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ABSTRACT

In warming climates, soil water content (SWC) may act as an important factor in determining belowground carbon dynamics in boreal forests. Here, we estimated the respiration and nonstructural carbohydrate (NSC) concentrations of tree roots in a mature Scots pine (*Pinus sylvestris* L.) stand in southern Finland during two growing seasons with contrasting weather conditions. Root respiration was estimated with four different methods: 1) incubating excised roots, 2) partitioning forest floor respirations with root exclusion, or 3) based on temperature response functions and 4) modelling with the whole-tree carbon model 'CASSIA'. In addition, we conducted a drought experiment in a greenhouse to determine the effect of reduced soil-water availability on respiration by incubating soil and roots of Scots pine saplings.

We observed that the respiration of incubated roots of Scots pine saplings and soil decreased with drying after excluding the effect of temperature on respiration (R_{RES}), soil being more sensitive to drought than roots. Similarly, R_{RES} of incubated roots in the field was significantly decreased by lowered SWC, whereas respiration of the entire root system estimated with other methods was clearly higher in dryer and warmer than moister and cooler year. Nevertheless, incubated roots excavated from the topsoil are most affected by drying soil, which might not reflect the response of the entire root system. R_{RES} of incubated roots was negatively associated with root fructose and glucose concentrations. At the same time, root fructose, glucose and sucrose concentrations were negatively associated with SWC due to their role in osmoregulation. Thereby it seems that R_{RES} does not directly follow the changes in NSCs despite the apparent correlation. Our study highlights the responsive nature of root carbon dynamics in varying weather events that should be taken into account in estimating and modelling the impacts of warming climate.

1. Introduction

Global warming increases photosynthetic production (*i.e.* gross primary production; GPP) in the boreal zone (Briceño-Elizondo et al., 2006; Jansson et al, 2008), which may further enhance the allocation of carbon (C) to roots and C sequestration in soils. Increased soil temperatures (T_S) may also increase the activity of tree roots (*i.e.* respiration) (Pregitzer et al., 2000; Wang et al., 2014). Since the transpirational demand of plants increases with increased temperatures (Kirschbaum, 2000) and

warming may result in drying of northern soils (Dai, 2013), soil water content (SWC) may become a more important factor in the future than at present determining terrestrial C exchange in boreal forests (Liu et al., 2019). Drought stress can weaken or even cause mortality of mature trees in areas with intense droughts (Allen et al., 2010). Although Northern European forests rarely experience hot, dry summers, several examples from recent years (such as 2018) do exist (Lindroth et al., 2020; Peters et al., 2020). Due to global warming, heat waves may become more frequent and last longer during growing seasons (IPCC,

2014a), while rain intensity and distribution throughout the year may change (IPCC, 2014b). Growing seasons may begin earlier, and the snow-cover period may shorten in spring in the Northern Hemisphere (Collins et al., 2013) thereby increasing the length of the growing season. Warming and lengthened growing seasons may induce changes in tree phenology and increase forest net C uptake (McMahon et al., 2010; Keenan et al., 2014). Meanwhile, the T_S in winter may be decreased, due to delayed snowfall and reduced insulation by snow (Halim & Thomas, 2018). All these may also affect root growth dynamics, such as root phenology (timing of root growth), root turnover and length of the growing period, which may eventually alter belowground C allocation and sequestration. Therefore, a comprehensive understanding of root C dynamics under warming and drought conditions is needed to better predict future changes in C balance, and the linkage between aboveground processes with belowground processes is needed to estimate how the warmer climate and altered water availability affect tree growth and vitality.

Roots, especially fine roots, are an important component of the belowground C cycle, since two-thirds of the net primary production (NPP) of trees can be allocated to roots (Helmisaari et al., 2002). Trees allocate C to roots and symbiotic mycorrhizae for growth, metabolic processes and for sustaining nutrients and water acquisition (Read, 1991). In turn, heterotrophic microbes benefit from plant-derived C from roots and mycorrhizae by stimulating the decomposition of soil organic matter (SOM) (Bengtson et al., 2012). T_S is the most important driver for soil respiration, although recent studies have found that temperature alone is insufficient for predicting variation in soil respiration on the diurnal or seasonal scales, even in the Boreal Zone (Makita et al., 2018). Tree root respiration (R_R) may be phenological in nature, controlled by allocation of photosynthates to roots during the growing season (Pregitzer et al., 2000; Moyano et al., 2008; Heinemeyer et al., 2012; Hopkins et al., 2013). Nonstructural carbohydrates (NSCs) are used not only for growth, energy metabolism (e.g. respiration) and C storage (Hartmann & Trumbore, 2016; Martínez-Vilalta et al., 2016; Schiestl-Aalto et al., 2019; Collalti et al., 2020), but also for other purposes in trees. For example, soluble sugars (mainly glucose, fructose and sucrose) are used to regulate water and osmotic potential in plant cells (Hartmann & Trumbore, 2016). In the fine roots of trees, soluble sugars are used in maintaining water uptake via osmoregulation, whereas starch plays a role as a long-term storage substance and buffer against stress in coarse roots (Dietze et al., 2014; Hartmann & Trumbore, 2016). NSC increases can be observed under drought conditions (Körner, 2003; Salmon et al., 2020), even though long-term NSC depletion can be a sign of a decline in plant vitality.

R_R is an important indicator for root C dynamics and overall tree metabolisms. However, since roots are in direct contact with the soil, it has been challenging to measure only the R_R or respiration of heterotrophic soil microbes (R_H), such as bacteria and mycorrhizal fungi. To study these processes under field conditions, the C fluxes from various sources should be separated. Several ways for estimating R_R are available; e.g. various physical separation methods and isotopic approaches (Hanson et al., 2000). R_R can also be directly measured from living or excised roots (Rakonczay et al., 1997; Burton & Pregitzer, 2003; Makita et al., 2012, 2013), which should result in estimates with only minor contribution by microbes on the root surface and without larger contribution of microbes in the rhizosphere (Makita et al., 2013). This can be further used for separating the contribution of root-associated mycorrhizae to R_R (Kelting et al., 1998). The root exclusion method, or so-called 'trenching', has long been used to physically separate R_H from total soil respiration to obtain the contribution of R_R (Hanson et al., 2000; Kuzyakov, 2006). In trenching, the roots are cut around the plot, and new ingrowth of roots is prevented by installing root-impermeable material, e.g. with a deep-reaching collar or mesh fabric. In comparison to other methods (e.g. isotopic analysis), trenching is reasonably simple and inexpensive, although it is laborious when constructed. In addition to the physical methods, R_R has been estimated by taking advantage of

the different temperature responses of R_R and R_H (e.g. Reichstein et al., 2005; Pumpanen et al., 2008, 2015). In the method by Pumpanen et al. 2015, R_R is assumed to originate from the photosynthates allocated to roots and to respond to changes in the assimilation of C by the vegetation and R_H from the decomposition of detrital matter. Thereby, R_R changes concomitantly with the seasonal changes in temperature on a longer time scale, whereas, R_H responds on a shorter time scale, for example, to weekly temperature variation. Furthermore, R_R can be estimated using whole-tree C balance modelling (e.g. the dynamic growth model 'carbon allocation sink source interaction' (CASSIA); Schiestl-Aalto et al., 2015), in which the photosynthetic products are allocated in NSC storage, growth and respiration of various tree parts, including the roots. Nevertheless, all these methods include limitations and uncertainties and thus, their comparison would allow further consideration of their applicability.

Our aims were to a) determine the dynamics of R_R and NSC in tree roots of a boreal Scots pine (Pinus sylvestris L.) forest, b) to test the performance of the root module in the CASSIA model, and c) to determine the soil-moisture response of soil R_H and R_R of Scots pine saplings in a controlled drought experiment. For the purpose, we formulated the following research questions:

1) Does the decreased SWC reduce R_R of:
   a) mature trees in the current climate?
   b) saplings in controlled drought?
2) How strongly are NSC concentrations in mature tree roots associated with SWC?
3) What are the pros and cons of the various methods used to study R_R and how do these affect the applicability of the methods under different conditions?

We addressed these questions with a combination of different experimental approaches and modelling. First, we studied the questions under field conditions in the mature Scots pine stand in southern Finland during two growing seasons with contrasting weather conditions. We used three different measurement-based methods in the Scots pine stand to reveal the R_R under natural conditions and compared these to an estimate obtained with the CASSIA model, and determined NSC of the tree roots. Secondly, we performed the controlled greenhouse experiment in which we studied the soil-moisture response of bulk soil R_H and R_R of 7-yr-old Scots pine saplings. The controlled experiment allowed a detailed analysis over moisture response, whereas the field measurements revealed the natural response of mature trees to soil moisture that decreases slowly starting from the surface of the soil.

2. Methods

2.1. Greenhouse experiment

2.1.1. Experimental setup

A controlled drought experiment was conducted with Scots pine saplings in a greenhouse at the Viikki campus, University of Helsinki (60°14'N, 25°01'E) in southern Finland for 6 weeks from June to July in 2018. The saplings were sown in 2009 in the nursery garden at the Haapastensyrjä breeding station in Läyliäinen, southern Finland. The saplings were grafted with four different Scots pine clones in 2011 and grown in pots with peat-based soil, which was limed and fertilized when necessary. Since the clones were grafted onto stems (Supplementary Fig. S1), the foliage were similar in the clones, yet the root systems were not. Therefore the roots were not treated by clones, but as individuals in later analysis. The saplings were moved to the greenhouse in May 2018 repotted in 7.5-l pots that were filled with additional peat-based soil. At the time of the experiment, the saplings were 7 yr old, their height was 75–154 cm and the diameter of the bottom of the stem 11.8–32.2 mm (see more details in Supplementary Table S1). The saplings were evenly distributed between the treatments.

All the saplings were acclimated in the greenhouse for several weeks and watered. The saplings were circulated regularly within their own tables to balance the light levels between individuals. The sampling was destructive, *i.e.* different individuals, but the same four clones, were measured each week. The saplings were divided into three groups with different watering treatments: 1) control saplings (CO) that were watered three times a week to field capacity (FC), 2) saplings that were left to dry (DR) and 3) saplings that were first left to dry, but rewatered (RE) when the water potential of the needles was down to –2.2 MPa. In practice, rewatering was conducted one day before measurements in the fifth week (July 8), when the RE saplings were manually watered until the soil was fully moist. After rewatering, the RE saplings were also watered the same way as the CO saplings. Until the rewatering, four clones per CO and DR treatment were measured once per week, since until this point the treatments between DR and RE did not differ. For the remaining 2 weeks of the experiment, saplings of the RE treatment were also measured as described above, and only three saplings per week were measured in the DR treatment.

