

Temperature effects on root exudation in mature beech (*Fagus sylvatica* L.) forests along an elevational gradient

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Research Article

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Abstract

Aims: Root exudation is an important process with presumed large impact on soil biological activity and nutrient cycling. Recent advances in *in situ*-measurement techniques have enabled deeper insights into the impact of tree root exudation on rhizosphere processes, but the abiotic and biotic controls of exudation rate remain poorly understood. We explored the temperature dependence of exudation of mature beech (*Fagus sylvatica* L.) trees.

Methods: We measured fine root exudation in seven beech forests along an elevational gradient (310-800 m a.s.l.) and related C-flux rates to prevailing and mean summer temperatures (MST), soil moisture, soil chemistry, and root morphological traits.

Results: Average mass-specific exudation (averaged over all sampling dates) ranged from 12.2 $\mu\text{g C g}^{-1} \text{h}^{-1}$ to 21.6 $\mu\text{g C g}^{-1} \text{h}^{-1}$ with lowest rates measured at highest elevations and peak rates at mid elevation (490 m). Regression analyses showed a highly significant positive effect of prevailing air and soil temperature on exudation rates ($p < 0.01$) with an average increase by 2 $\mu\text{g C g}^{-1} \text{h}^{-1}$ per 1 °C temperature increase, identifying temperature as the most influential factor among the tested climatic, edaphic and root morphological parameters. Relations to soil moisture, mean annual precipitation, topsoil N concentration (all negative), and specific root length and soil pH (positive) were less tight, the relation to MST insignificant.

Conclusions: Root exudation of beech trees is to a considerable extent controlled by prevailing temperature, whereas the dependence on the site's average summer temperature is weak.

Introduction

Climate warming affects biogeochemical processes and the carbon (C) cycle of forests through various direct and indirect interactions, notably temperature effects on photosynthesis, plant respiration, soil organic matter (SOM) decomposition, nutrient diffusion in soil and root nutrient uptake kinetics, and the abundance and activity of mycorrhizal partners (Norby et al. 2007; Yin et al. 2013b). While much experimental research with trees addressed warming effects on photosynthesis, respiration, belowground C allocation and fine root dynamics (Bai et al. 2010; Graham et al. 2014; Liang et al. 2013; Majdi and Ohrvik 2004) and soil microbial activity and SOM decomposition (von Lützow and Kögel-Knabner 2009), much less is known about the temperature dependence of rhizodeposition, i.e. the loss of carbon from roots (Yin et al. 2013b). Rhizodeposition, which includes exudation (the leakage of soluble organic compounds from living root cells), the secretion of mucilage and other organic substances, and the shedding of dead cells and tissues from roots, represents a net flow of C from roots to the soil which can have profound effects on C and nutrient cycling in forest ecosystems (Jones et al. 2009). Root exudation is thought to be a key mediator in plant-soil interactions that influences the composition and activity of soil microbial communities and thus soil enzyme activity, SOM decomposition and nutrient cycling (Cheng et al. 2014; Fransson and Johansson 2010; Jones et al. 2004; Pausch and Kuzyakov 2017). Despite its important role, the C flux rate with root exudation is usually rather small (Jones et al. 2004; Meier et al. 2020; Nguyen 2003; Pinton et al. 2007), comprising an estimated 5–21 % of net primary productivity in forest ecosystems (Badri and Vivanco 2009; Knnenberg and Phillips 2017) or up to a third of the photosynthetic carbon gain of tree saplings (Liese et al. 2018). It is not well understood, how a temperature increase is influencing exudation rate, as this C flux is dependent on a multitude of biotic and abiotic factors and is rarely quantified under field conditions (Yin et al. 2013). It in fact remains the most uncertain part of the C cycle in ecosystems, especially in mature forests (Pausch and Kuzyakov 2017).

Biotic factors influencing root exudation are photosynthetic carbon gain and carbohydrate allocation to roots, plant phenology and development stage, root morphology (notably specific root length, SRL), the type of mycorrhizal fungi, and the presence of pathogenic soil microbes (Kuzyakov 2002; Meier et al. 2013; Neumann and Römheld 2007; Tückmantel et al. 2017), as well as root membrane conductivity and the root – soil solute concentration gradient as factors determining basal exudation (Jones et al. 2004). Abiotic factors that have been found to influence root exudation rate include radiation through its influence on photosynthesis (Nakayama and Tatenno 2018), deficiency of nutrient elements such as P and N (Meier et al. 2020; Yin et al. 2014), soil moisture (Jakoby et al. 2020; Preece et al. 2018), and temperature (Yin et al., 2013a). The few studies that have addressed temperature effects on tree root exudation employed either experimental soil warming of seedling cultures (Karst et al. 2017; Uselman et al. 2000; Yin et al. 2013 a, b), or compared trees of the same species at sites differing in mean temperature (Yang et al. 2020). While most studies indicate higher exudation under elevated temperatures, higher exudation at lower temperatures was also reported (Karst et al. 2017; Yang et al. 2020). Due to the multitude of possible influential factors and the difficulties associated with measuring tree root exudation under field conditions, our understanding of climate warming effects on exudation is very limited. This weakens our capacity to predict changes in soil biological activity and C and nutrient cycling in forest soils in a future warmer world.

