrained	-1,09±0,22***	-1,31±0,24***	-1,35±0,24***	-1,41±0,22***	-1,4±0,19***
K		-0,6±0,21***	-0,58±0,23	-0,4±0,26	-0,48±0,21
Mg		0,38±0,11***	0,43±0,13***	0,36±0,13***	$0,12\pm0,11$
P		$0,23\pm0,11$	$0,26\pm0,11$	$0,11\pm0,11$	$0,02\pm0,11$
Species: birch			$0,08\pm0,25$	$-0.08\pm0.23$	-0,64±0,22***
Species: pine			$0,26\pm0,34$	-0,25±0,36	-0,34±0,32
Species: spruce			$0,14\pm0,25$	-0,19±0,26	-0,67±0,22***
Country: LT				-0,19±0,28	$0,23\pm0,33$
Country: LV				$0,38\pm0,2$	1,2±0,26***
pH					$0,01\pm0,17$
Corg					0±0
N					-0,01±0,02
Ca					$0,03\pm0,01$
BA					-0,01±0,01
A					0±0
AIC	10177	10167	10172	10170	10164
BIC	10208	10215	10239	10249	10279
logLik	-5084	-5075	-5075	-5072	-5063
R <sup>2</sup> marginal	0,75	0,77	0,77	0,77	0,78
R <sup>2</sup> _conditional	0,79	0,79	0,78	0,78	0,78

Table S-1040: Mean estimated annual cumulative total respiration (t CO2-C ha<sup>-1</sup> year<sup>-1</sup>) and biomass (t dm. ha<sup>-1</sup>) in the study sites. Abbreviations: Rtot – total respiration, Rhet – heterotrophic respiration, aGV and bGV – aboveground and belowground biomass of herbaceous ground vegetation, respectively, FRP – fine root production, fLF – foliar fine litter, cLF – coarse woody litter; NE – not estimated, NA – biomass not present or in negligible amounts. \*Used as soil CO2 input influx values for soil CO2 balance estimation; \*\* assuming 100% moss cover.

Study site	Rtot	Rhet	aGV*	bGV*	Shrubs	FRP*	Moss**	Moss production**	fLF*	cLF
EEC104	6.04	7.62	0.93	NE	0.08	NE	7.20	1.24	3.80	1.30
EEC105	4.76	9.48	1.10	NE	0.07	NE	5.63	1.08	3.31	1.72
EEC106	6.07	7.95	0.38	NE	0.43	NE	3.61	1.11	3.63	1.51
EEC108	5.08	12.77	0.83	NE	0.73	NE	NE	0.92	4.44	1.88
EEC109	7.61	10.36	0.70	NE	0.51	NE	2.75	0.26	3.12	0.53
LTC104	3.92	-	NA	5.11	2.72	5.00	5.93	2.08	3.77	0.62
LTC105	6.45	-	2.19	4.10	NA	1.29	NA	NA	3.19	0.13
LTC106	7.59	-	0.50	3.52	1.35	1.25	NA	NA	4.15	0.25
LTC108	2.98	-	3.49	1.69	2.37	NE	0.96	NE	4.37	1.87
LTC109	4.04	-	5.78	2.91	6.18	NE	NA	NA	2.20	0.83
LVC104	6.59	12.45	2.60	0.72	NE	2.10	NE	0.35	4.09	0.03
LVC105	9.01	15.78	2.47	1.43	NE	1.40	NE	0.74	4.03	0.45
LVC106	10.50	18.03	3.23	1.83	NE	2.96	NE	1.68	2.76	0.44
LVC107	5.36	7.57	1.49	3.02	NE	5.57	NE	NA	3.98	1.08
LVC108	7.25	17.25	1.82	2.19	NE	0.94	NE	1.20	2.67	0.35
LVC109	6.89	13.72	1.12	1.36	NE	0.64	NE	NA	3.33	1.52
LVC110	5.43	13.92	1.12	2.43	NE	2.89	NE	0.32	4.00	1.33
LVC111	5.51	17.42	0.82	2.38	NE	1.51	NE	1.18	3.11	0.47
LVC112	6.60	12.50	1.67	3.11	NE	NE	NE	NA	1.52	0.27
LVC115	6.61	10.76	1.44	2.48	NE	1.92	NE	NA	2.57	0.86
LVC116	5.65	11.66	3.22	6.12	NE	NE	NE	1.06	1.42	0.34
LVC308	6.27	7.72	3.15	1.36	NE	NE	NE	0.75	1.85	0.33
LVC309	3.98	12.90	1.61	3.29	NE	NE	NE	NA	2.09	0.33
LVC311	4.57	18.30	1.51	3.69	NE	NE	NE	NA	1.68	0.24
LVC312	5.27	9.49	2.14	1.62	NE	NE	NE	0.83	0.94	0.21
LVC313	4.96	11.11	3.98	3.08	NE	NE	NE	NA	2.96	0.45
Drained	6.21±0.43	11.68±1.72	1.82±0.52	$2.89 \pm 0.85$	$0.84 \pm 0.45$	2.53±0.77	$5.02\pm0.87$	0.98±0.25	3.22±0.44	$0.73 \pm 0.27$
Undrained	4.38±1.20	14.37±3.97	2.35±1.61	2.42±0.84	4.27±2.5	1.08±0.57	NE	1.01±0.23	2.53±1.06	0.78±0.62

