New Phytologist

Research

Carbon allocation to root exudates is maintained in mature temperate tree species under drought

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Received: *6 December 2021*
Accepted: *4 April 2022*

New Phytologist (2022) **235:** 965–977
doi: 10.1111/nph.18157

Key words: belowground carbon allocation, carbon partitioning, experimental drought, *Fagus sylvatica* (European beech), fine-root exudation, *Picea abies* (Norway spruce), rhizosphere, temperate forest C budget.

Summary

- Carbon (C) exuded via roots is proposed to increase under drought and facilitate important ecosystem functions. However, it is unknown how exudate quantities relate to the total C budget of a drought-stressed tree, that is, how much of net-C assimilation is allocated to exudation at the tree level.
- We calculated the proportion of daily C assimilation allocated to root exudation during early summer by collecting root exudates from mature *Fagus sylvatica* and *Picea abies* exposed to experimental drought, and combining above- and belowground C fluxes with leaf, stem and fine-root surface area.
- Exudation from individual roots increased exponentially with decreasing soil moisture, with the highest increase at the wilting point. Despite *c*. 50% reduced C assimilation under drought, exudation from fine-root systems was maintained and trees exuded 1.0% (*F. sylvatica*) to 2.5% (*P. abies*) of net C into the rhizosphere, increasing the proportion of C allocation to exudates two- to three-fold. Water-limited *P. abies* released two-thirds of its exudate C into the surface soil, whereas in droughted *F. sylvatica* it was only one-third.
- Across the entire root system, droughted trees maintained exudation similar to controls, suggesting drought-imposed belowground C investment, which could be beneficial for ecosystem resilience.

Introduction

In recent years, important processes controlling ecosystem carbon (C) dynamics and plant susceptibility to drought have been identified in the rhizosphere – the interface between plant roots and the soil environment (Finzi *et al.*, 2015; Joseph *et al.*, 2020; Williams & de Vries, 2020). In this narrow zone, plants interact with their environment by releasing root exudates, which fulfill fundamental roles in the regulation of microbial growth (de Graaff *et al.*, 2010), the liberation of C from protective associations with minerals (Keiluweit *et al.*, 2015), maintenance of soil hydrological properties (Carminati *et al.*, 2016) and communication with plants and other organisms (Bais *et al.*, 2006). Collectively, these interactions facilitate water and nutrient acquisition (Coskun *et al.*, 2017; Williams *et al.*, 2021), microbiome selection (van Dam & Bouwmeester, 2016) and plant species interactions (Ehlers *et al.*, 2020) that can alleviate plant stress (Vives-Peris *et al.*, 2020). Potential shifts in C allocation to exudates in drought-exposed ecosystems can affect many of the processes influenced by root exudates. However, although drought is a major natural risk that threatens the functionality of long-living ecosystems such as forests in the 21st century (IPCC, 2018), we do not know how shifts in C allocation to root exudates in response to soil water limitation are related to tree C budgets.

Trees respond to reduced water supply by modifying their belowground C allocation (Ruehr *et al.*, 2009; Hagedorn *et al.*, 2016; Hommel *et al.*, 2016) and potentially increase root exudation rates (Karst *et al.*, 2017; Karlowsky *et al.*, 2018; Preece *et al.*, 2018; de Vries *et al.*, 2019; Jakoby *et al.*, 2020). However, most studies only use single root branches – defined as ephemeral terminal branch orders – to describe a plant's exudation behavior, which does not consider changes in root growth, distribution, and longevity that can also be significantly altered under drought (Nikolova *et al.*, 2020; Zwetsloot & Bauerle, 2021). Allometric scaling of root exudates from a single root branch to the entire root system, while accounting for changes in root production and

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longevity, can advance our understanding of species-specific belowground C allocation patterns during periods of drought and improve terrestrial biosphere models (Fatichi *et al.*, 2019). In combination with an assessment of aboveground net-C assimilation, calculating the balance of belowground C allocation dynamics can identify whether trees 'invest' in the production of root exudates under drought.

Belowground C allocation has been assessed in pot experiments with small annual or perennial species (Kaštovská *et al.*, 2015; de Vries *et al.*, 2019) and tree saplings (Hagedorn *et al.*, 2016; Preece *et al.*, 2018). However, findings from these experiments cannot be easily translated to mature forest ecosystems. Soil water dynamics deviate drastically not only between homogenized and naturally developed field soils but also between surface soil and subsoil. Consequently, it is difficult to simulate exudation dynamics in artificial setups, and field-based studies are required to understand how an entire root system responds to drought. Previous studies addressing the impact of drought on root exudation failed to include measurements across different soil depths, although general vertical variations in exudation rates were identified (Finzi *et al.*, 2015; Tückmantel *et al.*, 2017). However, altered root distribution patterns with depth may affect root-system-level exudation and consequently whole-tree C budgets. Stable-isotope labeling studies have allowed C-flux integration over the entire rooting zone but this was usually achieved by tracing belowground C allocation via microbial activity (Joseph *et al.*, 2020; Gao *et al.*, 2021). As microbial respiration is hampered under drought (Moyano *et al.*, 2013), tracing C via microbial activity may hide potential increases in exudation, particularly if vertical variations occur. To scale root exudates to C-allocation dynamics in a forest ecosystem, vertically separated *in situ* exudate capture, combined with belowground root abundance is needed.

Root growth and exudation responses to water limitation may vary among tree species according to their drought susceptibility. Shallow-rooting species can be particularly vulnerable to drought; for example, when exposed to seasonal drought, *Picea abies* (L.) Karst., one of central Europe's most abundant and economically important tree species (Caudullo *et al.*, 2016) had a five-fold higher mortality rate compared with *Fagus sylvatica* L. (Pretzsch *et al.*, 2020), a broadleaf species representing the widespread natural vegetation in central Europe (Fang & Lechowicz, 2006). Each species exhibited different root responses to drought, with *F. sylvatica* having an inherently deeper root system (Schmid & Kazda, 2002), reduced fine-root diameter and increased specific root area to improve water uptake (Comas *et al.*, 2013; Hertel *et al.*, 2013; Nikolova *et al.*, 2020). By contrast, *P. abies* did not respond to soil moisture deficit by growing new, deeper roots but instead prolonged existing fine-root life span (Zwetsloot & Bauerle, 2021). It is likely that earlier seasonal transpiration by *P. abies* compared with deciduous *F. sylvatica* results in lower soil moisture under *P. abies* throughout the year (Grams *et al.*, 2021). Thus, the potential lack of access to water from deeper soil and overall lower soil moisture may amplify the susceptibility of *P. abies* to drought. Given the potentially crucial role of root exudates in response to water limitation, greater root exudation by both *F. sylvatica* and *P. abies* would be anticipated at root branches located in dry soils. In *P. abies*, prolonged root-system life span in dry surface soils may imply higher exudation across a larger proportion of *P. abies* root systems. By contrast, for the more dynamic root system of *F. sylvatica*, overall exudation amounts are harder to predict.

In this study, we utilized a novel throughfall exclusion experiment in a mature temperate forest, which imposed 5 years of severe drought during the entire growing season, to test if the allocation of photosynthates to root exudation increases under drought. We combined vertically distributed *in situ* root exudation measurements with fine-root surface area observations throughout the soil profiles of mature *P. abies* and *F. sylvatica* trees to identify C partitioning at the whole-tree level. We hypothesized, first, that roots in dry surface soils exude more C than roots in deeper moist soils and that root exudation rates are negatively correlated with soil water content across root-accessible soil depths. Therefore, allocation of C to exudates will be greater for the more drought-susceptible *P. abies* than for *F. sylvatica*. Second, we hypothesized that at the tree level, the proportion of C exuded by roots increases relative to net-photosynthetic C assimilation, which could be considered as a greater investment into root exudation in water-limited trees.