In this study, we investigated changes in root exudation in seven mature forests of European beech (*Fagus sylvatica* L.) along an elevation transect (310–800 m a.s.l.) on acid soil with the aim to explore the responses of root exudation of this tree species to both prevailing thermal conditions and varying mean annual temperature (8.4–6.0°C) at the site. Earlier research in Central European beech forests with an *in-situ* cuvette-based method showed that mass-specific exudation rates decrease greatly from the topsoil to the subsoil (Tückmantel et al. 2017) and increase with soil acidity and N deficiency of the soil (Meier et al. 2020). To capture the assumed temperature effects, we here extend the sample of studied beech forests to an elevational transect from the colline to the montane zone with a marked temperature decrease and precipitation increase, while keeping soil chemistry sufficiently constant. The studied beech forests are thus exposed to increasingly warmer and drier summers in downslope direction, as is predicted to happen in the course of climate warming in the 21st century in Central Europe and elsewhere (Kaspar et al. 2017). By employing a space-for-time substitution, this setting may allow rough estimates of anticipated future changes in tree root exudation with climate warming. Besides temperature, we measured soil moisture and several soil chemical parameters as well as fine root biomass (FRB) and fine root morphological traits in order to relate exudation to possible controlling abiotic and biotic factors. We hypothesized that the root exudation rate increases with increasing prevailing temperature due to an overall increase in the tree's metabolic activity, while the cumulative annual C flux with exudation is primarily determined by growing season length, which increases from the colder to the warmer stands.

Material And Methods

Study sites, climate, and geology

The study was conducted in seven European beech forests of mature age along an elevational gradient between 310 and 800 m a.s.l. on the eastern slopes of the Rothaar Mountains in the state of Hesse, central Germany. The elevation transect had a length of approx. 30 km in east-west direction and ranged from the colline/submontane to the montane belt, covering a gradient in mean annual temperature (MAT) of about 2.4°C. All stands belonged to the Luzulo-Fagetum forest community (beech forests on acidic soils) and stocked on acidic bedrock (Triassic sandstone or Paleozoic clay shale) in level to slightly inclined terrain (Table 1). In the forests, study plots of 30 m x 30 m size were selected in sections with closed canopy. While mean diameter at breast height (DBH) varied only between 32 cm and 45 cm in the seven stands and tree ages ranged mostly between 100 and 180 years according to information of the forest offices, stem density was more variable (150–578 stems ha⁻¹). Mean tree height as measured in 15 trees per plot with a Vertex III height meter (Haglöf, Längsele, Sweden) with at least three measurements taken per tree from different directions decreased with elevation from 33 m to 20 m. The study region has a cool-temperate humid climate with MAT decreasing from 8.4 to 6.0°C and mean annual precipitation (MAP) increasing from 600 to 1200 mm yr⁻¹ from the colline to the montane zone (German Weather Service, period 1981–2010; Table 1).

During the study period from spring 2014 to winter 2015, air and soil temperature were continuously measured in 2015 with iButton sensors (Maxim, Dallas, USA) installed at 1.5 m height above the forest floor and in the topsoil (3 cm depth) in the seven stands. The sensors were read every 60 min. Dendrometer tapes (type D1, UMS, Munich, Germany; precision of 0.1 mm) were permanently installed at 1.5 m height on 15 trees per plot to determine DBH and annual stem diameter increment through annual DBH recording.