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Table S<sub>2</sub>1144: Summary of soil CO<sub>2</sub> balance (mean  $\pm$ CI, t CO<sub>2</sub>-C ha<sup>-1</sup> year<sup>-1</sup>) estimation results. As soil earbon-CO<sub>2</sub> inputs-influx only ground vegetation, fine roots of trees and foliar fine litter is considered. Soil earbon-CO<sub>2</sub> balance is calculated by subtracting mean Rhet' from mean soil CO<sub>2</sub> inputinflux.

Drainage status	Country	Tree specie	Soil CO2 input	Rtot	Rhet`	Soil CO <sub>2</sub> balance
	EE	Mean	5.33±3.03	5.91±1.38	3.91±1.08	1.42±3.22
	LT		$6.06 \pm 3.33$	5.99±4.66	3.97±3.63	2.09±4.93
	LV		$5.28 \pm 0.86$	6.75±1.12	$4.56\pm0.87$	$0.72 \pm 1.22$
Drained	Mean		5.56±3.35	$6.22 \pm 4.9$	4.15±0.89	$1.41 \pm 1.70$
	Mean	Alder	5.01±2.06	6.34±2.45	4.24±1.91	0.77±2.81
		Birch	4.44±0.9	$6.8 \pm 0.77$	$4.60\pm0.60$	-0.16±1.08
		Pine	$6.75\pm1.92$	$5.23 \pm 0.45$	$3.38 \pm 0.35$	$3.37{\pm}1.95$
		Spruce	5.41±1.57	$6.99 \pm 1.98$	4.75±1.54	$0.66\pm2.20$
		Mean	$5.4\pm0.82$	$6.34{\pm}1.81$	$4.24 \pm 0.98$	1.16±2.44
	LT		5.73±1.73	3.51±1.04	2.04±0.81	3.69±1.91
Undrained	LV	Mean	$3.69{\pm}1.04$	5.24±1.37	$3.39{\pm}1.07$	$0.30\pm1.49$
	Mean		4.71±0.68	$4.38 \pm 0.33$	$2.72\pm1.32$	$2.00\pm3.32$
		Alder	4.57±1.25	5.47±2.80	3.56±2.18	1.01±2.51
	Mean	Birch	4.84±2.49	4.25±2.48	2.61±1.93	2.23±3.15
		Spruce	$3.7 \pm 1.22$	$4.6 \pm 1.60$	2.89±0.57	$0.81 \pm 1.35$
		Mean	4.37±1.81	4.77±2.49	3.02±1.21	1.35±1.91

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# Supplementary figures

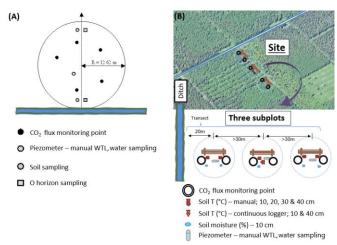


Figure S-1: Sampling design

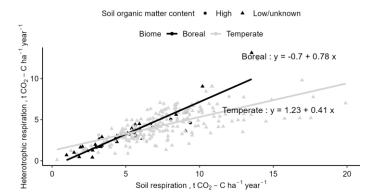


Figure S-2: Relationship between total soil respiration and soil heterotrophic respiration in forests according to previous studies (Jian, J. et al., 2021).

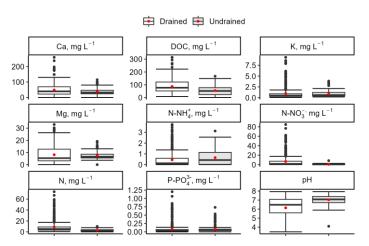
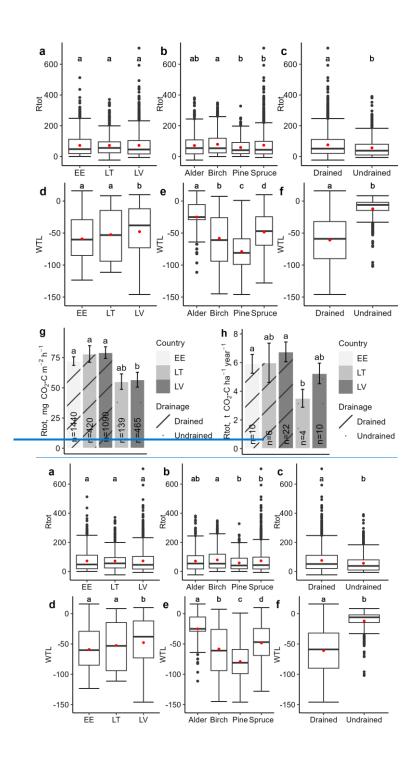


Figure S-3: Variation of soil water chemical properties.