Materials and Methods

Site description

Sampling occurred at the 'Kranzberg Forest Roof' (KROOF) long-term drought experiment located in southern Bavaria, Germany (48°25.2′N, 11°39.7′E). Drought was imposed on six throughfall exclusion plots (sizes between 110 and 200 m^2; Grams *et al.*, 2021) via automated understory roofs that withheld throughfall during the growing season (April to November). On average, roof closure withheld *c.* 70% of total annual precipitation during 5 years of simulated drought (Grams *et al.*, 2021). Six additional plots without roofs served as nondroughted controls. The mixed stands comprised large groups of *F. sylvatica* L. (90 ± 4 yr old) surrounded by *P. abies* (L.) Karst. (70 ± 2 yr old) trees. Each plot consisted of a *F. sylvatica* and a *P. abies* cohort with three to six individuals each (Grams *et al.*, 2021). The soil at the site originated from Loess over Tertiary sediments and was classified as haplic Luvisol (FAO Classification) with moder type humus. Sediments form a loamy dense layer at *c.* 50 cm depth that is difficult for roots to penetrate, so that > 90% of roots are found at depths of 0–50 cm (Häberle *et al.*, 2012). Soil pH was in the range 3.8–4.6 (*P. abies*, 4.1; *F. sylvatica*, 4.5) and C : N ratios typically decreased with depth and were higher under *P. abies* (14.4 ± 0.6) than under *F. sylvatica* (12.5 ± 0.4; Supporting Information Table S1). During the sampling period (26 May–03 June 2019), relative humidity and temperature at 2 m height were 82.9 ± 0.4% and 16.8 ± 0.1°C, respectively. Above the canopy, photosynthetically active radiation (PAR) was 655.5 ± 17.9 μmol s^{-1} m^{-2} during the day (recorded every 10 min). Precipitation amounted to 17.7 mm during sampling (withheld on droughted plots). Soil moisture was assessed across

the soil profile as volumetric soil water content (SWC in vol.-%) using time domain reflectometry sensors (TDR; Campbell Scientific, Logan, UT, USA) installed vertically at depth increments of 0–7, 7–30 and 30–50 cm.

Root exudate collection and analysis

We sampled intact root branches in each of three drought and three control plots in previously installed root window boxes (40 cm long, 40 cm wide, *c.* 50 cm high; $n = 3$ per plot) that allowed access to roots without disturbing the experimental site. Root branches, comprising first- to third-order roots attached to a single transport root, were randomly selected for sampling (Fig. S1). Sampled root branches had an average weight of 0.20 ± 0.02 g, an average fine-root (≤ 2 mm diameter) surface area of 17.15 ± 1.83 cm^2 and 23.9 ± 4.5 tips cm^{-2} root surface area (Table S2). We sampled exudates from root branches growing in surface soils at the interface between the organic layer and mineral soil (0–7 cm depth) and the mineral soil (7–30 cm depth) according to Phillips *et al.* (2008). Briefly, root branches were carefully excavated, and the soil was gently removed with tweezers and by rinsing with a nutrient solution to limit osmotic stress (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.15 mM $MgSO_4$, 0.3 mM $CaCl_2$). We excluded dead roots and roots that did not pass a vitality check (i.e. no lateral roots present or black tissue color) from sampling and evaluation. Afterwards, root branches were left to recover for 48 h in a 1 : 1 mixture of sand and native soil from the site, cleaned again, and placed into 30 ml glass syringes containing sterile glass beads simulating a physical soil environment. Syringes were flushed three times with the nutrient solution and then equilibrated for 48 h, flushed again, and left wrapped in aluminum foil and covered with leaf litter. After another 48 h, we extracted root exudates trapped in the syringes using a membrane pump after adding 30 ml nutrient solution. We sampled 36 root branches in total, 18 from *F. sylvatica* and 18 from *P. abies*, at either 0–7 cm or 7–30 cm soil depth (Table S3). Blank syringes ($n = 4$) with glass beads, flushed with nutrient solution but without root branches, served as a reference. Root exudates were filtered through sterile syringe filters (0.22 µm, Rotilabo® MCE; Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and stored at 4°C until analysis. All consumables were acid-washed in 1% HNO_3 before use. Root exudation below 30 cm soil depth was estimated from minirhizotron and soil water content data (see the section on 'Exudation at the root system and tree level').

Exudate samples were quantitatively analyzed for total non-purgeable organic carbon concentration (TOC) with a multi N/C 2100 S (Analytik Jena GmbH, Jena, Germany). The method included the removal of total inorganic carbon by adding 50 µl 2 M HCl and flushing with synthetic air (180 s). The detection limit was 69.8 µg C l^{-1}.

Root characteristics

All root branches were harvested after exudate collection and scanned at 1200 dpi (Epson Perfection 4990 Photo; SEIKO Epson Corp., Tokyo, Japan). Root-surface area and the number of root tips were determined using WinRhizo (WinRhizo Pro 2016a; Regent Instruments Inc., Quebec, QC, Canada). Root branches were dried and total dry biomass was recorded. Measured exudate TOC was expressed per root surface area with a diameter ≤ 2 mm (henceforth: fine roots) of each branch, to correspond to sampled roots from soil coring (see the section on 'Exudation at the root system and tree level'). We also related exudation rates to the dry biomass of the branches (Fig. S2) and to absorptive-root density (Fig. S3), calculated as the number of root tips per unit of total surface area of the root branches (Table S2). Similar trends with treatment and depth were observed regardless of which parameters were used for normalization.

Assessment of C fluxes and parameters for scaling to the rooting zone and the tree level

C assimilation

To quantify C assimilation, light-saturated (photosynthetically active photon flux density = 1500 µmol m^{-2} s^{-1}) gas exchange rates (A_{sat}) were determined at 400 ppm carbon dioxide (CO_2) concentration for two trees per species and plot using an open gas-exchange system (LI-6800; Li-Cor Inc., Lincoln, NE, USA) over 2 wk in June 2019. Gas exchange rates were modeled for leaves in the shade crown for both species and six different needle ages for *P. abies* (Tables S4–S6). Light response curves were derived for leaves in the sun and shade crowns of *F. sylvatica* and *P. abies*, assuming steady assimilation at respective light saturation points (Larcher, 2001; Matyssek, 2010), a linear decrease between light saturation and light compensation and leaf respiration below light compensation (Methods S1). Assimilation rates were derived from light response curves during each 10-min interval when PAR was measured during exudate sampling. Daily assimilation rates were calculated assuming constant light conditions within these 10-min intervals. The total leaf area for *F. sylvatica* and *P. abies* was calculated using allometric equations determined individually for both species based on tree diameter and tree height (Patzner (2004); Table S7). No reduction in the leaf area was detected for *F. sylvatica* or the shade crown of *P. abies* in drought plots, while the leaf area in the sun crown of *P. abies* trees in drought plots was *c.* 50% lower compared with trees on control plots (data not shown) and the reduction was considered in our calculations accordingly. To obtain daily C assimilation per tree, leaf areas of the shade and sun crown were multiplied with assessed assimilation rates. Daily C assimilation was summed for all trees per species and plot and divided by plot size (Grams *et al.*, 2021) to obtain assimilation per species and m^2 and day, assuming each species occupied 50% of the plots as species distribution was uniform (Grams *et al.*, 2021).

Stem respiration

Stem respiration (µmol CO_2 m^{-2} stem area s^{-1}) was measured on two *F. sylvatica* and two *P. abies* trees per plot using custom-built chambers (60–204 cm^2) that were sealed to the stem at 1 m

height with Terostat-IX (Henkel AG & Co. KGaA, Duesseldorf, Germany). Respired CO_2 was measured with a Delta Ray Isotope Ratio Infrared Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in 5-min intervals during days and nights when C assimilation measurements took place. The cumulative daily stem respiration was calculated for each tree on days when C assimilation was measured, and scaled to the total tree stem area based on tree diameter and height (based on a conical tree shape; McDaniel *et al.* (2012); Rance *et al.* (2012); Table S7), assuming unchanged respiration rates along the stem (see Supporting Information Methods S1).