Soil chemical analyses

To characterize soil chemistry, each five topsoil samples were collected with a 6.6 cm-diameter corer at random position in the study plots in summer 2015. Subsequently, the thickness of the organic layer was measured in the cored hole. All soil samples were separated into organic layer and mineral topsoil material (0–10 cm), transferred to the laboratory in a cooling box, sieved (< 5 mm for organic layer material; < 2mm for mineral soil), and stored in polyethylene bags at 4°C for further processing. Subsamples were analyzed in field-moist condition for pH (measured in H₂O: 10 g fresh soil in 25 ml deionized water, or in CaCl₂: 10 g soil suspended in 0.01 M CaCl₂) after 1 h of equilibration. Additional subsamples were dried (60°C, 48 h), ground, and analyzed for total carbon and nitrogen concentrations through gas chromatography with an elemental analyzer (vario EL III, Elementar Analysensysteme GmbH, Hanau, Germany). Since all soils were highly acidic, total C content was assumed to be organic C (SOC). The total P content was determined by ICP-OES analysis (Perkin Elmer Optima 5300 DV) after acid-pressure digestion (65% HNO₃ at 195°C for 6 h) of the ground soil samples. Plant-available phosphorus was estimated with the resin bag method according to Bowman and Cole (1987) using Dowex 1 x 8–50 anion exchange gel (Dow Water& Process Solutions, USA) that was placed for 16 h in a solution of 1 g of soil material suspended in 30 ml water (Sibbesen 1977). Extracted P was re-exchanged with NaCl and NaOH solutions and the P concentration measured in a spectrophotometer (Libra S 21, Biochrom, UK) at 712 nm after adding 5 mM hexaammonium-heptamolybdate solution (Murphy and Riley 1962). The water content of the topsoil was determined gravimetrically in each five soil samples collected at random position in the plots every month from March to December 2015. The sampling in August and September was conducted synchronously with the collection of root exudates.

Fine root biomass and root morphology

In November 2018, each 12 root samples were taken at random locations in the 30 m x 30 m plots using a soil corer (6.6 cm in diameter) and the material separated into organic layer and mineral topsoil (0–10 cm) material. Samples were transported in a cooling box to the laboratory where they were kept at 4°C and processed within four weeks. Only fine roots (diameter < 2 mm) of beech were considered in the analysis. All fine root segments were picked out by hand and sorted into live and dead fine root mass under a stereomicroscope (40x magnification). Root vitality was assessed by means of root color and structure of the root surface, root elasticity and turgescence, branching structure, and the degree of cohesion of cortex, periderm, and stele (for criteria, see Persson, 1978; Meier & Leuschner, 2008). Standing FRB was expressed as profile total (organic layer and uppermost 10 cm of mineral soil; in g m^{-2}). Specific root length (SRL, m g^{-1}), specific root surface area (SRA, $\text{cm}^2 \text{g}^{-1}$), root tissue density (TD, mg cm^{-3}), and root tip frequency (RTF, number of root tips per fine root mass; n g^{-1}) were determined for the root material using a flatbed scanner and the software WinRhizo (Réagents Instruments, Quebec, Canada). Fine roots used for exudate collection were clipped off and measured for the total root surface area of the root segment. Root biomass was determined by drying (48h, 78°C) and weighing the sorted root samples.

Root exudate collection

Root exudates were collected in three sampling campaigns in July of 2014, August 2015, and September 2015 in nine soil pits excavated at each site in at least 3 m distance to the nearest mature beech tree, employing the cuvette-based *in situ*-collection approach (after Phillips et al. 2008; Freschet et al. 2021). Beech fine root strands were carefully extracted from the topsoil and cleaned with fine forceps and deionized water. The terminal strand sections (average cumulative length of all parts of the strand c. 16.5 cm and mean diameter c. 0.45 mm) were embedded in root cuvettes filled with sterile 2 mm-diameter glass beads to simulate the porosity of the soil and mechanical impedance in a matrix free of carbon. The beads covering the root were moistened with a carbon-free nutrient solution (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).

The roots in the cuvettes were equilibrated for 48 h before flushing the cuvettes with a diluted nutrient solution under low pressure. New carbon-free nutrient solution was added and after an equilibration of approx. 24 h, these trap solutions were collected using low-pressure vacuum. These solutions were subsequently filtered through sterile glass fiber filters (GE Healthcare Life Sciences Whatman, Glass Microfibre Filters, Grade GF/F) and stored at -20°C until further analysis. Control samples were taken from rootless cuvettes treated similarly. The samples were analyzed for their dissolved organic carbon using a total carbon analyzer (Shimadzu TOC-L CPH/CPN; Shimadzu Scientific Instruments, Duisburg, Germany). Taking fresh root biomass as a calculation basis, net mass-specific exudation rates ($\mu\text{g C g}^{-1} \text{h}^{-1}$) and annual C fluxes with exudation per root mass or ground area ($\text{mg g}^{-1} \text{yr}^{-1}$, $\text{g C m}^{-2} \text{g}^{-1}$) were calculated, the latter by multiplying daily exudation rates by the length of the growing season (defined here as the number of days with average temperatures over 10°C). To estimate growing season length for the seven sites, we used gridded temperature data provided by the German Weather Service (DWD). Growing season length decreased with decreasing MAT from 170 to 125 d between 310 and 800 m a.s.l. (Table 1).