The air temperature (T_A) and relative humidity (RH) inside the greenhouse were measured at 15 min intervals with thermometers and psychrometers (Priva Hortimation; Priva B.V., De Lier, the Netherlands). The water potential of the saplings was measured weekly before sampling between 12 PM and 4 PM from the needles with a pressure bomb (PMS6000D; PMS Instrument Company, Albany, OR, USA).

2.1.2. Root and soil incubations

For the incubations, we took one bulk soil sample from each pot with an auger and cut one individual root from the root system as a whole with root tips (see Supplementary *Fig. S2A, B*). The excess soil was manually removed from the root samples with caution to avoid damage to the root or rhizome. Root samples were only cut once to minimize additional respiration from cut surfaces, although Makita *et al.* (2013) concluded that respiration rate was significantly higher when the samples were cut more than 10 times. They also concluded that microbial respiration from the soil attached to the roots had minimal effect on respiration after brushing (Makita *et al.*, 2013). The diameter of the root samples at the thickest point was approximately 1–2 mm and the full length of the sample root was approximately 10–15 cm. The soil samples were collected in plastic cups for the incubation. The soil and root samples were weighed before incubation to determine the fresh weight of the samples, with the exception of the roots on the first sampling day.

The soil and root samples were incubated separately, put in multi-layer 1-l gas-sampling bags within 5 min after taking or cutting to prevent a major decrease in carbon dioxide (CO_2) emissions. The supplementary material provides a schematic illustration and a picture of the entire incubation system (Supplementary *Fig. S2C, D*). The bag was sealed, emptied of air and refilled with 500 ml ambient air. The CO_2 concentration in the bag was measured at ambient temperature with a nondispersive infrared sensor (GMP343; Vaisala Oyj, Vantaa, Finland) every 5 s for a total of 15 min in each measurement. The air in the chamber bag was circulated ($0.5\,l\,min^{-1}$) during the measurement with a small pump (NMP 830 KVDC B; KNF Neuberger, Freiburg, Germany). The incubations were conducted at the same time each week in the afternoon. The ambient temperature in the greenhouse was stable during the sampling, but varied from week to week (see Supplementary *Fig. S3A*).

After incubation, the root samples were put into 15-ml plastic tubes and kept in the cold for approximately 1 h before heating in a microwave oven (1 min, 600 W) to stop the enzymatic activity. The root samples were stored in a freezer (–18 °C) until they were freeze-dried (SciQuip Ltd., Merrington, Shropshire, UK) for 3 days (72 h). Thereafter, the root samples were reweighed for dry weight. The soil samples were put in paper bags and dried for at least 24 h at 60 °C in an oven to determine the dry weight of the samples. The moisture content (%) of the root and soil samples was calculated as the ratio between the difference between the fresh and dry weight, and fresh weight.

The CO_2 efflux was calculated from the increase in CO_2 concentration inside the bag by linear fitting with time. Only measurements between 1 min and 5 min were used in the analysis, since it may need time to acclimate when the chamber bag is closed and measurement initiated. Since the size of the samples varied, we derived mass-based respiration rates using the dry weight of the individual root or soil sample.

2.1.3. Temperature responses of carbon dioxide fluxes and residual respiration

The dependency of respiration rate ($R(t)$) on temperature is most often described with the exponential function:

$$R(t) = r_0 Q_{10}^{\frac{T(t)}{10}}, \tag{1}$$

where r_0 is the basal respiration rate at 0 °C temperature, Q_{10} the temperature coefficient and $T(t)$ the air or soil temperature at measurement time t. First, we fitted Q_{10} and r_0 separately for bulk soil and root CO_2 fluxes in the CO treatment and used the Q_{10} and r_0 values obtained for predicting the respiration of the DR and RE treatments (*Eq. 1*). Then, we tested the possible association between respiration and soil moisture by reducing the predicted values ($R_i(t)$) from the measured CO_2 fluxes ($f_i(t)$) within treatment i (*Eq. 2*) and comparing these residual respirations (R_{RES}) with soil moisture:

$$R_{RES_i}(t) = f_i(t) - R_i(t). \tag{2}$$

2.2. Field experiments

The R_R at the field site was determined using three different measurement-based methods: 1) root incubation (Section 2.2.3), 2) root exclusion (Section 2.2.4) and 3) separating the components of the forest floor respiration (R_{FF}) based on short- and long-term temperature responses using automated chamber measurements (Section 2.2.7). In addition to these measurement-based methods, R_R was also determined 4) by modelling with whole tree C balance model CASSIA (Section 2.2.8).

2.2.1. The study site

The field experiments were conducted in a mature Scots pine stand at the Station for Measuring Ecosystem-Atmosphere Relations (SMEAR II) located near Hyytiälä forestry field station (61°51'N, 24°17'E) in southern Finland (Hari and Kulmala, 2005). The stand was established in 1962 by sowing, and classified as *Vaccinium* type with medium fertility (Cajander, 1926). The stand is mixed with scarce undergrowth Norway spruce (*Picea abies* (L.) H. Karst.) and sparse mature deciduous trees, such as downy birch (*Betula pubescens* Ehrh.), silver birch (*B. pendula* Roth) and European aspen (*Populus tremula* L.). The mean height and the diameter (at breast height) of the dominant Scots pines (with diameter > 15 cm) were 18.6 m and 18.5 cm, respectively, and density of the dominant trees 683 stems per ha and 1177 stems per ha, including also smaller trees (< 15 cm) in 2016 (Schiestl-Aalto *et al.*, 2019). The vegetation on the forest floor is characterized by ericaceous dwarf shrubs, such as bilberry (*Vaccinium myrtillus* L.), cowberry (*Vaccinium vitis-idaea* L.) and heather (*Calluna vulgaris* (L.) Hull), mosses such as Schreber's big red stem moss (*Pleurozium schreberi* (Brid.) Mitt.), dicranum mosses (*Dicranum* Hedw. sp.), and splendid feather moss (*Hylocomium splendens* (Hedw.) Schimp.), and herbs and grasses, *e.g.* wavy hairgrass (*Deschampsia flexuosa* (L.) Trin.). The soil is a Haplic podzol and the soil depth 0.5–0.7 m.

The site is characterized by a boreal climate with cool summers and cold winters. The mean annual temperature is 3.5 °C, while the mean monthly temperature varies from −7.7 °C in February to 16.0 °C in July and from 8.8 °C to 16.0 °C during the growing season (May–September) (years 1980−2009, Pirinen *et al.*, 2012). The mean annual rainfall at the site is 711 mm, distributed rather evenly throughout the year (Pirinen *et al.*, 2012).

Continuous measurements were conducted at the SMEAR II station. The net ecosystem CO_2 exchange (NEE) was measured with a closed-path eddy-covariance system above the stand at a height of 24 m in 2017 and 2.5 m higher in 2018. The instrumentation is described in detail in Vesala *et al.* (2005) and calculations and corrections to the NEE fluxes in Kolari *et al.* (2009). In practice, the NEE was partitioned into total ecosystem respiration (TER) and GPP. GPP was derived from the NEE and TER, as follows:

$$GPP = -NEE + TER \qquad (3)$$

The T_A was measured with a resistance thermometer (Pt100 sensor) at 1 min intervals at a height of 16.8 m. The thermal time (*i.e.* the effective temperature sum; degree-day, °Cd), was calculated as the sum of the daily average temperatures above 5 °C from days when the average temperature was permanently more than 5 °C.

The T_S was measured at 15 min intervals with temperature sensors (KTY81-110 thermocouples; NXP Semiconductors, Eindhoven, the Netherlands) from five locations in the soil A-horizon (approx. 2–5 cm in depth) and upper B-horizon (approx. 9–14 cm in depth). The soil-moisture content (SWC) was measured at 30 min intervals in the soil B-horizon (approx. 14–25 cm in depth) with time-domain reflectometry (TDR100; Campbell Scientific Inc., Logan, UT, USA) and a Delta-T Theta probe soil-moisture sensor (Delta-T Devices Ltd., Cambridge, UK) at 15 min intervals in the soil B-horizon (approx. 10 cm in depth). The meteorological and soil data were averaged for daily averages.

2.2.2. Chamber measurements of soil CO_2 efflux

The intact R_{FF} (CO_2 efflux) was measured with manual and automated chambers at the study site. The automated measurements were conducted with two chambers during May–October 2017 and with three chambers during June–November 2018. In addition, we used measurements conducted with three automatic chambers during April–November in 2013–2015 in modelling. The automated chamber was a transparent box (20 × 20 cm in area and 25 cm in height) made of 6-mm acrylic (see Supplementary *Fig. S4A*) and covered with aluminium foil to exclude the light. The air in the chamber was circulated with a small fan during the measurement, and T_A was monitored in the chamber with a thermocouple type K sensor. The chamber is described in detail in Pumpanen *et al.* (2015). The chamber was placed on an aluminium frame (7 cm in height) and tilted to the side of the frame (opened) between the measurements by an electric motor (E192.24.625; Micro Motors, Mid Glamorgan, UK). The chamber enclosed all the natural ground vegetation (*e.g.* mosses, dwarf shrubs, herbs) inside the frame. The CO_2 concentration in the chamber was measured with a nondispersive infrared sensor (GMP343; Vaisala Oyj, Vantaa, Finland) at 5-sec intervals for closures that lasted for 3.5 min every 30 min.

The R_{FF} was also measured with a manual chamber at 14 permanently installed collars at 2–4-week intervals from April to September 2017 and from April to November 2018, using a standard closed-chamber technique (Pumpanen *et al.*, 2015). The cylindrical chamber was 19.7 cm in diameter and 23.9 cm in height, equipped with a small fan and covered with aluminium foil to exclude sunlight. During the measurement, the chamber was placed on the collar, enclosing all the natural ground vegetation inside the collar. The CO_2 concentration in the chamber headspace was measured with the same infrared sensor CO_2 probe (Vaisala Oyj, Vantaa, Finland) as with the automated measurements for 5 min.