Soil and root respiration

Soil respiration rates were used to estimate the microbial response to drought and to calculate root respiration. Soil CO_2 efflux (μmol m^{-2} plot area and s^{-1}) was measured via permanent soil collars (PVC pipe, 20 cm inner diameter, 12 cm height), which were inserted *c.* 2 cm deep into the soil and sampled every 30 min to 1 h per tree species for 7 d in each plot ($n = 1$–3 per species and plot) using a multiplexed automated soil chamber system (Li-Cor-8100M; Li-Cor), and the daily sum was calculated per plot (see Supporting Information Methods S1). We averaged the daily sums of 7 d measurement periods per plot and species to calculate the contribution of root respiration to total soil respiration using estimates from the site, that is, 50% for *F. sylvatica* and control *P. abies* trees and 40% for *P. abies* trees on drought plots (Nikolova, 2007).

Exudation at the root system and tree level

Fine-root biomass, surface area and number of tips per plot were assessed using two soil cores (34 mm diameter) per species and plot in October 2018. Cores were taken randomly within the rooting zones of each species and divided into two depth increments (0–7 and 7–30 cm mineral soil depth; Nickel *et al.*, 2018). Fine roots (≤ 2 mm) were extracted from cores by washing with tap water and separated by species under a stereomicroscope. Fine roots were scanned and analyzed for surface area and the number of tips using WinRhizo, and subsequently dried to assess dry fine-root biomass. Fine-root surface area (Fr_{sa}) per m^2 for each species and soil depth was calculated from fine-root surface area per soil core (Fr_{core}), using the core volume (V_{core}) and the respective thickness of the soil increment (7 cm for 0–7 cm and 23 cm for 7–30 cm soil depth):

$$Fr_{sa} = \frac{Fr_{core}}{V_{core}} \times \text{depth}\,(0.07\,\text{m}/0.23\,\text{m}) \times 10\,000\,(\text{m}^2\,\text{m}^{-2})$$

The total number of fine-root tips m^{-2} was calculated using the same function, that is, by dividing root tips per soil core by core volume and multiplying by soil increment thickness.

Although most fine roots of both species were in the upper 30 cm (Zwetsloot *et al.*, 2019), we estimated fine-root surface area at 30–50 cm soil depth to integrate over the entire rooting zone (Häberle *et al.*, 2012). As no soil cores were taken to this depth, we analyzed images from minirhizotron tubes (six per plot, capturing roots of both species and each reaching a vertical depth of 50 cm), taken every 2 wk during the growing season, and once a month during the winter months with a minirhizotron camera (BTC-100X Camera; Bartz Technology, Carpinteria, CA, USA; Zwetsloot *et al.*, 2019; see supplements). We analyzed the number of root tips from minirhizotron images for the 7–30 cm and 30–50 cm depth layers, respectively, and calculated their ratio to estimate fine-root surface area below 30 cm. There were 1.9 times more tips at 7–30 cm than at 30–50 cm for *F. sylvatica*, and 12.4 times more tips for *P. abies*. Using these factors, the total number of root tips for the 30–50 cm soil was calculated from the number of root tips obtained from cores:

$$\text{Tips}_{30-50} = \frac{\text{Tips}_{7-30}}{1.9/12.4}$$

A nonlinear regression between the number of fine-root tips and fine-root surface area ($Fr_{sa} = 8.1 \times \text{Tips}^{0.3}$, $R^2 = 0.4$, $P < 0.001$) was then used to estimate fine-root surface area at 30–50 cm depth.

To obtain root-system level exudation (g C m^{-2} d^{-1}), fine-root surface area (m^2 m^{-2}) was multiplied by exudation rates of the individual root branches (g C cm^{-2} d^{-1}; Fig. 1). We used the relationship between soil water content and exudation rates across both species at 0–30 cm (Fig. 2c) to estimate exudation rates based on soil water content at 30–50 cm depth. Finally, to assess whole-tree C exudation, we calculated root-system exudation per m^2 plot surface area (Ex_{fra}) as a relative proportion of net-C assimilation:

$$Ex_{fra} = \frac{\sum \text{Exudation}\,(0-50\,\text{cm depth})}{\text{Net ass.}\,(\text{ass.} - \text{stem resp.} - \text{root resp.})} \frac{(\text{g C m}^{-2}\,\text{d}^{-1})}{(\text{g C m}^{-2}\,\text{d}^{-1})}$$

Statistics

All statistical analyses were conducted in R (v.3.6.3; R Development Core Team, 2020) in the RStudio environment (v.1.2.1335; R Development Core Team, 2020). We used linear mixed-effects models ('lme' function in the nlme package; v.3.1–137, Pinheiro *et al.*, 2018) with plot as random effect to test the relationship between dependent variables (exudation, assimilation, respiration, root characteristics) and independent variables (soil depth, treatment (control or drought) and species). The significance of individual terms and interactions of independent variables was determined by likelihood ratio tests using the ANOVA function. Pairwise *post hoc* testing of significant terms and interactions was performed using the 'emmeans' function (emmeans package v.1.5.2–1; Searle *et al.*, 1980). Differences were considered as significant at $P < 0.05$. We checked if the model assumptions of homoscedasticity ('leveneTest' function in the car package, v.2.1–2; Fox & Weisberg, 2019) and normal distribution of residuals ('shapiro.test') were met and transformed dependent variables, where necessary. We performed a nonlinear regression ('nls') to fit a power function for the relationship

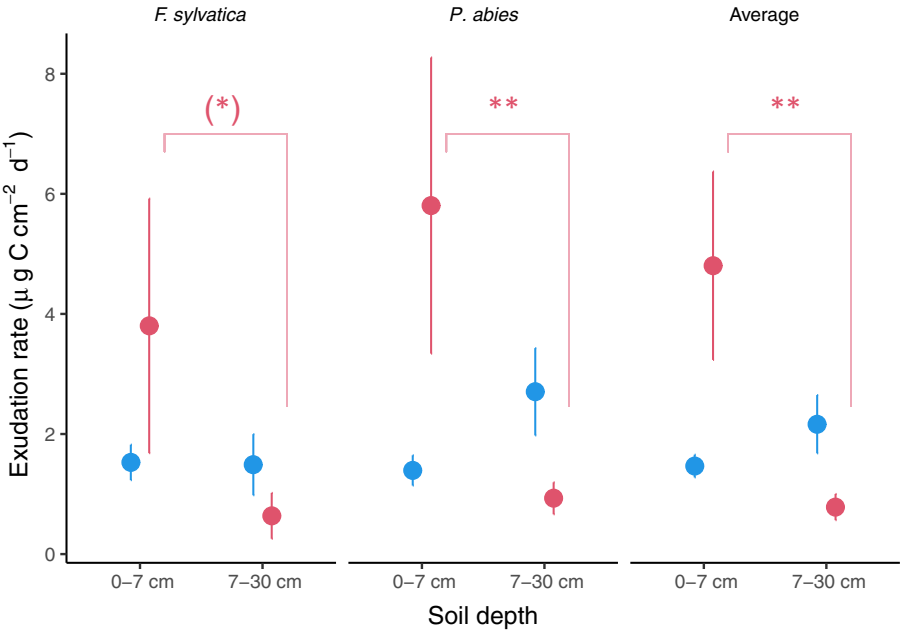

Fig. 1 Fine-root exudation rates (branch-level exudation) per fine-root surface area in *Fagus sylvatica* and *Picea abies* and average values over both species in control (blue) and drought (red) plots in the 'Kranzberg Forest Roof' (KROOF) experiment. Significant differences between 0–7 cm and 7–30 cm soil depths for the drought plots are indicated with red asterisks: (*), $P = 0.1$; **, $P < 0.01$. Symbols and whiskers indicate means ± SEs for $n = 3$ plots per treatment.

between root exudation rates and soil water content. The coefficient of determination and *P*-value for the regression were estimated from power transformation and linear regression of the data. Finally, we assumed that the maximum curvature of the power function represented the highest increase in exudation with SWC. Therefore, we calculated the first derivation of the power function and, using the 'optimize' function (STATS package, v.4.0.4), assessed the maximum curvature of the power function as a threshold for increased exudation with SWC. Results are presented as means ± 1 SE for $n = 3$ plots per treatment and species.