Statistical analyses

All statistical analyses were conducted with SPSS software. The data was tested for fit to normal distribution using a Shapiro-Wilk test. Normally distributed data were tested for homogeneity of variances with a Levene test. Site differences between means of edaphic (total C and N content, C/N-ratio, pH (H_2O), pH (CaCl_2), total and plant-available P content) and root morphological variables (TD, SRA, SRL, and RTF), and exudation rates (net mass-specific exudation rate and annual C flux with exudation) were examined with one-way analysis of variance for parametric data and a Kruskal-Wallis test for non-parametric data. ANOVAs were followed by a Scheffé or a Dunnett T3 test, if homogeneity of variances was not given. Kruskal-Wallis tests were followed by pairwise comparisons to locate differences.

Pearson correlations were used for investigating the relation between root exudation rate and elevation, climatic and edaphic variables, and root morphological traits. If data were non-normally distributed, Spearman rank correlation analysis was employed. Correlations were tested for the variables long-term mean temperature and precipitation, average summer temperatures of 2014 and 2015, soil water content at the date of exudate sampling, and air and soil temperatures averaged over the seven days prior to sampling. The p-values were adjusted by the Benjamini-Hochberg procedure for multiple testing.

Multiple regression analyses with backward variable elimination were conducted to test for significant independent predictors of root exudation rates and the estimated annual C flux with exudation per m^2 ground area. In the initial model, prevailing temperature, soil moisture, stem density, pH, soil C, N and P content, and the four root morphological traits were included. At each elimination step, the variable showing the smallest contribution to the model was deleted until all variables remaining in the model produced significant *F* statistics. The p-values were calculated via the bootstrapping method because most of the data showed no fit to normal distribution. Variables were tested for multi-collinearity and were excluded when they were highly correlated and collinearity diagnostics (variance inflation factor and tolerance) were critical.

Results

Elevational change in climatic and edaphic conditions

During the measuring years, annual mean air temperature (weather station data) decreased at a lapse rate of c. 0.57°C per 100 m from 9.9 to 7.5°C in 2014 (and from 9.3 to 6.9°C in 2015) along the elevational gradient. Both years were on average by about 1.5°C (2014) and 1.0°C (2015) warmer than the long-term average of the 1981–2010 period (Table 1). Annual precipitation increased from 706 mm at 310 m to 1115 mm at 800 m in 2014, and from 571 to 1054 mm in 2015, corresponding to average increases by 83 and 99 mm per 100-m increase in elevation during the two study years.

The recording of climatic conditions in the stands directly before exudate sampling in summer 2015 gave smaller air temperature (T_a) and soil temperature (T_s) decreases with elevation during the sampling campaigns (T_a decrease by 0.37 and 0.53°C 100 m⁻¹ and T_s decrease by 0.41 and 0.45°C 100 m⁻¹ in August and September 2015, respectively; Fig. 1a). The amount of precipitation recorded one week prior to each sampling campaign showed a very slight decrease with elevation in 2014, with the lowest amount measured at the highest sites at 800 m (244 mm) and 690 m (260 mm) and the highest amount at the intermediate sites at 490 m (291 mm) and 560 m (281 mm). Precipitation increased with elevation during the August and September 2015 sampling campaigns (Fig. 1b). Gravimetric soil water content (SWC) of the organic layer plus uppermost mineral soil (0–10 cm) determined for the sampling in August and September 2015 roughly doubled between the lowest and highest sites from 15–25 vol. % at the low-elevation sites to 38–47 vol. % at the high-elevation sites (Fig. 1c). In summer 2015, SWC increased along the gradient from 46.9% at 310 m to 67.0% at 800 m in the organic layer, and from 22.8–43.6% in the mineral topsoil (Table 2).

Table 2

Soil physical and chemical characteristics of the organic layer and the mineral topsoil (0–10 cm) of the seven beech forests along the elevational gradient (means and SE in brackets). Results for soil water content (SWC) are the means of gravimetric determination on nine sampling dates with n = 5 samples per site. Different upper-case and lower-case letters indicate significant differences between sites at p < 0.05 (p-values adjusted by the Benjamini-Hochberg procedure for multiple comparisons).