The CO_2 efflux was calculated from the increase in CO_2 concentration inside the automated and manual chambers by linear fitting against time. Only measurements between 45 sec and 4 min, and between 40 sec and 190 sec were used in the analysis for the manual and automatic chambers, respectively. If the concentration increase was clearly saturating after some tens of ppm, the measurement was discarded, because it indicated leakage (*e.g.* the chamber was not properly placed on the collar).

2.2.3. Incubation and nonstructural carbohydrate content of excised roots

The tree roots were excavated from the soil of the Scots pine stand approximately 100 m from the main experimental area to prevent disturbance of the study site. Three root samples were excavated from depths of approximately 5–15 cm from the ground level at 2–4-week intervals from April to November in 2017–2018. The sample roots (Supplementary *Fig. S4B*) were cut from coarse roots, usually including the root tips, and manually cleaned of excess soil as in the greenhouse (see section 2.1.2). The diameter of the root samples at the thickest point was approximately 1–2 mm and the full length of the sample root was approximately 15–20 cm. The sample roots were cut with only one cutting surface to minimize additional respiration from the extra cuts (Makita *et al.*, 2013). The incubation protocol in the field was similar to that used in the greenhouse experiment. The CO_2 fluxes (*i.e.* respiration) were calculated, and afterwards the root samples were processed in a manner similar to that of the incubated root samples of the greenhouse experiment. T_A and T_S were used for temperature fitting when calculating R_{RES} (see section 2.1.3; *Eq. 2*).

The dry-mass-based respiration of excised roots was upscaled to the ground area, using an estimate for daily living pine root biomass at the study site (SMEAR II). The daily living root biomass per ground area was calculated for each day of the year, using the estimates of total pine root biomass and daily growth at the study site. The total pine root biomass (diameter < 5 mm) was on average 221 g m^{-2} (Ding *et al.*, 2021), and the daily root growth dynamics measured at the site in 2018 (Ding *et al.*, 2020) were used for the study years, whereas the turnover rate of the roots was assumed to be 1 yr^{-1}.

The concentration of analysed soluble sugar compounds (alpha and beta glucose, fructose, sucrose and raffinose) and starch of the incubated root samples from the field were later analysed at the Natural Resources Institute, Helsinki, Finland (LUKE), as described in detail in Schiestl-Aalto *et al.* (2019). In practice, the soluble sugar concentrations after extraction were analysed with a gas chromatograph (Agilent 7890B GC; Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a mass spectrometer (Agilent 5977A MSD; Agilent Technologies Inc., Santa Clara, CA, USA), and starch with a spectrophotometer (Shimadzu UV-2600; Shimadzu Co., Kyoto, Japan). The NSC concentrations were determined per dry weight.

2.2.4. Root exclusion and plot-specific measurements

The R_R can be estimated from the total R_{FF} by removing the R_H and ground vegetation respiration (R_{GV}) from it. To separate these different forest floor components, root exclusion or so-called 'trenching' plots were established at the study site in June 2017. First, a trench was dug around the round plot with a diameter of approximately 0.6–0.7 m, and to a depth of at least 40–50 cm, most of the times all the way to the bedrock. All the roots, mainly of trees and dwarf shrubs, growing into the plot were cut. A nylon mesh fabric with a pore size of 1 μm (LK-Suodatin Oy, Tampere, Finland), was installed around the plot (see Supplementary *Fig. S4C*). The mesh enables water and nutrients to flow into the plot, but prevents the ingrowth of roots and mycorrhizal fungal hyphae. After mesh installation, the trench around the plot was refilled with soil. The soil inside the plot was left untouched, but all shoots of the ground vegetation were cut out. In all, six trenching plots were established around the site in two areas with three trenching plots per area.

As the tree roots were excluded and ground vegetation was cut from the trenched plots (*Fig. 1*), the CO_2 flux measured from these plots comprises mainly of R_H. However, these plots include also an increased number of decaying residual roots, and due to the lack of water uptake by the plants, soil moisture and T_S in these plots may have altered (*Fig. 1*). To minimize the effect of decaying residual roots in this study, we determined the respiration of dead roots (R_{DR}) by measuring root decomposition in the soil, which was further reduced from the CO_2 fluxes measured. In the following sections, we describe in detail how we monitored the CO_2 fluxes and environmental factors at these plots and eliminated the effect of residual roots and altered drivers in the
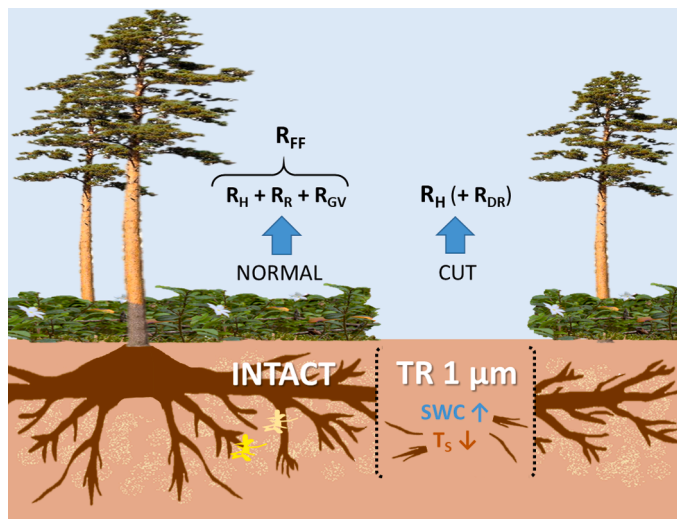
Fig. 1. Forest floor respiration (R_{FF}) measured from the intact spots with tree roots and their root-associated mycorrhizal fungi, and normal ground vegetation. The total R_{FF} contains respiration of heterotrophic microbes (R_H) and tree roots (R_R) in soil and respiration of ground vegetation (R_{GV}). R_H and respiration of dead residual roots (R_{DR}) measured from trenching plots (TR with 1μm mesh) without ground vegetation. R_{DR} was afterwards reduced from respiration of trenched plots. Photos of Scots pine and ground vegetation by Juho Aalto.

trenching plots.

Manual soil respiration (CO_2 efflux) measurements at the trenching plots (see Supplementary *Fig. S4D*) were conducted in 2017–2018 every 2 weeks from April to August and once per month in September, October and November from six plots in 2017 and four plots in 2018. In some trenching experiments CO_2 efflux measurements are initiated after one or more years after the trenching. However, here we started the measurements earlier to prevent the increased CO_2 emissions due to Gadgil effect, which has previously shown to be significant after three years on our site (Ryhti *et al.*, 2021). Soil respiration was measured, using the same manual chamber method that was used in the intact spots and described earlier, but the height of the chamber we used here was 19.9 cm or 23.9 cm, and the CO_2 concentration in the chamber was measured every 5 sec for 5 min or 15 min in 2017 and 30 min in 2018, except for the first measurement day when the measurement was for 5 min. The flux calculated from the CO_2 concentration change inside the chamber headspace between 1 min and 4 min was used in the analysis. However, in a few cases when the early measurement seemed unstable, we used measurements after 5 min, keeping the time of the fitting the same (3 min), and checked that the level of the flux was comparable to that of others.

T_S was measured at 4-hr and 1-hr intervals in 2017 and 2018, respectively, at all trenching plots with temperature sensors (iButton Thermochrons; Maxim Integrated, San Jose, CA, USA) from mid-July 2017 to July 2018. The thermochrons were placed at the interface of the organic layer and mineral soil surface. Hourly values were interpolated for 2017 (in 2018, the measurements were already hourly), and the daily mean was calculated for the daytime hours 9 AM–4 PM, because the CO_2 effluxes were measured during that time. The daily T_S values for each plot were derived for missing days to cover the entire year, using the linear correlation between the daily means measured continuously with the thermocouples at the study site and the daily plot-specific daytime means of the CO_2 efflux measurement days.

The SWC of the trenching plots were measured with a PR2 profile probe soil-moisture sensor and recorded with an HH2 moisture meter (Delta-T Devices Ltd., Cambridge, UK) biweekly or monthly at the same time as the CO_2 flux measurements were conducted. Since the tubes were not installed precisely at the same depth (due to *e.g.* shallow bedrock or large rocks), the exact measurement depths varied between

the plots. In the further analysis, we selected SWCs that were measured at 5–15 cm depths in the soil for each plot. The measured SWC readings revealed clearly the seasonal pattern in the soil moisture, but the absolute values differed between the plots even when continuous measurements showed values of field capacity. Therefore, the baseline levels of the measurements were adjusted according to the continuous SWC measurements at the study site. In practice, we derived the difference between the values measured at each plot and field capacity, which was then added to all the measured values. These values were then used to estimate the daily soil moisture for each plot by using linear relationship between the continuous moisture measurements and plot-specific soil moisture values.

2.2.5. Decomposition of residual roots in trenched plots

We assumed that during the construction of the trenching plots in July 2017, all roots of trees died in the root exclusion, as did the dwarf shrub, herb and grass roots when the ground vegetation was cut, and began slowly to decompose. Therefore, nylon mesh bags (LK-Suodatin) with a pore size of 1 mm were filled with 1.5 g (dry weight) of Scots pine roots (with a diameter < 2 mm) and placed between the organic layer and mineral soil surface in each trenched plot in mid-July 2017. The first two root litter bags were collected from each plot in April 2018 and the second two in November 2018. The first decomposition period was from July 2017 to April 2018 and the second from April to November 2018.

The root litter mass-loss rate in the trenched plots was 30.3% at the time of the first collection and 37.7% at the time of the second collection, resulting in 30.3% and 10.5% decrease in the root mass between the placing of the litter bags in soil and the first and second bag collection, respectively. These values were used for the decomposition rate of roots in the 1–2-mm size class, whereas the decomposition in the smallest size class (< 1 mm) was assumed to be 30% times as fast as for 1–2-mm size class for the whole study period resulting in a comparable decomposition rate to Ryhti *et al.* (2021). The mean annual decrease for 5–10-cm-diameter roots was 3.4% (Palviainen and Finér, 2015), which we used for the largest size class (mean diameter 7.5 cm) for the whole study period. Then, we estimated the decomposition rate for the first period for the size classes 2–5 mm, 5–10 mm and 10–20 mm with a fitted power equation (for more details see Ryhti *et al.* (2021)). The annually decomposed root mass and CO_2 emissions caused by the decomposition of dead residual roots (R_{DR}) were estimated and removed from the measured CO_2 fluxes according to Ryhti *et al.* (2021), using the periods and values for 2017–2018 described above. Even though some of the trenched roots may stay alive for a while whereas the roots in the litter bags start decomposing immediately, most of the active roots are fine ones with short natural lifetime. Therefore we assume that this method provides a good enough estimate of CO_2 release related to root decomposition.