Results

Soil water content

Volumetric SWC was lower in drought plots than in control plots for both species, but the difference was only significant at 0–7 cm depth (Table 1). Under drought, *P. abies* trees tended to have the lowest SWC across all soil depths and 0–7 cm soils were significantly drier than the deeper 7–30 and 30–50 cm soils under both species (Table 1). In the control plots, SWC at 0–7 cm depth was lower than SWC below 30 cm but neither differed from SWC at 7–30 cm (Table 1).

Exudation rates of single root branches

Neither biomass nor fine-root surface area of root branches differed between species, treatments or depths, whereas root tip abundance and estimated absorptive-root density were overall higher in *F. sylvatica* than in *P. abies* (Table S2). Exudation rates were significantly higher in the dry 0–7 cm soil than in the more moist 7–30 cm soil, for both species in drought plots (Figs 1, S1, S2). Exudation rates per fine-root surface area were 3.8 ± 2.1 µg C cm^{-2} d^{-1} in 0–7 cm depth and 0.6 ± 0.4 µg C cm^{-2} d^{-1} in 7–30 cm depth for *F. sylvatica* ($P = 0.1$) and 5.8 ± 2.5 µg C cm^{-2} d^{-1} in 0–7 cm and 0.9 ± 0.3 µg C cm^{-2} d^{-1} in 7–30 cm for *P. abies* ($P < 0.01$; Fig. 1). In the control plots, where the vertical SWC distribution was more homogeneous, exudation rates did not differ across soil depths for either species. Average exudation rates per fine-root surface area did not differ between drought plots and control plots. However, in the drought plots, there was a strong trend towards increased exudation in the 0–7 cm depth and decreased exudation in the 7–30 cm depth compared with controls (Fig. 1).

Exudation rates of root branches per fine-root surface area declined with increasing SWC across treatments and soil depths in *P. abies*. Although a similar trend of declining exudation with increasing SWC was detected in *F. sylvatica*, the relationship was not statistically significant (Fig. 2). Overall, root branches exuded more C at lower SWC than at higher SWC under drought (Figs 2, S4). In both species, a single root branch in the driest 0–7 cm soil exuded substantially higher amounts of C than all other root samples (Fig. 2). However, there were no distinctive features to these roots – other than being in the driest soils – that would justify removing them from the dataset. Interestingly, expressing exudation rates per number of root tips (Fig. S5) brought the exudation rate in the *F. sylvatica* root branch with the highest exudation rate closer to the mean values of the other root branches, supporting our assumption that the high exudation rates were reliable. Owing to the high variability in a few data points, we also ran the regression analyses without the two high-exuding branches in the driest soils and obtained a similar relationship between root exudation and SWC regardless of whether or not these two data points were included in the model (Fig. S6). We identified a SWC threshold (the maximum curvature of the power function) at which exudation rates increased, which was similar for both species: 9.1 vol% SWC for *P. abies* and 8.3 vol% for *F. sylvatica* (Fig. 2). This SWC

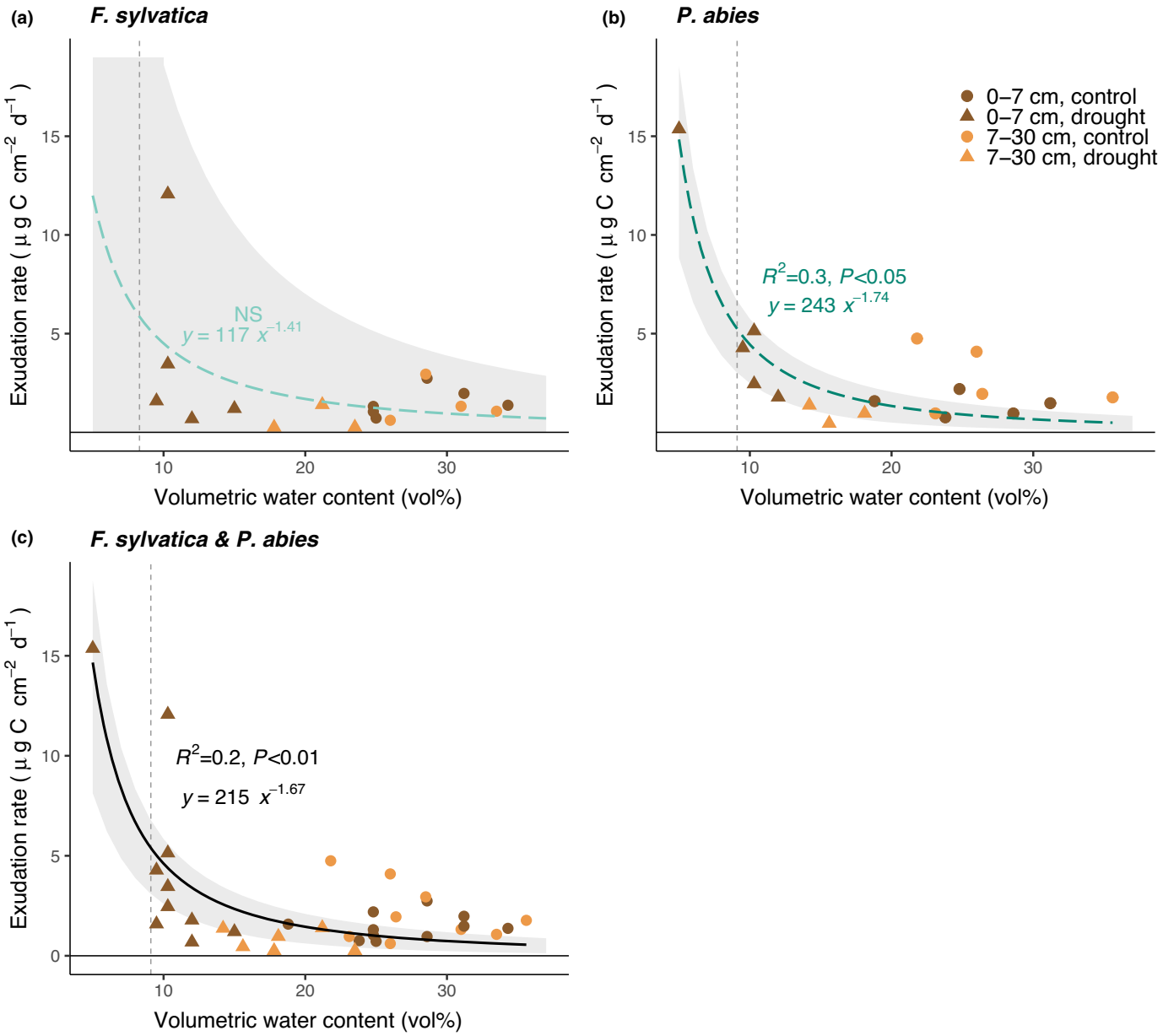

Fig. 2 (a–c) Relationships between exudation rate per fine-root surface area and volumetric soil water content (SWC) across treatments for root branches of *Fagus sylvatica* (a), *Picea abies* (b) and both species combined (c). Circles indicate control plots and triangles indicate drought plots. Dark brown symbols indicate 0–7 cm soil depth and light brown symbols indicate 7–30 cm soil depth. Regression lines (with gray shading indicating 1 SE) are given (note that the regression for *F. sylvatica* is not significant and the SE area exceeds the frame of the graph). The dashed gray line marks the maximum curvature of the regression at 8.3 vol% (*F. sylvatica*) and 9.1 vol% SWC (*P. abies* and both species combined), respectively, indicating increased exudation below these SWCs. R^2 and *P*-values for the regression were calculated from power transformation and linear regression of the data.

threshold was in the range of the permanent wilting point of the soil on the site (7.4–13.5 vol%; Grams *et al.*, 2021).