Elevation	m a.s.l.	310	380	490	560	600	690	800
Parent material		Sandstone	Sandstone	Clay shale	Clay shale	Clay shale	Clay shale	Clay shale
Geological epoch		I BU	I BU	I K	u D	u D	m D	m D
Org. layer thickness	cm	4.5 (0.26)	5.2 (0.35)	7.7 (0.42)	7.8 (0.39)	7.9 (0.66)	10.9 (0.65)	10.5 (0.49)
Org. layer mass	[kg m ⁻²]	7.97 (1.05)	7.56 (1.18)	8.41 (0.86)	11.05 (1.01)	11.21 (1.76)	13.90 (1.38)	15.03 (2.44)
Texture								
0–10 cm		Sandy loam	Sandy loam	Sandy loam	Loamy silt	Loamy silt	Silt loam	Sandy Loam
Bulk density								
Org. layer	[g cm ⁻³]	0.18 (0.02)	0.13 (0.02)	0.12 (0.01)	0.15 (0.01)	0.14 (0.02)	0.12 (0.01)	0.14 (0.02)
0–10 cm	[g cm ⁻³]	1.32 (0.02)	1.36 (0.04)	1.11 (0.03)	1.07 (0.07)	0.99 (0.11)	0.75 (0.06)	0.76 (0.04)
SWC								
Org. layer	[wt %]	46.9 (2.1)	41.4 (1.9)	59.1 (1.6)	57.8 (1.6)	52.4 (1.7)	61.3 (1.2)	66.9 (1.1)
0–10 cm	[wt %]	22.8 (0.9)	18.7 (0.8)	26.1 (1.3)	27.1 (1.3)	29.4 (1.1)	38.1 (0.7)	43.6 (1.4)
pH (H ₂ O)								
Org. layer		4.3 ^A (0.03)	4.7 ^A (0.38)	4.2 ^{AB} (0.02)	3.9 ^B (0.07)	4.2 ^{AB} (0.03)	4.2 ^{AB} (0.07)	4.1 ^{AB} (0.04)
0–10 cm		3.7 ^b (0.02)	4.6 ^a (0.09)	3.8 ^a (0.06)	3.8 ^a (0.12)	3.7 ^b (0.09)	3.7 ^b (0.03)	3.7 ^b (0.18)
pH (CaCl ₂)								
Org. layer		3.7 ^A (0.05)	4.3 ^A (0.34)	3.6 ^A (0.05)	3.3 ^B (0.09)	3.4 ^A (0.06)	3.3 ^B (0.04)	3.3 ^B (0.13)
0–10 cm		3.4 ^{ab} (0.04)	3.5 ^{ab} (0.05)	3.5 ^a (0.03)	3.3 ^{ab} (0.05)	3.4 ^{ab} (0.03)	3.3 ^{ab} (0.02)	3.1 ^b (0.18)
C _{tot}								
Org. layer	[mg g ⁻¹]	143.7 ^D (9.6)	175.7 ^{CD} (8.4)	255.4 ^{AB} (11.6)	253.6 ^{AB} (12.1)	215.0 ^{BC} (10.8)	276.5 ^A (14.2)	304.4 ^A (10.5)
0–10 cm	[mg g ⁻¹]	26.1 ^c (1.7)	28.1 ^c (1.4)	52.9 ^b (7.2)	63.3 ^b (7.8)	56.9 ^b (3.6)	82.4 ^a (3.3)	111.8 ^a (9.4)
N _{tot}								
Information on soil texture was provided by the local forestry offices (Waldeckische Domänialverwaltung).								
Geological epochs: I BU – lower Bunter (Triassic), I K – lower Keuper (Triassic), u D or m D - upper/middle Devonian shale.								
P _{resin} – resin-exchangeable P.								