2.2.6. Estimating respiration with root exclusion

Since the chamber measurements of the trenched and intact plots were conducted on slightly different days and the trenched plots were moister than the intact soil, we also needed daily CO_2 effluxes for days that were not measured. These were estimated using soil moisture, T_S and an empirical equation fitted to the available measurements. We utilized equation used by Mäkelä *et al.* (2008) and Ryhti *et al.* (2021) where the measured CO_2 efflux ($f_i(t)$) at plot i was assumed to be driven by the temperature (T) and moisture (M) in the soil as follows,

$$f_i(t) = \left(1 + \left(\frac{1 - RWC_i(t)}{\propto}\right)^v\right)^{-1} r_{0_i} Q_{10_i}^{\frac{T_i(t)}{10}}, \tag{4}$$

where α and v are empirical parameters determining the response of CO_2 efflux to soil moisture, r_0 the basal respiration rate at 0 °C temperature, Q_{10} the temperature response coefficient, $T_i(t)$ the T_S in soil horizon B and RWC the relative water content at moment t. RWC at plot i was calculated according to Mäkelä *et al.* (2008) as follows:

$$RWC_i(t) = \frac{M_i(t) - WP}{FC - WP},\qquad(5)$$

where $M_i(t)$ is the SWC in soil horizon B at plot i, FC the field capacity (0.33 m^3 m^{-3}) and WP the wilting point (0.07 m^3 m^{-3}) according to Ilvesniemi *et al.* (2010). First, we estimated parameters r_0 and Q_{10} by setting $v = 11.27$ and $\alpha = 1.062$ (according to Mäkelä *et al.* (2008)) with the nls-function in (R Core Team, 2019), using the 'port' algorithm. Then, we estimated the daily CO_2 fluxes (R_i) at each plot i, using these plot-specific parameters (r_0 and Q_{10}) in *Eq. 4*. For the trenching plots, we used the mean soil-moisture value of the automated measurements at the site to overcome the potential effects of altered soil moisture on the CO_2 emissions due to root exclusion (see SWC in *Fig. 3D*).

We assumed that the trenched plots contained only heterotrophic microbes and their respiration (*i.e.* R_H) after the roots were excluded from the plots and ground vegetation was cut. The R_{GV} was modelled for 2017–2018, using the measurements from the trenching experiment at the study site (SMEAR II) conducted in 2013–2015 by Ryhti *et al.* (2021). The daily CO_2 fluxes of the ground vegetation were estimated for the study years by estimating the parameters for 2013–2015 with *Eq. 4*, and using them the same way as for the trenched plots.

The R_R was separated by removing the mean R_H of the trenching plots and mean R_{GV} from the mean total R_{FF} measured on the intact spots at the study site as follows:

$$R_R = R_{FF} - R_H - R_{GV}\qquad(6)$$

2.2.7. Estimation of respiration components based on temperature responses

The temperature responses of the CO_2 effluxes in the automated chambers on different temporal timescales were studied to separate R_R from the R_{FF}, according to Pumpanen *et al.* (2015). The method is based on the assumption that the apparent seasonal temperature response is higher than the short-term response, due to the phenology of the vegetation, subsequent changes in the assimilation of C and the allocation of photosynthates belowground, all of which contribute to R_R. Following Reichstein *et al.* (2005), we assumed that the short-term temperature responses fitted over the 7-day periods represented the CO_2 efflux of heterotrophic microbes decomposing SOM (R_H) and long-term temperature response fitted over the growing season represented the total CO_2 efflux both autotrophs and heterotrophs (*i.e.* R_{FF}). The R_H in chamber i was calculated as follows:

$$R_{H_i}(t) = r_{0i} Q_{10h_i}^{\frac{T_i(t)}{10}},\qquad(7)$$

where R_H is the heterotrophic respiration at moment t, r_0 the average 7-day temperature response fitting in the second week of May 2013–2015, Q_{10h} the mean Q_{10} value over the 7-day periods and $T(t)$ the soil temperature at moment t. Since the automated CO_2 flux measurements were initiated in early June or mid-May in the study years when T_S had already risen, we used an average of r_0 in 2013–2015 fitted over the 7-day period starting in the second week of May for both study years. The R_{FF} for each chamber i was calculated in a manner similar to that in *Eq. 7*, but using parameters Q_{10} and r_0 of the long-term fitting over the growing season. R_R was separated by removing the calculated R_H from R_{FF} as follows:

$$R_{R_i}(t) = R_{FF_i}(t) - R_{H_i}(t).\qquad(8)$$

2.2.8. Whole-tree carbon balance model CASSIA

A dynamic tree growth model CASSIA (Schiestl-Aalto *et al.*, 2015) was used to simulate R_R. In CASSIA, which was previously validated for our study site (Schiestl-Aalto *et al.*, 2015, 2019), photosynthesized C is allocated to the tree organs (*i.e.* foliage, xylem, fine roots) for growth, metabolism, C storage and mycorrhizal symbionts. Continuous measurements of T_S and SWC at SMEAR II were used as inputs for root growth and maintenance respiration of roots. As in other inputs, GPP

and T_A at 16.8 m (see section 2.2.1) measured at the station were used. Root growth in the model was updated according to Ding *et al.* (2020), who quantified the response of daily growth rate of pioneer and fibrous roots to T_S and SWC with data of root growth collected with scanner images at SMEAR II. R_R was calculated as the sum of maintenance and growth respiration. Maintenance respiration is dependent on T_S and SWC, and growth respiration is proportional to the growth rate.

2.3. Statistical analysis

Pearson's correlation analysis and linear regression analysis ($P < 0.05$) were used to test the dependence between R_{RES} (*i.e.* the difference between the measured and predicted respiration values, *Eq. 2*) of incubated bulk soil and roots and the NSC concentrations in the root samples, and SWC. In addition, the associations between T_A and T_S, SWC, NSC concentrations and incubated respiration variables, and between the three methods of determining R_R were tested, using Pearson's correlation analysis. The Shapiro–Wilk test ($P < 0.05$) for small sample sizes (< 50) was used to test normality of the variables (Supplementary *Table S2*). Correlation and linear regression analysis were carried out using the R program (R Core Team, 2019).

3. Results

3.1. Controlled drought with Scots pine saplings

The soil moisture clearly decreased with advancing drought, but recovered to the initial levels after rewatering (Supplementary *Fig. S3C*). It also decreased slightly in the CO treatment with continuous watering, and during the last 2 weeks, the moisture in the CO soil was lower than in the RE treatment (Supplementary *Fig. S3C*). Root moisture remained rather high during the experiment in all treatments; however, the data are unavailable for the first sampling day (Supplementary *Fig. S3D*). Root moisture was highest in the CO treatment, lowest in the DR treatment and increased after rewatering in the RE treatment (Supplementary *Fig. S3D*). The needle water potential of the CO saplings was higher than in the other treatments, except on the last sampling day, when the water potential of the RE saplings increased to a similar level (Supplementary *Fig. S3E*). The water potential in the DR treatment was lowest and decreased notably as drying advanced (Supplementary *Fig. S3E*). T_A of measurements days varied from 21 °C to 36 °C during the experiment (Supplementary *Fig. S3A*).

The mean bulk soil R_{RES} between the observed respiration and fitted temperature response tended to decrease slightly over time in all the treatments. However, it clearly showed the steepest decrease with continuous drying (DR) (*Fig. 2A*), indicating that the temperature response alone resulted in overestimation of the rate of bulk soil respiration in the later phase of the experiment. The mean bulk soil R_{RES} of RE was similar to the CO level (*Fig. 2A*). The mean root R_{RES} was mostly slightly higher in the CO treatment than in the other treatments (*Fig. 2B*). The bulk soil R_{RES} samples were positively associated with soil moisture in the CO and DR treatments (*Fig. 2C*). The root R_{RES} was positively correlated with soil moisture only for the DR treatment (*Fig. 2D*).