Root exudation and carbon allocation at the root system and the tree level

Fine-root surface area did not differ between treatments (Table 2). However, for both species there was a trend towards a smaller proportion of fine-root surface area at 0–7 cm depth in the drought plots, while the proportion of fine-root surface area at 7–30 cm and 30–50 cm soil depth was greater compared with the controls (Table 2).

Scaled to the root-system level, fine-root exudation across all soil depths did not differ between species or treatments (Fig. 3a). Fine-root exudation of *F. sylvatica* trees was 0.099 ± 0.023 g C m^{-2} d^{-1} in control plots and 0.106 ± 0.037 g C m^{-2} d^{-1} in drought plots, whereas fine-root exudation of *P. abies* amounted to 0.091 ± 0.021 g C m^{-2} d^{-1} in control and 0.119 ± 0.044 g C m^{-2} d^{-1} in drought plots (Fig. 3a).

Table 1 Soil volumetric water content (SWC in vol%) per soil depth increment (0–7 cm, 7–30 cm, and 30–50 cm) under *Fagus sylvatica* and *Picea abies* trees on control and drought plots in the 'Kranzberg Forest Roof' (KROOF) drought experiment.

Species	Treatment	0–7 cm	7–30 cm	30–50 cm
Fagus sylvatica	Control	28.1 (1.6)$^{a\ A}$	29.8 (1.4)$^{a\ AB}$	34.8 (2.2)$^{a\ B}$
	Drought	10.4 (1.3)$^{b\ A}$	20.1 (0.9)$^{ab\ B}$	28.3 (1.3)$^{ab\ C}$
Picea abies	Control	25.1 (1.8)$^{a\ A}$	26.9 (2.0)$^{ab\ AB}$	31.2 (2.0)$^{ab\ B}$
	Drought	8.9 (1.1)$^{b\ A}$	18.0 (1.4)$^{b\ B}$	22.2 (3.3)$^{b\ B}$

SWC was measured on 27 May, before exudate sampling. Lowercase letters indicate significant ($P < 0.05$) differences between species and treatments within each soil depth increment (0–7, 7–30, and 30–50 cm, respectively). Capital letters indicate significant differences between soil depths within the same species and treatment. Values are given as means with SEs for $n = 3$ plots per treatment.

Table 2 Fine-root (≤ 2 mm) surface area and depth distribution of *Fagus sylvatica* and *Picea abies* trees on control and drought plots in the 'Kranzberg Forest Roof' (KROOF) drought experiment.

			Fine root distribution (%)		
Species	Treatment	Fine-root area ($m^2\ m^{-2}$)	0–7 cm	7–30 cm	30–50 cm
Fagus sylvatica	Control	8.7 (1.1)	33.5 (7.4)	40.1 (6.4)	26.4 (0.9)
	Drought	9.0 (0.9)	19.5 (5.7)	51.5 (6.2)	29.1 (1.3)
Picea abies	Control	5.4 (0.6)	43.4 (16.4)	37.4 (13.1)	19.2 (3.3)
	Drought	4.1 (0.8)	34.3 (11.9)	46.1 (12.4)	19.6 (1.2)

Fine-root distribution (as % of the total fine-root surface area) is given across the soil profile in three depth increments. Note that fine-root abundance at 30–50 cm depth was modeled from minirhizotron regression data (see the section on 'Exudation at the root system and tree level' and Supporting Information Methods S1). There were no significant differences between treatments. Values are given as means with SEs for $n = 3$ plots per treatment.

The amount of C exuded at the root-system level did not change with soil depth for *F. sylvatica*, but there was a trend towards higher exudation rates below 30 cm depth in drought (0.022 ± 0.003 g C $m^{-2}\ d^{-1}$) than in control plots (0.013 ± 0.002 g C $m^{-2}\ d^{-1}$, Figs 3a, 4). In drought plots, *P. abies* tended to exude more at 0–7 cm and 30–50 cm depths (0.079 ± 0.050 and 0.016 ± 0.005 g C $m^{-2}\ d^{-1}$, respectively) than in control plots, whereas exudation at 7–30 cm depth (0.024 ± 0.014 g C $m^{-2}\ d^{-1}$) was lower than in control plots (0.047 ± 0.025 g C $m^{-2}\ d^{-1}$, $P > 0.05$; Figs 3a, 4).

During early summer, both *F. sylvatica* and *P. abies* trees in drought plots assimilated less than half the C of trees in control plots. Assimilation of *F. sylvatica* was 25.5 ± 4.8 g C $m^{-2}\ d^{-1}$ in control plots and 12.7 ± 3.9 g C $m^{-2}\ d^{-1}$ in drought plots ($P = 0.05$), whereas *P. abies* assimilated 22.5 ± 2.4 g C $m^{-2}\ d^{-1}$ in control plots and 8.3 ± 0.7 g C $m^{-2}\ d^{-1}$ in drought plots ($P < 0.05$). At the tree level, stem respiration did not differ between species but there was a trend towards higher stem respiration in *F. sylvatica* in control plots (3.0 ± 0.5 g C $m^{-2}\ d^{-1}$) than in drought plots (0.8 ± 0.2 g C $m^{-2}\ d^{-1}$; $P = 0.07$) and stem respiration also tended to be higher in control *P. abies* (4.6 ± 1.0 g $m^{-2}\ d^{-1}$) than in *P. abies* in drought plots (2.8 ± 0.7 g $m^{-2}\ d^{-1}$, $P = 0.1$; Fig. 4). Root respiration of *F. sylvatica* in control plots (3.8 ± 1.1 g $m^{-2}\ d^{-1}$) was significantly higher than root respiration in drought plots (1.4 ± 0.5 g $m^{-2}\ d^{-1}$, $P < 0.05$) and somewhat higher than that of *P. abies*. Roots of *P. abies* in control plots (2.9 ± 0.9 g $m^{-2}\ d^{-1}$) tended to respire more than roots in drought plots (0.7 ± 0.1 g $m^{-2}\ d^{-1}$; $P = 0.1$; Fig. 4). Net assimilation was higher in control plots than in drought plots in both *F. sylvatica* (18.7 ± 4.0 g C $m^{-2}\ d^{-1}$ in control plots and 10.6 ± 3.5 g C $m^{-2}\ d^{-1}$ in drought plots; $P = 0.1$) and in *P. abies* trees (15.1 ± 2.2 g C $m^{-2}\ d^{-1}$ in control plots and 4.8 ± 0.4 g C $m^{-2}\ d^{-1}$ in drought plots; $P = 0.07$; Fig. 4). The proportion of net-C assimilation allocated to root-system exudation (Ex_{fra}) during early summer in *F. sylvatica* trees was $0.5 \pm 0.1\%$ in control plots and doubled to $1.0 \pm 0.1\%$ of net assimilation in drought plots ($P = 0.1$; Fig. 3b, 4). In *P. abies* trees, $0.7 \pm 0.2\%$ of net-C assimilation was allocated to root exudates in control plots, whereas in drought plots the proportion of net-C assimilation allocated to fine-root exudation increased more than three-fold ($2.5 \pm 1.0\%$; $P < 0.05$; Fig. 3b, 4).

Discussion

Our study aimed to investigate whether tree species increased C allocation to root exudation in response to drought, both at the individual root level and at the whole-tree level. Consistent with our first hypothesis, *P. abies* root exudation rates increased with decreasing SWC, and root exudates in *F. sylvatica* showed a similar trend, indicating increased exudation rates of root branches in dry surface soils. When scaled to the whole-tree level, fine-root exudation did not differ between the control and drought treatments. However, the proportion of net-C assimilation partitioned to root exudation was significantly higher for trees under drought, supporting our second hypothesis that the belowground investment increases when water becomes limited. We found stronger evidence to support both hypotheses in the more drought-susceptible *P. abies*, but *F. sylvatica* showed similar trends.