Elevation	m a.s.l.	310	380	490	560	600	690	800
Org. layer	[mg g ⁻¹]	7.8 ^D (0.46)	8.8 ^D (0.36)	14.1 ^{BC} (0.56)	13.1 ^{BC} (0.61)	11.9 ^C (0.51)	15.4 ^{AB} (0.7)	16.7 ^A (0.57)
0–10 cm	[mg g ⁻¹]	1.6 ^c (0.12)	1.4 ^c (0.09)	3.2 ^b (0.36)	3.1 ^b (0.34)	3.5 ^b (0.18)	4.7 ^a (0.17)	6.1 ^a (0.48)
C/N								
Org. layer	[g g ⁻¹]	18.6 ^{AB} (0.37)	19.8 ^A (0.42)	17.9 ^{BC} (0.21)	19.3 ^A (0.22)	17.7 ^{BC} (0.28)	17.9 ^{BC} (0.23)	18.3 ^{ABC} (0.14)
0–10 cm	[g g ⁻¹]	15.2 ^d (0.51)	19.8 ^{ab} (0.75)	16.2 ^{cd} (0.51)	19.9 ^a (0.40)	16.3 ^{cd} (0.30)	17.5 ^{bc} (0.16)	18.3 ^{ab} (0.23)
P _{tot}								
Org. layer	[mg g ⁻¹]	0.82 (0.04)	0.96 (0.04)	0.92 (0.04)	0.92 (0.04)	0.87 (0.04)	0.90(0.04)	0.83 (0.05)
P _{resin}								
0–10 cm	[mg g ⁻¹]	0.04 ^a (0.003)	0.01 ^b (0.003)	0.01 ^b (0.003)	0.01 ^b (0.002)	0.02 ^b (0.002)	0.01 ^b (0.001)	0.03 ^a (0.003)
Information on soil texture was provided by the local forestry offices (Waldeckische Domänialverwaltung).								
Geological epochs: l BU – lower Bunter (Triassic), l K – lower Keuper (Triassic), u D or m D - upper/middle Devonian shale.								
P _{resin} – resin-exchangeable P.								

Among the most conspicuous changes observed in soil chemical and physical factors was the doubling in organic layer thickness from about 5.5 cm at 310 m to 10.5 cm at 800 m a.s.l., which was associated with an increase in organic layer mass from 8.0 kg m⁻² at 310 m to 15.0 kg m⁻² at 800 m (Table 2) and concomitant increases in organic layer C and N stocks. The organic carbon concentration (SOC) in the organic layer material increased from 143.7 mg g⁻¹ at 310 m to 304.4 mg g⁻¹ at 800 m, and that of the mineral topsoil from 26 mg g⁻¹ at 310 m to 111.83 mg g⁻¹ at 800 m. In parallel, total N concentration increased in the organic layer from 7.8 mg g⁻¹ at 310 m to 16.7 mg g⁻¹ at 800 m, and in the mineral topsoil from 1.64 mg g⁻¹ at 310 m to 6.05 mg g⁻¹ at 800 m. Soil C/N ratio and P content showed no elevational trends in both layers (Table 2).

Elevational change in fine root biomass and root morphology

FRB in the organic layer increased with elevation largely in parallel with the increasing forest floor depth from 2.2 g m⁻² at 310 m to 24.0 g m⁻² at 690 m (significant relation; $r = 0.51$, $p < 0.01$), revealing a positive relation to MAP and a negative one to MAT (Table 3). FRB in the mineral topsoil (0–10 cm) varied between 9.3 and 48.9 g m⁻² without a clear elevational trend (Fig. 2). Both FRB components were negatively related to the P concentrations in the organic layer (P_{tot}) and mineral soil (P_{resin}) but were unrelated to soil C/N ratio. None of the examined root morphological parameters (SRL, SRA, TD, RTF) changed significantly with elevation, nor were influenced by climatic or soil chemical variables (Table 3).

Table 3

(a) Results of Spearman rank correlation analyses on the dependence of fine root biomass (FRB), root morphological parameters (specific root length - SRL, specific root surface area - SRA, root tissue density - TD and root tip frequency - RTF), mass-specific root exudation rate, and extrapolated annual C flux with exudation on elevation, mean annual precipitation (MAP), mean annual temperature (MAT), multiannual summer temperature (MST), mean soil water content (SWC) in summer, precipitation immediately before sampling, and air and soil temperature 7 days prior to sampling, and various soil chemical parameters in the organic layer, mineral topsoil (0–10 cm) and of the pooled organic layer and mineral topsoil (n = 5 measurements per layer and site, n = 2 sampling dates in 2015). (b) Results of Spearman rank correlation analyses on the dependence of fine root biomass (FRB), root morphological parameters (SRL, SRA, TD and RTF), and mass-specific root exudation rate and extrapolated annual C flux with exudation on stem density, SRL, SRA, TD and RTF. Given are the Spearman correlation coefficients and the significance of the relationship (*: $p < 0.05$, **: $p < 0.01$). Positive correlations are indicated by positive r values, negative ones by negative r values. p -values were adjusted by the Benjamini-Hochberg procedure for multiple comparison. Correlation coefficients > 0.5 are printed in bold.