3.2. Natural drought in the mature forest

3.2.1. Overview of environmental conditions

The T_A levels in spring and summer were considerably higher in 2018 than in 2017 (*Fig. 3A*). This resulted in wide differences in the effective temperature sums (*i.e.* sum of the daily average temperatures above 5 °C), which were 1040 and 1659 °Cd in 2017 and 2018, respectively. The T_S fluctuation lagged behind that of T_A, being lower than in the air in summer and higher during winter (*Fig. 3A*). The annual GPPs were comparable for the 2 yr (1204 g C m^{-2} yr^{-1} in 2017, 1249 g C m^{-2} yr^{-1} in 2018, *Fig. 3B*) and annual NEEs as well (–299 g C m^{-2} yr^{-1} in 2017, –324 g C m^{-2} yr^{-1} in 2018, *Fig. 3B*). The mean R_{FF} measured with
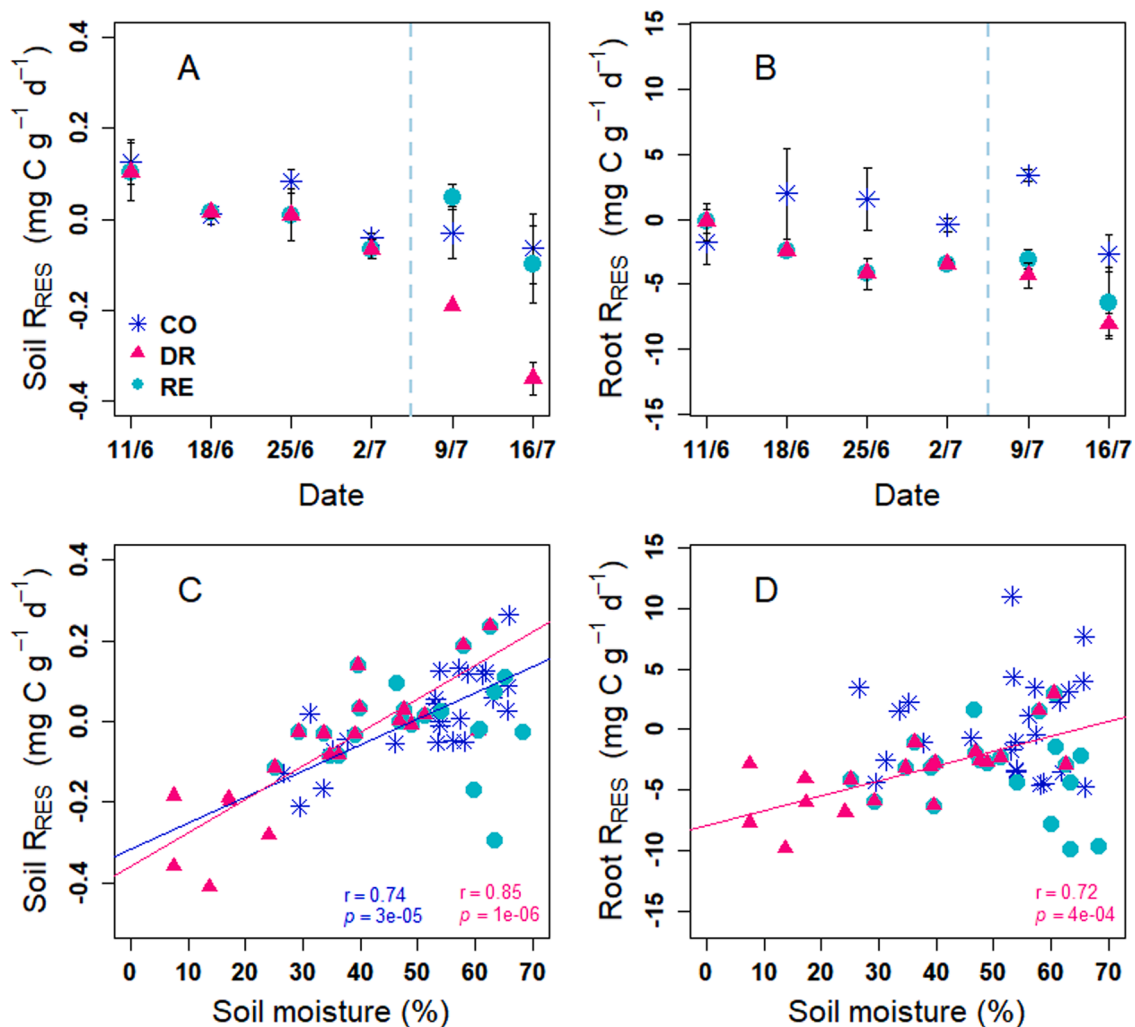
Fig. 2. Residual respiration (R_{RES}) between the measured and fitted temperature response ± standard error (SE) of the incubated bulk soil (A) and incubated roots (B), bulk soil R_{RES} (C) and root R_{RES} against soil moisture (D) in the control (CO) (blue), drying (DR) (pink) and rewatered (RE) (light blue) treatments in the 6-week drying experiment in June–July in the greenhouse experiment. The dashed light blue line in the A and B panels indicates initiation of rewatering (RE treatment). The DR and RE treatments were equal before the re-watering, which caused the overlap of results. The solid lines represent linear regression ($P < 0.05$), where the association between the means of the various treatments and soil moisture was calculated, using Pearson's correlation.

chambers at the study site was higher in 2018 than in 2017, but the difference between the study years was not as clear at the trenching plots containing only R_H (Fig. 3C). The year 2017 was evenly moist while in 2018, the SWC clearly decreased during summer (Fig. 3D). The mean SWC in the trenched plots was higher in 2018 than in the continuously measured SWCs in intact plots at the study site. Similar differences were not seen in 2017.

3.2.2. Respiration and nonstructural carbohydrates of incubated roots

The respiration of the incubated roots showed a clear annual pattern in 2017 whereas in 2018, the seasonal changes were not as notable (Fig. 4A). The raffinose concentration was clearly higher in spring and autumn than in summer, and rather similar in both years (Fig. 4B). In contrast, the fructose and glucose concentrations were on average higher in 2018 than those in 2017 (Fig. 4B). The annual pattern of the fructose and glucose concentrations also contrasted between the study years, decreasing during summer 2017 and increasing during summer 2018 (Fig. 4B). The sucrose, glucose and fructose (SGF) concentrations combined and sucrose concentration showed no pattern during year 2017, while having an increasing trend in 2018 (Fig. 4C). The starch concentrations in general exceeded those of the SGF. Starch was lowest in spring and showed no clear interannual differences between the study

years (Fig. 4D).

The raffinose concentrations in the incubated root samples were positively correlated with SWC throughout the study years (Fig. 5B, Table 1), but showed a stronger negative correlation with T_S (Table 1). The glucose, fructose and sucrose concentrations showed a significant negative correlation with SWC throughout the study years (Fig. 5C, D, E; Table 1), but not with T_S (Table 1). The correlation between the starch concentration and SWC was not significant ($P = 0.058$) (Fig. 5F); however, the starch concentration of the roots did correlate with T_S (Table 1).

The residuals of the measured respiration of incubated roots and the temperature response (R_{RES}; Eq. 2) were positively associated with the SWC when fitted with T_A (Fig. 5A) throughout the study years (Table 1). R_{RES} fitted with T_A was negatively associated with the glucose and fructose concentrations in the roots throughout the study years (Table 1). R_{RES} calculated with T_S was negatively correlated with the glucose, fructose, SGF and soluble carbohydrate concentrations (Table 1), whereas there was no significant correlation between R_{RES} and raffinose or starch concentration in the roots (Table 1).