Lower soil water content promotes C exudation of root branches

Various studies have found elevated exudation when roots were exposed to dry soil (Karlowsky *et al.*, 2018; Preece *et al.*, 2018; de Vries *et al.*, 2019; Jakoby *et al.*, 2020). Accordingly, we hypothesized that exudation rates would be highest from roots exposed to the lowest SWC. Supporting this hypothesis, we found significantly higher exudation rates for both species in the drier surface soil under drought, whereas exudation rates in the moister control plots, where vertical differences in SWC were less distinct, did not differ across soil depths (Fig. 1; Table 1). These trends persisted regardless of whether exudation was normalized by root biomass or absorptive-root density (Figs S1, S2). However, and in contrast to previous studies (Finzi *et al.*, 2015;

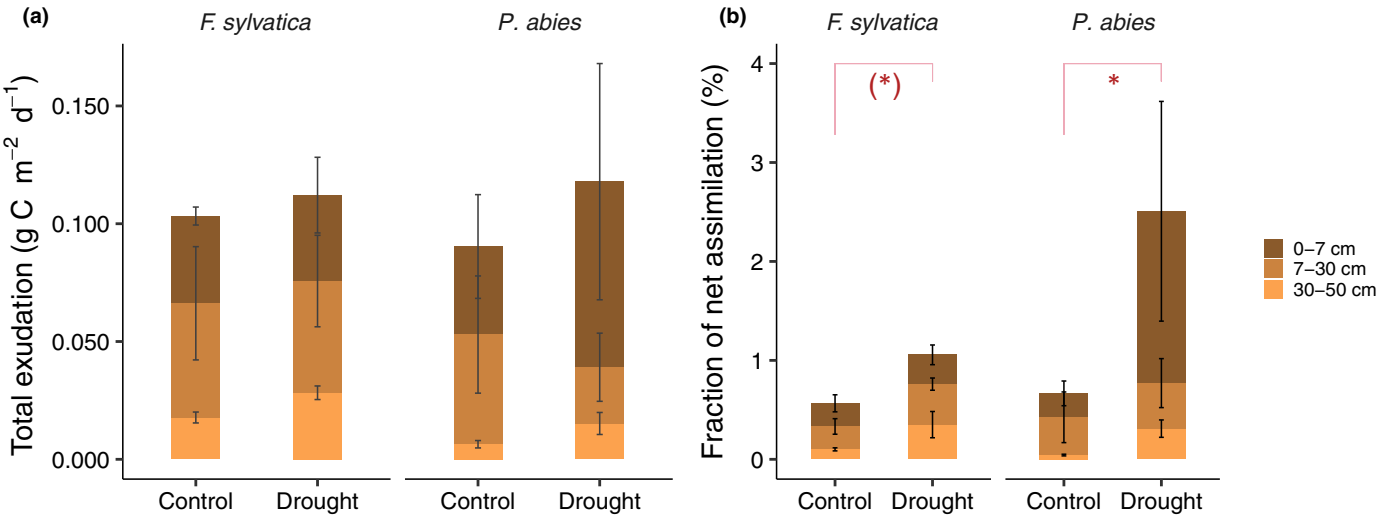

Fig. 3 (a, b) Fine-root exudation of *Fagus sylvatica* and *Picea abies* trees integrated over three rooting depths in the 'Kranzberg Forest Roof' (KROOF) experiment as total fine-root exudation (root-system level exudation) in g C m^{-2} plot surface area d^{-1} (a), and as a fraction of net assimilation of the trees (tree-level exudation: Ex_{fra}, in %) (b). Significant differences are highlighted: (*), $P = 0.1$; *, $P < 0.05$. Bars and whiskers indicate means ± SEs for $n = 3$ plots per treatment. Note that values for 30–50 cm soil depth were modeled from minirhizotron and soil water content data (see the section on 'Exudation at the root system and tree level' and Supporting Information Methods S1). Exudation data were integrated over a 2 wk period in early summer.

Tückmantel *et al.*, 2017), root exudation tended to increase with depth under control conditions, which may reflect site-specific soil texture characteristics (Grams *et al.*, 2021). We found a threshold at low SWC where root exudation rates increased sharply (9.1 vol% SWC for *P. abies* and 8.3 vol% for *F. sylvatica*; Fig. 2), which corresponded to the wilting point in the loess-dominated silty soil at the study site (Grams *et al.*, 2021), suggesting that trees were stimulated to release exudates when water availability became severely limiting. However, it is unlikely that exudation rates increase indefinitely with decreasing SWC, as there is evidence that root exudation is eventually reduced under severe drought (Williams & de Vries, 2020), for example, when roots lose contact to the soil. However, given that the SWC in the rhizosphere is less dynamic and probably higher under drought than the SWC of nonrooted soil (Carminati, 2013; Holz *et al.*, 2018), the SWC of the rhizosphere may differ from the bulk soil measurements captured by the TDR method used in this study. Thus, exudation may already be stimulated at higher rhizosphere SWC than the observed threshold indicates. Fine-scale spatiotemporal measurements in the rhizosphere could further elucidate the relationship between SWC and root exudation. Although, we found no changes in absorptive-root density with drought (Table S2), further studies are necessary to identify whether and how root morphology interacts with root exudation under drought (Wen *et al.*, 2022).

As we did not sample root exudates from dead roots and excluded roots that did not pass the vitality check, the presented exudation rates only reflect those in vital tree roots. Nonetheless, the *in situ* exudate capture approach provides a reasonable measure of soluble C input to the rhizosphere under drought, a fraction of C that is disregarded when belowground C allocation is solely traced via respiratory losses from soil. As soil microbial activity declined under drought (indicated by reduced soil CO_2 efflux; Table S6), increased exudation under low SWC might not be captured by measurements of respiratory losses or microbial biomass. For example, Joseph *et al.* (2020) reported that C mineralization was strongly reduced in soils below 15% SWC, which was close to the threshold at which we measured the highest exudation. Consequently, elevated exudation may contribute to C accumulation in the dry surface mineral soil, where large increases in C stocks at 0–5 cm depth were measured (M. Brunn *et al.*, unpublished).

Belowground C allocation at the root-system level is maintained under drought

Despite aboveground growth reduction and declining photosynthesis rates, several studies have reported increased belowground C allocation to roots under drought (Poorter *et al.*, 2012; Hagedorn *et al.*, 2016; Hommel *et al.*, 2016; Jakoby *et al.*, 2020). Although the opposite has also been shown (Ruehr *et al.*, 2009), these studies mostly measured C allocation as root growth or exudation at the root-branch level but did not assess whether C exudation at the root-system and tree levels also increased. Extending root C exudation to larger scales helps to identify processes related to the up- and downregulation of exudation at the whole-tree level and the linkage to rhizosphere characteristics (Prescott *et al.*, 2020; Schnepf *et al.*, 2022). Given the potential ecological benefits of belowground C allocation in the forest´s capacity to recover from drought (Hagedorn *et al.*, 2016) and for tree drought tolerance (Carminati *et al.*, 2016), we hypothesized that trees would increase the partitioning of C from net photosynthesis into root exudation under drought.