		FRB org.lay.	FRB 0-10cm	SRL	SRA	TD	RTF	Root Exudation rate	Annual C Flux
		(g m^{-2})	(g m^{-2})	(cm g^{-1})	($\text{cm}^2 \text{g}^{-1}$)	(mg cm^3)	(n g^{-1})	($\mu\text{g g}^{-1} \text{h}^{-1}$)	($\text{g m}^{-2} \text{yr}^{-1}$)
a)									
Elevation	(m a. s. l.)	0.510**	0.093	-0.032	0.032	-0.013	0.082	-0.305**	-0.126
MAP (1981–2010)	(mm)	0.510**	0.093	-0.032	0.032	-0.013	0.082	-0.305**	-0.126
MAT (1981–2010)	(°C)	-0.466**	0.104	0.038	-0.044	0.009	-0.069	0.305**	0.260**
MST (1981–2010)	(°C)			0.097	0.076	-0.005	0.003	0.291	0.181
Ann. precip. 2014, 2015	(mm)	-	-	-0.024	0.009	0.113	0.087	-0.288**	0.181**
Ann. temp. 2014, 2015	(°C)	-	-	0.049	-0.041	0.068	-0.039	0.313**	0.280**
Mean SWC (topsoil)	(wt%)	-	-	-0.014	0.077	-0.043	0.113	-0.495**	-0.458**
Actual precipitation	(mm)	-	-	-0.022	-0.016	0.182	0.051	-0.169	-0.038
Actual air temperature	(°C)	-	-	0.092	0.066	-0.116	-0.056	0.669**	0.143
Actual soil temperature	(°C)	-	-	0.090	0.057	-0.097	-0.060	0.656**	0.214
C_{tot}									
Org. layer	[mg g^{-1}]	0.496**	-	-	-	-	-	-	-
0–10 cm	[mg g^{-1}]	-	-0.023	-	-	-	-	-	-
Upper soil	[mg g^{-1}]	-	-	-0.042	0.038	-0.024	0.058	-0.303**	-0.166
N_{tot}									
Org. layer	[mg g^{-1}]	0.496**	-	-	-	-	-	-	-
0–10 cm	[mg g^{-1}]	-	-0.042	-	-	-	-	-	-
Upper soil	[mg g^{-1}]	-	-	-0.044	0.061	-0.055	0.058	-0.320**	-0.249
C:N									
Org. layer	[g g^{-1}]	0.037	-	-	-	-	-	-	-
0–10 cm	[g g^{-1}]	-	0.315	-	-	-	-	-	-
Upper soil	[g g^{-1}]	-	-	0.081	0.060	0.04	0.014	0.080	0.310
pH H ₂ O									

		FRB org.lay.	FRB 0-10cm	SRL	SRA	TD	RTF	Root Exudation rate	Annual C Flux
Org. layer		-0.373**	-	-	-	-	-	-	-
0–10 cm		-	0.103	-	-	-	-	-	-
Upper soil		-	-	0.015	-0.078	0.023	-0.068	0.198	0.180
pH (CaCl₂)									
Org. layer		-0.432**	-	-	-	-	-	-	-
0–10 cm		-	0.037	-	-	-	-	-	-
Upper soil		-	-	0.013	-0.052	0.006	-0.075	0.244**	0.224**
P_{tot}									
Org. layer	[mg g ⁻¹]	-0.345**	-	0.147	0.024	0.125	0.075	0.204	0.147
P_{resin}									
0–10 cm	[mg g ⁻¹]	-	-0.326**	0.061	0.015	0.145	0.105	0.020	-0.241**
b)									
stem density	(n ha ⁻¹)	-0.360**	0.073	0.081	0.014	0.029	-0.033	0.323**	0.298**
SRL	(cm g ⁻¹)	-0.020	0.046	-	-	-	-	0.250**	0.229**
SRA	(cm ² g ⁻¹)	0.068	0.049	-	-	-	-	0.293**	0.300**
TD	(mg cm ³)	-0.079	-0.096	-	-	-	-	-0.257**	-0.283**
RTF	(n g ⁻¹)	0.084	0.017	-	-	-	-	0.121	0.117

Elevational change in exudation rates and dependence of exudation on climate, soil chemistry and root morphology

Average mass-specific root exudation rate across the seven sites was $18.34 \pm 6 \mu\text{g g}^{-1} \text{h}^{-1}$ (Fig. 4a) with site means (averaged over all sampling dates) ranging from $12.2 \mu\text{g g}^{-1} \text{h}^{-1}$ at 690 m to $21.6 \mu\text{g g}^{-1} \text{h}^{-1}$ at 380 m a.s.l. While lowest specific exudation rates were measured at the highest sites 690 and 800 m ($12.2\text{--}15.0 \mu\text{g g}^{-1} \text{h}^{-1}$), variation among sites was large and peak rates were recorded at mid elevation (490 m: $22.7 \mu\text{g g}^{-1} \text{h}^{-1}$) and not at the lowest sites (Fig. 4a). Yet, the negative relation between mean exudation rate and elevation was highly significant ($r = -0.31$, $p < 0.01$; Table 3). C flux with exudation per ground area, calculated by multiplying specific exudation with FRB in the organic layer, was significantly higher above 400 m a.s.l. than at lower elevation (Fig. 4b).