3.2.3. Respiration of tree roots with different methods

The respiration derived from the excised root incubation method was notably lower from June to October than those from the other three
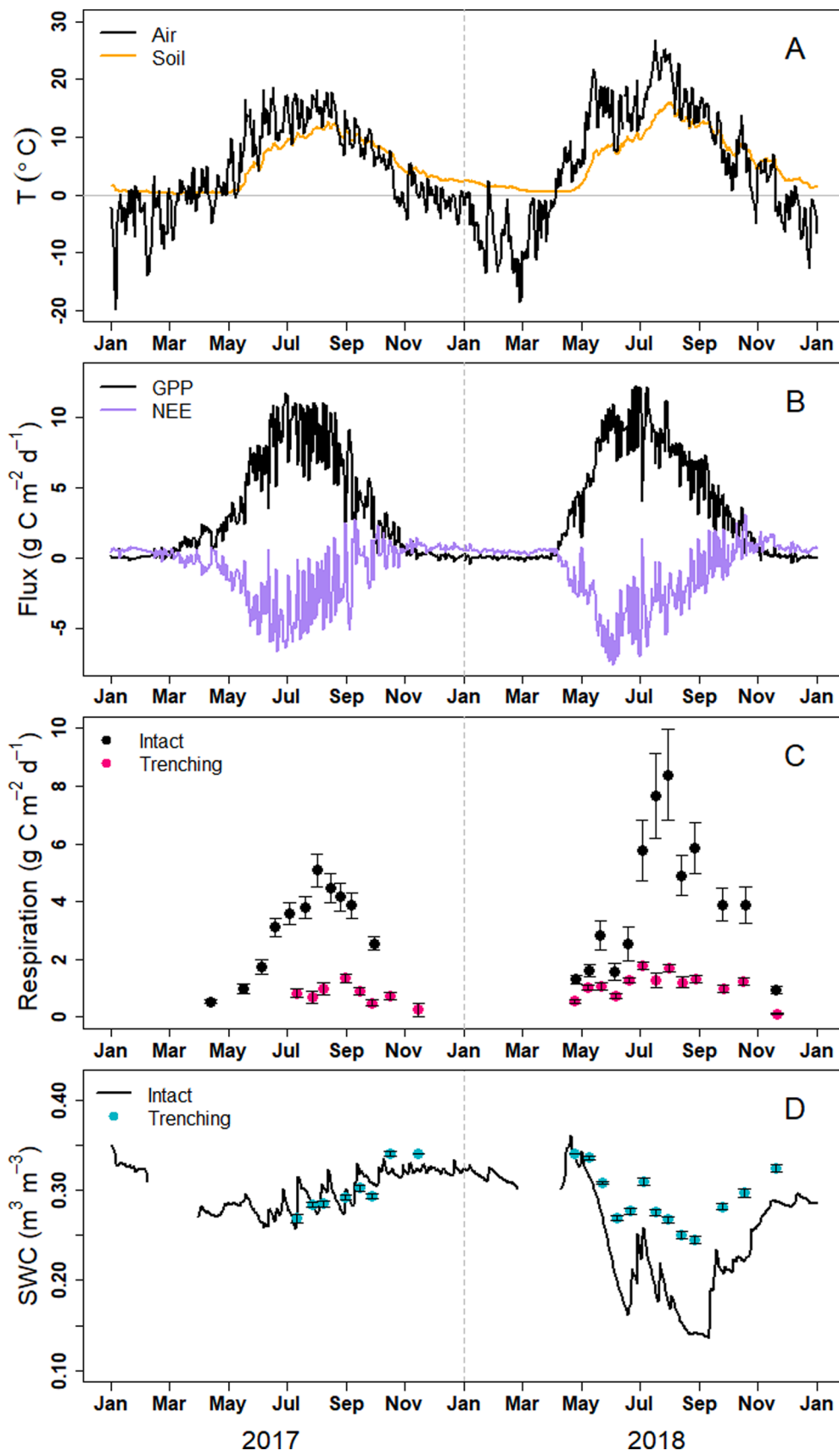
Fig. 3. Daily mean temperature (T) in the air (black) and soil B-horizon (at 9–14-cm depths) in orange (A), gross primary production (GPP) and measured net ecosystem carbon dioxide (CO_2) exchange (NEE) (B), mean forest floor respiration at intact plots ± standard error (SE) in black and the mean respiration of root exclusion plots (trenching) ± SE in pink (C), soil-water content (SWC) in the soil B-horizon (at 10-cm depth) at intact spots in black and in trenching plots ± SE in blue (D) at the field study site during 2017 and 2018.
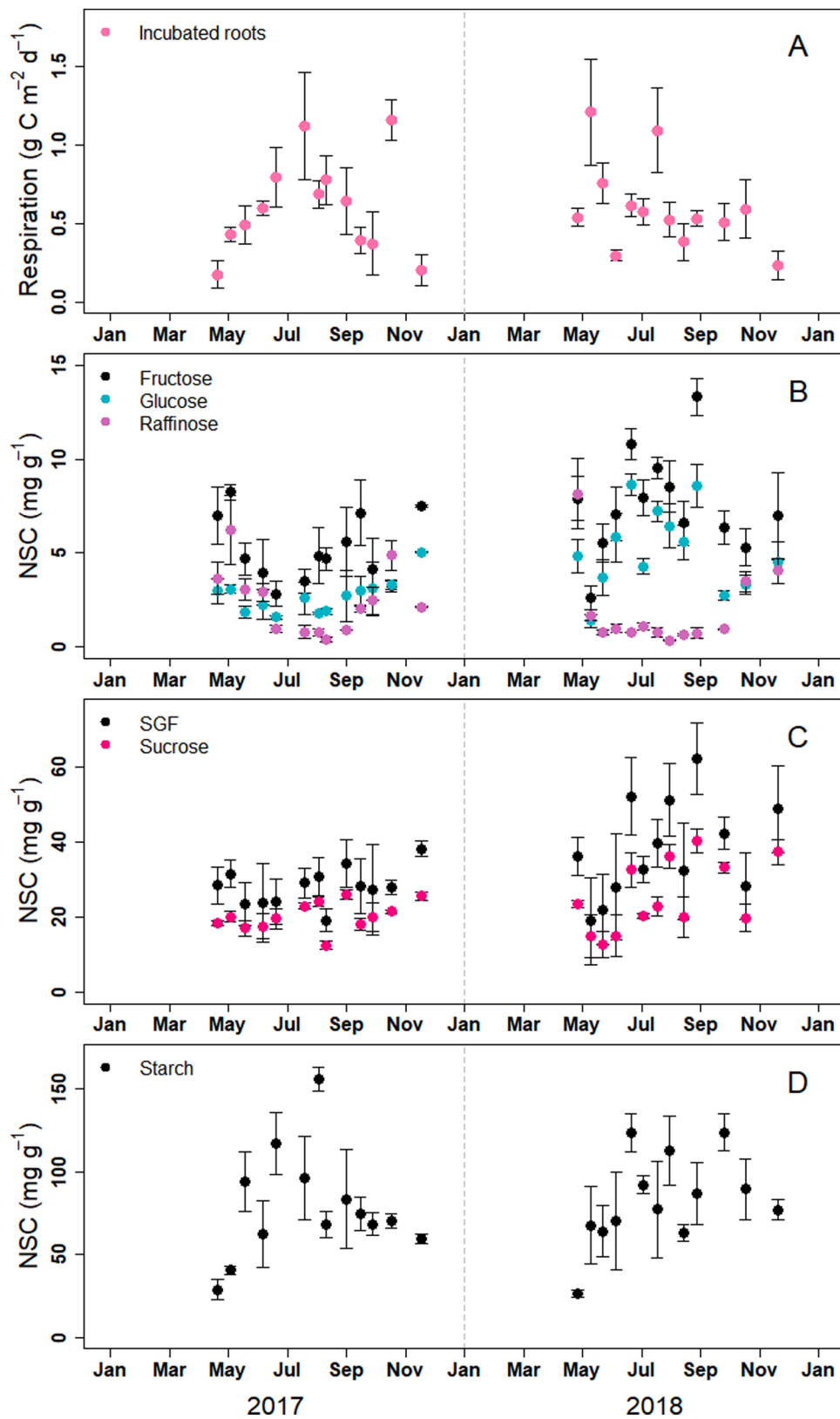
Fig. 4. Mean respiration of incubated roots ± standard error (SE) measured at ambient temperature in light red (A), mean concentrations ± SE of fructose, glucose and raffinose in black, light blue and purple, respectively (B), fructose, glucose and sucrose (SGF) combined and sucrose in black and pink, respectively (C) and starch in black (D) in incubated root samples at the field study site during 2017 and 2018.
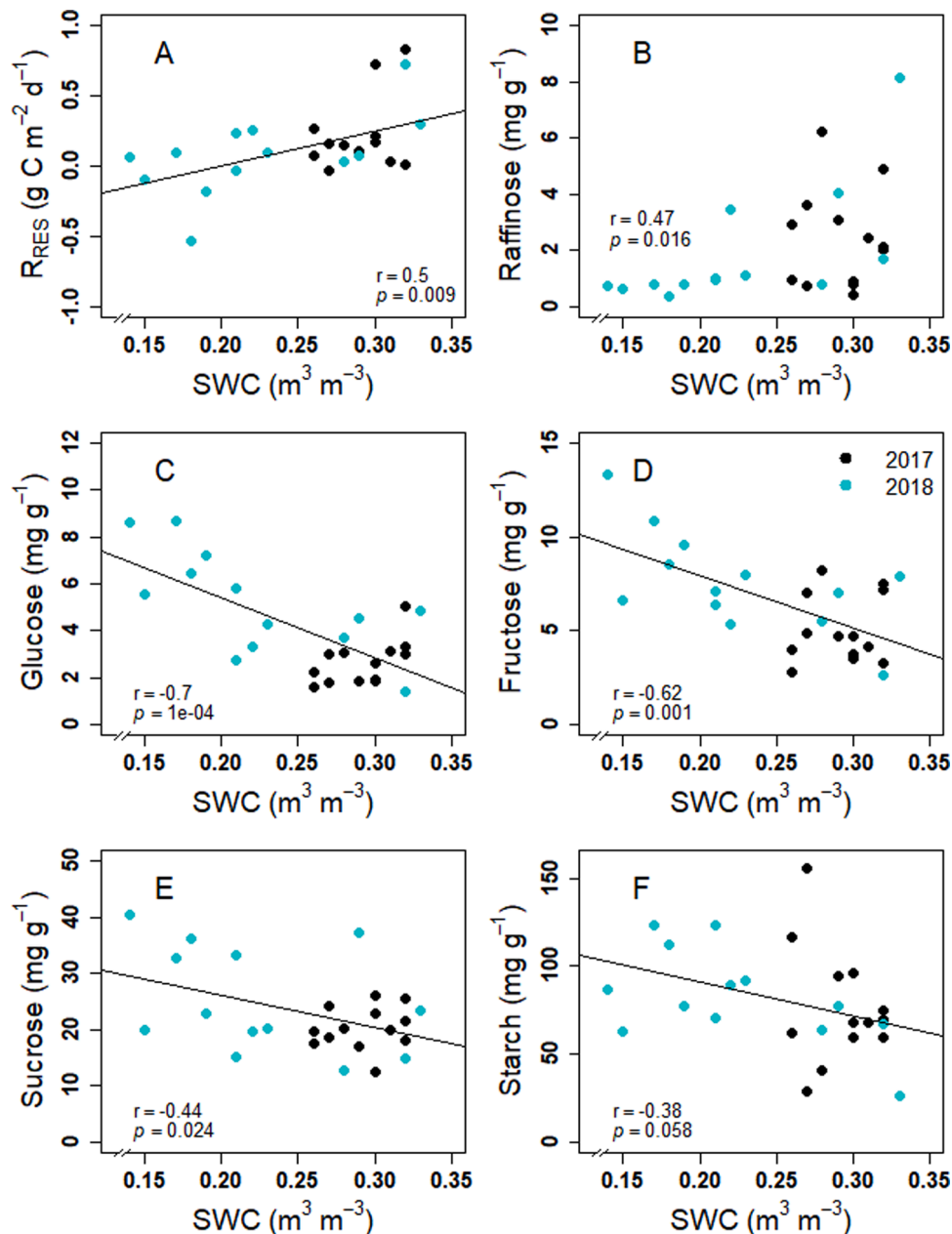
Fig. 5. Residual respiration (R_{RES}) between the measured and fitted air temperature response of the incubated roots against soil-water content (SWC) (A), raffinose (B), glucose (C), fructose (D), sucrose (E) and starch (F) concentrations in incubated roots against SWC at the field study site during 2017 in black and 2018 in light blue. The lines represent linear regression ($P < 0.05$), where the association between the means and SWC was calculated, using Pearson's correlation.

methods in both study years (*Fig. 6*), and there was no significant association between the incubated R_R and R_R estimated with the other methods (not shown). The R_R calculated by the root exclusion method agreed well with the R_R determined with the temperature response method ($r = 0.85$, $P < 0.001$) and the CASSIA model ($r = 0.86$, $P < 0.001$) (*Fig. 6*; Supplementary *Fig. S5A, B*). The temperature response method resulted in estimations of R_R similar to those of CASSIA (*Fig. 6*) and was strongly correlated with CASSIA ($r = 0.997$, $P < 0.001$).

Maximum photosynthesis, which is indicated by GPP, was rather similar in both study years, yet the photosynthesis rate began to increase slightly later, and increased faster in 2018 than in 2017 (*Fig. 3B*). However, the estimated aboveground (*i.e.* foliage, shoot, stem) growth and respiration began earlier, and the daily rates were higher according to CASSIA in 2018 than in 2017 (*Fig. 7*). The modelled R_R and root

growth were highest after the aboveground growth began to decrease in both years (*Fig. 7*). The growing period for modelled root growth was similar in both years (*Fig. 7*).