25 Figure S-44: Summary of water table level (WTL, cm) depth and and total respiration (Rtot, mg CO<sub>2</sub>—C m<sup>-2</sup> h<sup>-1</sup>) measurement results by country (a, d), dominant tree species (b, e) or drainage status (c, f), and mean measured (g) or annualized (h) total respiration stratified by both country and drainage status.

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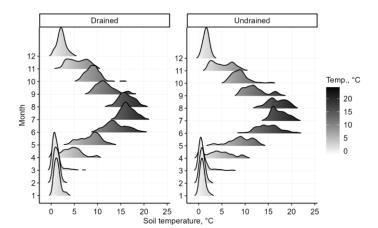


Figure S-5: Density plots of soil temperature at  $10\ \mathrm{cm}$  depth.

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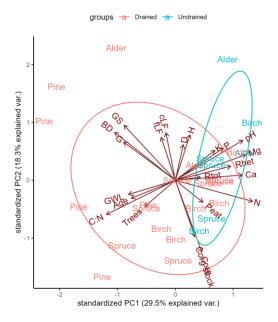


Figure S-6: PCA visualizing the covariation of the measured variables. Abbreviations: Age – stand age; BD – bulk density; C:N – ratio between organic carbon and nitrogen in soil; cLF – coarse woody litter; Corg stock – soil organic carbon stock; D – mean tree diameter; fLF – fine foliar litter; G – basal area; GS – growing stock; GWL – water table level; H – mean tree height; pH – soil pH value; Het – annual soil heterotrophic respiration; Rtot – annual total forest floor respiration; Trees – tree density; K, Ca, Mg, P, and Corg represent the content of potassium, calcium, magnesium, phosphorus, and organic carbon in the soil, respectively.

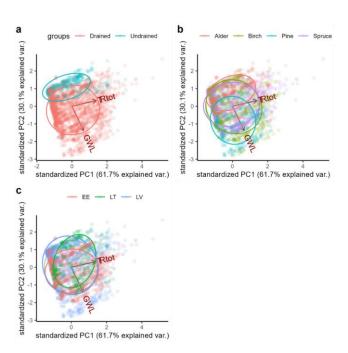


Figure S-7: PCA of total forest floor respiration (Rtot), soil temperature and WTL data. In figures a, b, c data are grouped by drainage status, dominant tree species and country, respectively.

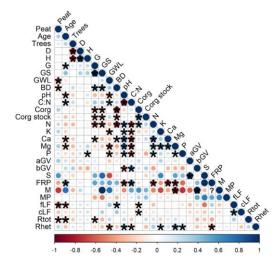


Figure S-8: Correlation matrix of annualized data (soil physical and chemical parameters at 0-30 cm depth). Abbreviations: Peat – peat (organic) layer depth; Age – stand age; Trees – tree density; D – mean tree diameter; H – mean tree height; G – basal area; GS – growing stock; GWL – water table level; BD – bulk density; pH – soil pH value; C:N – ratio between organic carbon and nitrogen in soil; Corg stock – soil organic carbon stock; K, Ca, Mg, P, and Corg represent the content of potassium, calcium, magnesium, phosphorus, and organic carbon in the soil, respectively; aGV, bGV, S, FRP, M, MP, fLF, cLF – biomass of aboveground herbaceous vegetation, shrubs, tree fine root production, moss, moss production, fine foliar litter, coarse woody litter, respectively; Rtot – annual total forest floor respiration; Rhet – annual soil heterotrophic respiration.

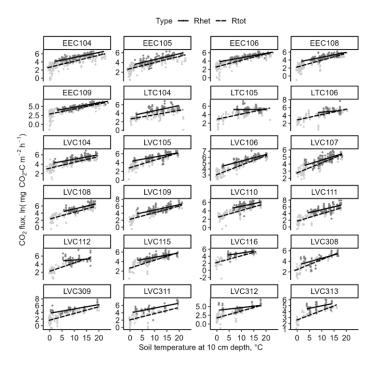


Figure S-9: Relationship between soil temperature and log-transformed soil heterotrophic (Rhet) and total (Rtot) respiration.