We found an overall reduction in net-C assimilation with drought for both species, > 40% in *F. sylvatica* and > 60% in *P. abies*. However, in contrast to declining aboveground

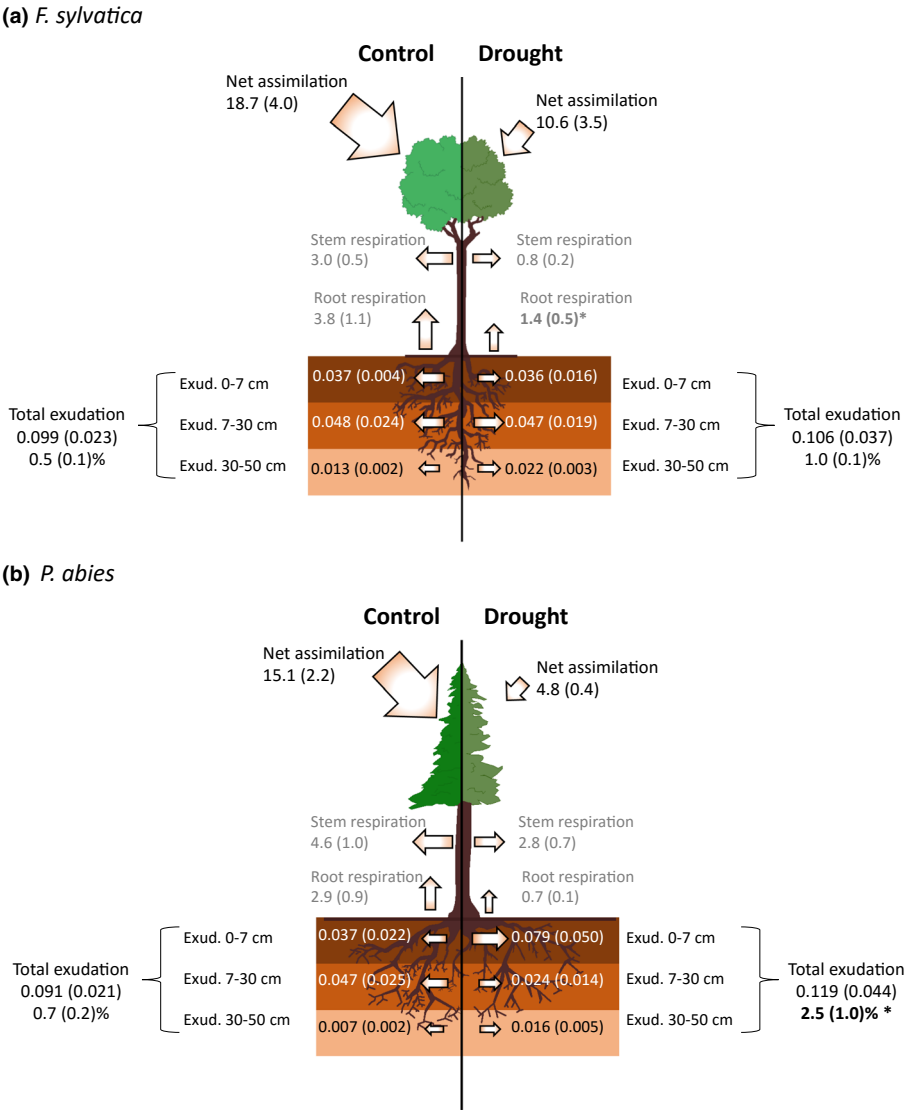

Fig. 4 (a, b) Carbon (C) fluxes in *Fagus sylvatica* (a) and *Picea abies* (b) on control (left) and drought plots (right) after 5 yr of repeated summer drought. Numbers next to the arrows show C fluxes in g C m^{-2} plot surface area d^{-1} (net assimilation, stem respiration, root respiration and root exudation). Respiration fluxes are shown in gray. Numbers next to the roots give the fine-root exudation separated by soil depth increments (dark brown, 0–7 cm; brown, 7–30 cm; light brown, 30–50 cm). Total exudation of the entire rooting zone and the proportion of net-C assimilation allocated to total exudation (assimilation – stem respiration – root respiration; see the section on 'Assessment of C fluxes and parameters for scaling to the rooting zone and the tree level' and Supporting Information Methods S1) are given next to the brackets. Note that values for 30–50 cm soil depth were modeled from minirhizotron and soil water content data (see the section on 'Exudation at the root system and tree level'). Bold numbers and asterisks indicate significant differences ($P < 0.05$) in scaled root respiration and proportion of net assimilation allocated to exudation between control and drought plots. Values are given as means with SEs for $n = 3$ plots per treatment. All data represent a 2 wk period in early summer.

C assimilation, belowground C release through fine-root exudation at the root-system level remained constant with drought (Figs 3a, 4), suggesting that the reduced fine-root surface area at 0–7 cm depth and increased fine-root surface area at 7–30 cm depth (Table 2) were compensated by higher exudation in surface and lower exudation in deeper soils. Nevertheless, the fraction of net-C assimilation allocated to root exudates doubled for drought-stressed *F. sylvatica* trees and tripled for *P. abies* (Figs 3b, 4), supporting our second hypothesis that trees under drought partition relatively more available C to root exudation at the tree level.

In our study, the proportions of net-C assimilation allocated to root exudation were only 0.6 ± 0.1% and 1.8 ± 0.6% in control and drought plots, respectively, which were below the 3–30% previously reported at other study sites for multiple species (Kuzyakov & Domanski, 2000; Jones *et al.*, 2009; Finzi *et al.*, 2015; Abramoff & Finzi, 2016; Gougherty *et al.*, 2018). Our observed exudation rates from root branches are in line with modeled or measured root exudation rates of diverse vegetation types (Finzi *et al.*, 2015; Dror & Klein, 2021; Rog *et al.*, 2021; Sell *et al.*, 2021), although they are at the lower end of reported values from comparable temperate forests (Tückmantel *et al.*, 2017; Meier *et al.*, 2020) and other ecosystems (summary provided by Gougherty *et al.* (2018). Discrepancies in exudate estimates across studies may arise as a result of methodological differences such as filter size variations (0.2 vs 0.7 μm) or the use of C-free materials (Gougherty *et al.*, 2018), bedrock characteristics (Meier *et al.*, 2020) or potential reuptake during longer collection periods (Oburger & Jones, 2018). In this study, we targeted low-molecular-weight substances of vital roots and thus excluded other rhizodeposits or volatile compounds (Delory *et al.*, 2016), which might account for a large fraction of previously reported root C deposition rates. Low root-system level exudation could also be related to physiological conditions varying throughout seasons, as exudates may not peak in early summer when we sampled, but in the late summer and autumn (Jakoby *et al.*, 2020), when fine-root production is higher (Abramoff & Finzi, 2016; Zwetsloot *et al.*, 2019). As net-C

assimilation is lower in autumn, the proportion of C allocated to root exudates might therefore be substantially higher towards the end of the growing season. Thus, the presented C fluxes may not reflect whole year dynamics but give an accurate approximation of relative and absolute exudation patterns of mature trees during early summer. We did not measure exudation or fine-root surface area in the deepest soil increment, but we ensured high scaling accuracy to the whole-tree level by observing and modelling C fluxes of different soil depths and entire above- and belowground compartments (see Methods S1 for further discussion on accuracy). Exudate C may have partially originated from tree C storage pools that were reduced under drought (Hesse *et al.*, 2021). However, there is indication of rapid belowground allocation of recently fixed C (Gorka *et al.*, 2019; Fossum *et al.*, 2022) and exudates at the experimental site contained at least 65–90% newly assimilated C (K. Hikino *et al.*, unpublished data).