4. Discussion

4.1. Respiration of tree roots

Respiration of tree roots is an important component in belowground C dynamics, since it accounts for almost half the respiration of the forest floor throughout the year in the boreal zone in general (Hanson *et al.*, 2000), as also demonstrated at our study site (Ryhti *et al.*, 2021). Soil warming may increase living root biomass (Leppälammi-Kujansuu *et al.*, 2013) and root development, but decrease fine-root longevity

Table 1

Pearson's correlations between air temperature (T_A) and soil temperature (T_S), soil-water content (SWC), glucose, fructose, sucrose, sucrose, glucose and fructose (SGF) combined, soluble carbohydrates (SGF + raffinose), raffinose and starch concentrations, incubated tree root respiration (R_R) and residual respiration (R_{RES}) of incubated roots between the measured and fitted temperature response with T_S (R_{RES} (T_S)) and with T_A (R_{RES} (T_A)) at the field study site. Significant correlations are marked in bold.

|	T_A	T_S	SWC	Glucose	Fructose	Sucrose	SGF	Soluble	Raffinose	Starch	R_R	R_{RES} (T_S)	R_{RES} (T_A)
T_A	1												
T_S	**0.69**[a]	1											
SWC	**−0.42**[c]	**−0.54**[b]	1										
Glucose	0.19	0.31	**−0.70**[a]	1									
Fructose	0.06	0.14	**−0.61**[a]	**0.87**[a]	1								
Sucrose	−0.09	0.28	**−0.44**[c]	**0.56**[b]	**0.58**[b]	1							
SGF	−0.01	0.29	**−0.59**[b]	**0.79**[a]	**0.81**[a]	**0.94**[a]	1						
Soluble	−0.12	0.15	**−0.51**[b]	**0.77**[a]	**0.80**[a]	**0.93**[a]	**0.98**[a]	1					
Raffinose	**−0.60**[b]	**−0.76**[a]	**0.47**[c]	−0.14	−0.02	−0.08	−0.09	0.09	1				
Starch	0.36	**0.56**[b]	−0.38	0.05	0.00	0.37	0.27	0.16	**−0.57**[b]	1			
R_R	**0.54**[b]	0.27	0.02	-0.19	−0.38	−0.21	−0.27	−0.30	−0.18	0.21	1		
R_{RES} (T_S)	0.09	−0.33	**0.46**[c]	**−0.39**[c]	**−0.49**[c]	−0.39	**−0.46**[c]	**−0.42**[c]	0.24	−0.10	**0.82**[a]	1	
R_{RES} (T_A)	−0.28	−0.29	**0.50**[b]	**−0.47**[c]	**−0.55**[b]	−0.24	−0.39	−0.34	0.26	−0.03	**0.62**[a]	**0.82**[a]	1

[a] $P < 0.001$
[b] $P < 0.01$
[c] $P < 0.05$
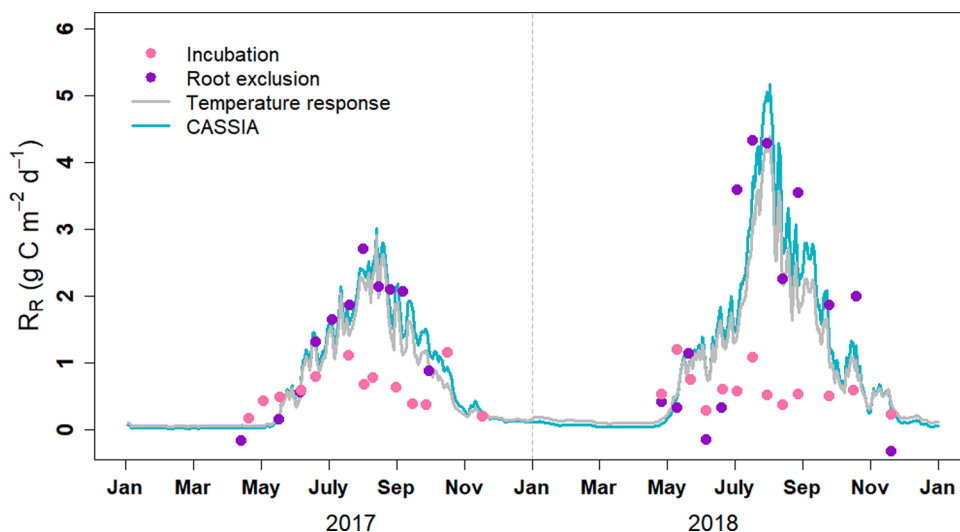
Fig. 6. Mean tree root respiration (R_R), using excised root incubation (pink), mean R_R partitioned from total forest floor respiration in the field, using the root exclusion method (purple), mean R_R between chambers partitioned with a temperature response approach from total forest floor respiration (grey) and R_R simulated with the whole-tree carbon (C) balance model 'CASSIA' (blue) at the field study site during the study years 2017 and 2018.
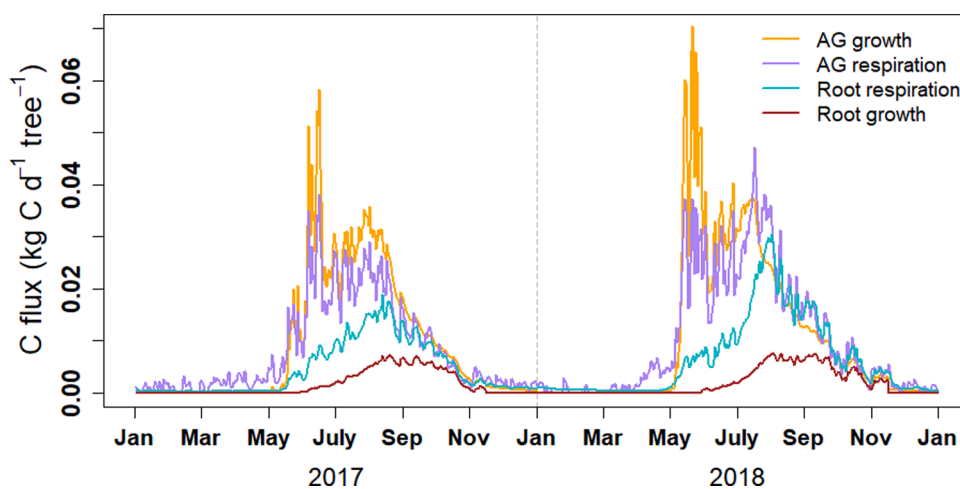

Fig. 7. Aboveground (AG) respiration, AG growth, root respiration (R_R) and fine-root growth simulated with the whole-tree carbon (C) balance model 'CASSIA' at the field study site during the study years 2017 and 2018. Aboveground includes foliage, shoots and stem.

(Leppälammi-Kujansuu *et al.*, 2014; Kilpeläinen *et al.*, 2019). Warming has also been shown to increase respiration of roots (Pregitzer *et al.*, 2000; Wang *et al.*, 2014). Indeed, the estimated R_R was higher in the field in the warmer year according to most of the methods used, as was also the measured R_{FF}. Likewise, Chi *et al.* (2021) found that forest floor respiration in mature Scots pine and mixed Norway spruce stands in Sweden was higher in the warmer, dryer year 2018 than in the cooler, wetter year 2017. Warming has been estimated globally to induce increases in forest floor respiration (Bond-Lamberty *et al.*, 2010,2018) and especially in its heterotrophic component (Wang *et al.*, 2014). However, warming-induced changes in soil moisture may alter respiration of heterotrophs and tree roots (Wang *et al.*, 2014).

Here, we showed that the decrease in SWC decreased R_R of roots sampled in the greenhouse and of mature trees in the field. Our results are in line with those of Burton and Pregitzer (2003), who found that dry conditions significantly reduced respiration rates of the excised roots of sugar maple (*Acer saccharum* Marshall) and red pine (*Pinus resinosa* Alton) in Michigan, USA. On the contrary, Mäki *et al.* (2022) found that R_R had negative relationship with soil moisture in southern boreal Scots pine stands, including our study site. Nevertheless, it is noteworthy that in our study, root samples for incubation were excavated at the field site from rather shallow mineral soil (approximately 5–15 cm in depth) that was drier than the deeper soil layers. Unfortunately, the fresh weights of the root samples were not measured at the field site; visually however, they seemed notably dry during the drier periods from June to August in 2018. Since the R_R rate derived from the incubated roots showed clearly lower rates than the other methods, it implies that the root actions in the topsoil do not represent the status of the entire root system, including the deeper soil layers, varying root types and symbiotic microbes. In practice, roots in the deeper soil layers usually do not experience such a notable decrease in the SWC as roots in the topsoil layer. Different tree species also have various strategies to response on drought stress by modifying their water uptake dynamics (Grossiord *et al.*, 2017), for example, by growing roots to access for deeper water sources (Mackay *et al.*, 2020). Thus, the total R_R in the entire soil column may not be hindered as much, as the significant association between R_R and SWC obtained in the topsoil would indicate. We further discuss the potential drawbacks of the incubation method later.

The shoot growth modelled with CASSIA began rapidly in spring in both years and strongly followed T_A, whilst a rapid increase was also evident for aboveground respiration, which was closely in line with the aboveground growth throughout the growing season. In contrast, R_R began to gradually increase after late May, but the root growth increased notably later after the aboveground growth began to decrease. During late autumn, the aboveground and belowground daily respiration values were similar and showed similar short-term dynamics. The discrepancy between aboveground growth and root growth was shown previously by Abramoff and Finzi, (2015), who reviewed that intensive root growth is initiated after intensive shoot growth has decreased in boreal trees. The same phenomenon was also observed at our study site by Ding *et al.* (2020).

4.2. Nonstructural carbohydrates in tree roots

As assumed, the root NSC concentrations were associated with SWC. Higher concentrations of glucose and fructose in the roots of mature trees were observed in the dry year 2018 than in the moist year 2017 during the summer months, indicating a sensitive response of glucose and fructose concentrations to changes in soil moisture. Likewise, Zang *et al.* (2014) observed a significant negative correlation between the fructose concentrations of fine roots and cumulated soil water potential for drought-stressed European beech (*Fagus sylvatica* L.) saplings. Accumulation of glucose and fructose in the roots was also reported for jack pine (*Pinus banksiana* Lambert) and white spruce (*Picea glauca* (Moench) Voss) seedlings after exposure to 7 days of water stress (Koppenaal *et al.*, 1991). Similarly, Rodriguez-Calcerrada *et al.* (2017)

found a negative correlation between root soluble sugar concentration and leaf or stem water potentials for *Ulmus minor* Mill. and *Quercus ilex* L. seedlings, which they assumed to be caused by osmoregulation. Moreover, we found negative correlations between the glucose and fructose concentrations and the R_R of mature tree roots in the field after removing the temperature response. Respiration consumes especially hexoses and therefore they are expected to decrease with increasing respiration rate, if the supply does not meet the consumption rate. However, taking into account the significant role played by glucose and fructose in osmoregulation, we can assume that the association observed between R_R and NSC is primarily driven by soil moisture, which affects them both. However, future studies are needed to determine the exact causality.