Our approach did not allow us to account for potential C fluxes to mycorrhizal fungi. However, root exudation in ectomycorrhizal trees under drought can be twice as high as under well-watered conditions (Liese *et al.*, 2018) suggesting preferential C allocation to exudation than to mycorrhizae. Although the rate of colonization for our exclusively ectomycorrhizal trees was comparable between control and drought plots, the number of vital ectomycorrhizal tips declined by > 70% after three years of drought at the experimental site (Nickel *et al.*, 2018). This decline was accompanied by changes in ectomycorrhizal species composition, suggesting a relative increase in more C-demanding ectomycorrhizal types able to forage long distances (Nickel *et al.*, 2018). Thus, it is unclear whether drought altered the partitioning of belowground C to exudates or mycorrhizae. Nonetheless, the presented rates reflect the soluble C that enters the rhizosphere. Although the proportion of net-assimilated C allocated to root exudation seems negligible in forest C budgets, after entering the soil, root exudate C can accumulate in dry soil and facilitate ecosystem functions (e.g. soil water storage or C sequestration; Sokol *et al.* (2019), thereby contributing to the belowground C sink strength of forests and acting as a component of drought resilience (Körner, 2015; Hagedorn *et al.*, 2016). The composition of exudates can also change with drought (Gargallo-Garriga *et al.*, 2018) and specific compounds in root exudates have been associated with complex and diverse roles, e.g. changing the quantity of osmolytes that maintain cell turgor under water stress, developing the soil structure (Ahmed *et al.*, 2014; Baumert *et al.*, 2018; Guhra *et al.*, 2022) and enabling microbial recruitment or selection (van Dam & Bouwmeester, 2016), which may ensure survival during periodic stresses (Huang *et al.*, 2019). Such changes in the metabolite composition of root exudates under drought could contribute to the increased belowground C allocation we measured here, presenting an intriguing avenue for further research.

Drought-susceptible *P. abies* has a greater belowground C allocation under water-limitation than *F. sylvatica*

Although both species showed similar patterns in exudation rates from individual root branches (Fig. 1) and at the root-system level (Fig. 3), we found relatively higher C allocation to root exudation in *P. abies* than *F. sylvatica* under drought (Fig. 3b). Greater tree-level exudation was mostly a result of the stronger decline in net-C assimilation in *P. abies* (> 60%) than in *F. sylvatica* (> 40%) under drought. Although both species maintained root-system level exudation at comparable rates throughout the soil profile, they showed different vertical distribution patterns: in *F. sylvatica*, root-system level exudation was homogeneously distributed through the soil profile, whereas *P. abies* released two-thirds of the allocated C into the surface soil under drought (Fig. 3). In addition, although both species reduced fine-root surface area in the surface soil, the decline in *P. abies* roots was less pronounced (Table 2), and exudation rates per fine-root surface area of root branches tended to be higher (Fig. 1). The decreased assimilation, respiration (Fig. 4; Table S6), and reduced growth (Pretzsch *et al.*, 2020; Grams *et al.*, 2021) of *P. abies* indicates that this species was more strongly affected by drought than *F. sylvatica*. It is therefore striking that *P. abies* allocated a relatively greater proportion of C belowground (Fig. 3b). However, our findings agree with the theory of Williams & de Vries (2020) that fast-growing species like *P. abies* increase relative exudation, while slower growing species like *F. sylvatica* maintain root exudation under drought (Williams & de Vries, 2020). Although the proportion of net-C assimilation allocated to root exudation in *P. abies* was greater than in *F. sylvatica*, assessing the benefits to the water balance or the ecosystem resilience of these species as a result of exudates was beyond the scope of this study. Whether tree-level C investment into root exudation is an active or passive process calls for finer-scaled manipulative experiments to identify mechanistic underpinnings. Alongside lower SWC, it should finally be noted that there may be several additional reasons for higher root exudation from *P. abies* in the surface soil. For example, soil–root nutrient concentration gradients may increase concentration-related diffusion under water limitation and contribute to elevated exudation (Canarini *et al.*, 2019; Butcher *et al.*, 2020). The low variation in absorptive-root density in *P. abies* compared with *F. sylvatica* (Table S2) further suggests limited morphological adaptation of *P. abies* roots to drought. Together with the observed prolonged life span of *P. abies* roots in the surface soil (Zwetsloot & Bauerle, 2021), overall root functionality might have been reduced (Vetterlein & Doussan, 2016; Nikolova *et al.*, 2020) and *P. abies* might have lost its capability to control C release to a greater extent than *F. sylvatica*. Although the relationships between root exudation, root morphology and root life span (both in general and under drought) require further study, our findings indicate that drought stress will have a greater impact on rhizosphere processes in *P. abies* than *F. sylvatica*.

Conclusions

Root-system and whole-tree level exudation during the study period in early summer were small compared with other assessed C fluxes and seemed negligible in the overall C budget of the forest. However, the observed elevated belowground C partitioning under drought may play a crucial role in ecosystem functioning and maintaining tree vitality, with the drought-susceptible

P. abies investing more C belowground under water limitation compared with *F. sylvatica*. Our findings pave the way for future work integrating the chemical composition of exudates, microbial and plant functional processes to evaluate the fate of root exudate C entering the soil, its spatiotemporal stability and its role in forest ecosystem drought resilience. Our findings encourage future studies to record belowground C allocation even under low microbial activity by including: *in situ* exudate collection during drought experiments; spatially explicit exudation measurements in naturally developed soil profiles; and calculations of tree-level exudation in mature forest. By integrating across different soil depths and using allometric scaling of the unique empirical dataset of the KROOF experiment, our study demonstrates that trees can maintain root exudation by increasing the proportion of net-C assimilates allocated to exudates under water limitation, suggesting novel strategies of up- and downregulating belowground C partitioning under drought. Given that there is large variation in how models estimate belowground C allocation under changing climate, our data provide valuable information about how temperate tree species partition assimilates into individual soil layers as well as to the entire rhizosphere under water limitation.

Acknowledgements

We are grateful to Gesine Preuss (University Koblenz-Landau, Germany) for technical assistance, Karin Meyer (University of Koblenz-Landau, Germany) for TOC analysis, Vincent Riedel (University of Würzburg, Germany), Romy Rehschuh (Karlsruhe Institute of Technology KIT, Germany) and Timo Knüver (University Innsbruck, Austria) for supporting soil respiration measurements, Marc Goebel (Cornell University, USA) and Maria Stange del Carpio (Technical University of Munich, Germany) for support with minirhizotron and root characteristics analyses. We thank Thorsten Grams (Technical University of Munich, Germany) for organizing access to the experimental site and the German Research Foundation (DFG) for project funding (PR555/2-1, PR292/22-1, GR 1881/5-1, MA1763/7-1, MA1763/10-1) as well as the Bavarian State Ministry for Nutrition, Agriculture and Forestry and the Bavarian State Ministry for Environment and Consumer Protection for providing funding for maintenance of the experimental site (W047/Kroof II). This research was further supported by a research program for junior scientists from the University of Koblenz-Landau, Germany.

Author contributions

MB, BDH and TLB designed the study. MB and BDH collected the data, developed hypotheses and the concept of the manuscript. MJZ and TLB helped with data analysis and interpretation. MJZ, FW, KP, KH, NKR and EJS contributed data and reviewed the manuscript draft. MB and BDH wrote the manuscript and all co-authors thoroughly revised and edited manuscript drafts. MB and BDH contributed equally to this work and share first authorship.

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Data availability

Data that support this study are available through Cornell University eCommons data repository at https://doi.org/10.7298/6r80-8a15.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Example of a sampled root branch.

Fig. S2 Exudation rates with drought per dry-root biomass.

Fig. S3 Exudation rates with drought per density of absorptive roots.

Fig. S4 Relationships between exudation rate per density of absorptive fine roots and volumetric soil water content for the drought treatment.

Fig. S5 Exudation rates per number of root tips related to volumetric soil water content.

Fig. S6 Relationships between exudation rate per fine-root surface area and volumetric soil water content separated for drought and control treatments.

Methods S1 Scaling approach.

Table S1 Edaphic conditions of the experimental site.

Table S2 Root characteristics of root branches.

Table S3 Number of root branches collected for exudation rates.

Table S4 Rates of light-saturated gas exchange (A_{sat}), and PAR light intensity at light saturation and light compensation for sun and shade leaves of *Fagus sylvatica* and *Picea abies*.

Table S5 Estimated decrease of A_{sat} in *Picea abies* trees with needle age.

Table S6 Rates of photosynthesis, stem, and soil and root respiration.

Table S7 Leaf and stem area used as parameters for scaling C fluxes.

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