In comparison, we noted that the sucrose concentrations in the roots showed weaker correlations with SWC than hexoses, possibly indicating a conversion of sucrose to hexoses. The cleavage of one molecule of sucrose into two molecules of hexose doubles the number of osmotically active substances in the root cells and thereby can facilitate water uptake and turgor increment under water stress (Kim *et al.*, 2000; Königshofer and Löppert, 2015). Taken as a whole, the sum of sucrose, glucose and fructose (SGF) correlated negatively with SWC, in line with previous findings of increased soluble sugar concentrations in roots under water stress (Kim *et al.*, 2000; Rogiers *et al.*, 2011; Galiano *et al.*, 2017).

In this study, the starch dynamics did not respond to SWC, which is in line with results reported for quaking aspen (*Populus tremuloides* Michx.) seedlings (Galvez *et al.*, 2011) and Scots pine saplings (Galiano *et al.*, 2017). However, depletion of root starch was observed upon advancing drought for Norway spruce saplings (Hartmann *et al.*, 2013). These contrasting findings may be associated with species-specific responses and differences in root growth demands and intensity of drought. In contrast, we observed higher starch content after the drier summer in 2018 than in 2017, and a weak negative correlation between SWC and starch content in the field. Since mature trees in the field were not under water stress, the response of the starch dynamic to SWC was more likely impacted by the negative correlation between SWC and T_S and the strong positive correlation between starch content and T_S. Similar result has also been found by Schiestl-Aalto *et al.* (2019) in the same site. As T_S strongly governed the rate of root growth at the study site (Ding *et al.*, 2020), the correlations observed may have indicated an association between root growth and root starch content, as observed by Wang *et al.* (2018) for mature Norway spruce. However, the correlation may be due to a similar effect of temperature on both variables. In all, these results support the earlier knowledge that root starch, as an important storage form of NSC in roots (Dietze *et al.*, 2014; Hartmann and Trumbore, 2016), functions as a C supply for sink activities, *e.g.* root growth (Noland *et al.*, 1996), and not as an osmoregulatory compound.

The raffinose content in roots was insensitive to changes in SWC during the dry period in 2018. The positive correlation observed between SWC and raffinose content in the roots during the whole study period was likely due to coincidence between higher SWC and lower T_S, and a strong negative correlation between T_S and raffinose concentration in the roots. Raffinose has shown to accumulate in Norway spruce roots when cooled, even if shoots were kept in a warm environment or removed (Wiemken and Ineichen, 1993).

4.3. Methodological differences in estimating respiration of tree roots

The estimated R_R values obtained with different methods in the field were largely compatible, except for the excised root incubation method. The overall R_R of the incubated roots was considerably lower during summer than obtained with other methods, which may indicate that this method was not successful for three main reasons: 1) it considered only the respiration and biomass of smaller roots (diameter < 5 mm), 2) the root samples were excavated from topsoil as discussed earlier, and 3) the daily living biomass of roots is an estimation, which may cause

uncertainties in the upscaling of the dry-mass-based respiration. Furthermore, additional uncertainty in this method was caused by the fact that the morphology and size of the root samples also varied between repetitions, because standardizing of these is difficult, which again may have caused differences in respiration between samples. Thus, normalization of the incubated R_R with the dry weight of the samples may not have been sufficient, since most of the variation in respiration of fine roots is explained by diameter, root tissue density, nitrogen (N) content or total root length per unit root mass, which also accounts for branching of the roots (Burton *et al.*, 2002; Makita *et al.*, 2009, 2012, 2016). The total root surface area could be one alternative measure for normalization, as used for stem and branch respiration by Sprugel (1990), but even that does not always reflect the amount of active root parts, because finer absorptive roots are often more active than coarse transport roots. We can expect microbial respiration to be minimal from excised root surfaces (Makita *et al.*, 2013), but with other methods, microbial rhizospheric respiration (*e.g.* root-associated mycorrhizae) may contribute to R_R. Root incubation in a controlled environment would allow determination of the detailed environmental responses of roots alone while avoiding, for example, the effect of temperature changes. However, controlled environments are difficult to arrange under field conditions, when measurement should be initiated as quickly as possible to prevent decay of the root sample. Anyhow, lifting root samples out from the soil causes bias in the respiration, since conditions in the soil and air differ (*e.g.* ambient temperature, moisture, pressure, and CO_2 concentration).

Root exclusion is often used to partition R_R and R_H, because it is quite inexpensive and an easy method to use after construction. However, the two respiration components are closely associated and mutually dependent (Bond-Lamberty *et al.*, 2004). For example, heterotrophic microbes benefit from plant-derived C from roots and mycorrhizae, while plants benefit from gaining nutrients by microbial decomposition (Read, 1991; Bengtson *et al.*, 2012). Therefore, the R_H measured may be somewhat smaller after root exclusion, which would further result in overestimation of R_R when partitioned. However, excluding roots and mycorrhizae from the system imparts competitive advantage to heterotrophs in the so-called 'Gadgil effect' (Gadgil and Gadgil, 1971; Fernandez and Kennedy, 2016) and may result in overestimation of R_H over time, which would, in turn, result in underestimation of R_R. Ryhti *et al.* (2021) found a clear indication of the Gadgil effect in trenched plots without ground vegetation, especially 3 yr after trenching. However, this was not detected in our study, since all measurements were collected less than 2 yr after conduction of the root exclusion. Various approaches have been used for taking into account the additional CO_2 emissions resulting from cut residual roots, due to increased C availability for microbial decomposition. For example, additional CO_2 emissions have been estimated with the modelling approach (Comstedt *et al.*, 2011; Savage *et al.*, 2018). Even though our estimate of additional CO_2 emissions by measuring root decomposition in the soil could be somewhat affected by the lack of a specific decomposition rate for mycorrhizal hyphae, and the fact that trenched roots may stay alive for a while whereas the roots in the litter bags start decomposing right after they have been buried, we expect this method to provide a sufficient estimate of the CO_2 emission. At the same time, reduced water uptake due to root exclusions increases the soil moisture in trenched plots, which may also alter respiration. To diminish the effect of higher SWC in the trenched plots, R_H was here modelled, using T_S and moisture of the intact soil (Savage *et al.*, 2018; Ryhti *et al.*, 2021). Here, the result nicely agreed with CASSIA and the temperature dependence method, but the root exclusion method still cannot be considered suitable for studying the detailed responses to changes in soil moisture.

In the temperature response method (Pumpanen *et al.*, 2015), R_R is calculated as the difference between total R_{FF} and R_H, which are parameterized and predicted, using the well-known Q_{10} temperature response function and different temporal scales. We used the mean of late spring r_0 values (Eq. 7) of previous years (2013–2015) in predicting

R_H, since the automated measurements were initiated later in the study years than usually. Therefore, T_S values were higher than normally during the first measurements in spring, and using these higher temperatures would have resulted in overestimation of R_H in early summer, followed by underestimation of R_R. In the method, only the temperature response is used in the fitting of CO_2 fluxes, while the response to SWC is not taken into account. This may have influenced *e.g.* the difference in estimated R_R between root exclusion and the temperature response method in 2018. In very severe moisture limitation, the short-term temperature responses could even be negative, obstructing calculations and preventing use of the method. The assumptions related to the temperature response of R_{FF}, where no seasonal thermal acclimation is considered, may be over-simplistic (Jarvi and Burton, 2013), and therefore not applicable in more extreme temperature conditions. However, the close consistency of this method with root exclusion and CASSIA model results suggests that in our conditions, the method succeeded in separating R_R from R_{FF}.

In the CASSIA model parameterization (Schiestl-Aalto *et al.*, 2015, 2019), C input results from the photosynthesis allocated to different tree organs, *e.g.* growth, metabolism, storage and mycorrhizal symbionts. R_R is estimated as a response to T_S and SWC, while parameterization is based on measurements from SMEAR II during 2002–2005 (Schiestl-Aalto *et al.*, 2019). Since the temperature response method, root exclusion method and modelling resulted in very similar estimates of R_R, we conclude that the CASSIA model seems to be a sufficient and inexpensive method for estimating the respiration rate of the entire site. However, as in every model, its behaviour is sensitive to site characteristics to which it has been constructed and parameterized, and therefore any generalization must be done with caution.

5. Conclusions

With global warming, soil-water availability may become a more important factor in determining soil C dynamics in boreal forests. Although, boreal forests have rarely experienced extremely hot and dry summers, severe heat waves and droughts have occur in recent years. While many studies examining drought effects on trees have been conducted under controlled laboratory conditions, C dynamics in the field with mature trees may act differently. Therefore, we studied belowground C dynamics of in the contrasting weather conditions in the mature Scots pine stand with addition of drought experiment with Scots pine saplings in greenhouse.

As assumed, the concentration of nonstructural C compounds in mature tree roots in the field increased with decreasing soil moisture, but in a compound-specific manner. Especially glucose, fructose and sucrose concentrations were higher in the drier, warmer year than in the moister, cooler study year, indicating osmoregulation in the roots. After removing the effect of temperature, root respiration was negatively associated with root fructose and glucose concentrations. However, we assume that low soil moisture drives the increase of these NSC compounds, and thereby respiration does not directly follow the changes in NSCs.

Here, we found that even though changes in soil temperature dominate the seasonal cycle of root respiration in mature Scots pine stand in the southern boreal zone, there was a significant, positive association with soil moisture. Additionally, similar relationship was also found in a controlled drought experiment with Scots pine saplings. The exceptionally dry conditions in 2018 in the field seemingly decreased the root respiration, at least in the topsoil layers. That does not necessarily reflect the response of the entire root system, as the soil moisture did not change drastically at lower soil depths. Although, root respiration was clearly higher in 2018 than in the cooler year 2017 due to higher soil temperatures, our results suggest that soil moisture is already an important factor in the current climate and will be a more important factor if droughts become more frequent and severe. Therefore, soil moisture should be taken into account in predicting current and future

root and belowground C dynamics.

The method used to study root respiration should be carefully selected, depending on the main aim, because the most specific methods may reveal detailed dynamics of single root types and locations, while coarser methods provide wider aggregates of various root and soil properties.

Declaration of Competing Interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2022.109040.

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