Estimated annual cumulative C fluxes with exudation per root mass, derived by multiplying average exudation rate with the number of days in the season with means $> 10^\circ\text{C}$, decreased significantly with elevation along the transect, from $80\text{--}85 \text{ mg g}^{-1} \text{yr}^{-1}$ at 310 m to $40\text{--}45 \text{ mg g}^{-1} \text{yr}^{-1}$ at 800 m a.s.l. (mean of the seven sites: $66 \pm 29 \text{ mg g}^{-1} \text{yr}^{-1}$; Fig. 4c). Estimating annual C flux per stand area by multiplying with FRB in the organic layer and mineral topsoil yields a different pattern along the transect. Highest stand-level C fluxes were calculated for 380 m (ca. $7 \text{ g C m}^{-2} \text{yr}^{-1}$) and 560 m (ca. $5 \text{ g m}^{-2} \text{yr}^{-1}$) with highest FRB, and lowest for 310 m and also 800 m (ca. $2 \text{ g m}^{-2} \text{yr}^{-1}$), but without a dependence on elevation (Fig. 4d). Averaged over all sites, we calculated a mean growing season C flux of $3.6 \pm 1.5 \text{ g m}^{-2}$.

The environmental factors with strongest influence on mass-specific root exudation rate were prevailing air and soil temperature with highly significant positive effects ($r = 0.66$, $p < 0.01$; Table 3a). In contrast, the relationships to MAT and MST and mean temperature of the measuring year were less tight ($r = 0.29\text{--}0.31$, $p < 0.01$). Close relations were also found for soil moisture (SWC) and MAP (both negative), and the N content of the upper soil (negative) and soil pH (CaCl₂) (positive), while soil P content and C/N ratio were not influential (Table 3a). Similarly, the annual stand-level C flux depended primarily on the prevailing temperature, followed with lower importance by soil moisture, annual air temperature and MAT, but not by MAP. Plotting exudation rate against the prevailing temperature or MST shows a

direct connection between the prevailing air temperature and root exudation (Fig. 5a), while the relation to MST (Fig. 5b) is weak ($r = 0.29$, $p > 0.05$). Annual fluxes did not show any significant relation to the prevailing temperature (Fig. 5c) or MST (Fig. 5d).

Mass-specific exudation rate was significantly positively influenced by SRL and SRA, but negatively by TD (Table 3b). RTF was not influential. Similar relations appeared also for the stand-level C flux. For stem density, a negative relation was found to FRB and a positive relation to root exudation and stand-level C flux.

Multiple regression analyses to separate intrinsic and abiotic drivers of root exudation

Multiple regression analyses on the influence of various climatic, edaphic, and root morphological factors on the exudation rate of beech indicate a predominant positive effect of the prevailing air temperature, followed by a positive effect of SRA (both at $p < 0.001$). The model explained 46% of the variance in exudation rate (Table 4). Annual C flux per ground area was found to depend primarily on soil moisture (negative effect; $p < 0.001$), SRL (positive effect; $p < 0.003$), and elevation (negative effect; $p < 0.001$), with 44% of the variance explained. None of the tested soil chemical variables had a significant effect on the exudation rate or the annual C flux.

Table 4

Multiple regression analyses with backward variable elimination on the effects of elevation, prevailing air temperature, soil water content (SWC), specific root area (SRA), and specific root length (SRL) on root exudation rates and the estimated annual carbon flux per m^2 ground area in the topsoil of the seven beech forests along the elevational gradient. The +/- signs indicate positive/negative correlation.

Model						
Y	R ²	P		Predictor	F	P
Exudation rate	0.46	< 0.001	+	Prevailing air temperature	69.7	< 0.001
			+	SRA	10.2	< 0.001
Annual flux	0.44	< 0.001	-	SWC	26.6	< 0.001
			+	SRL	11.8	0.003
			-	Elevation	7.3	< 0.001

Discussion

The dependence of root exudation on temperature and edaphic factors

The measured mass-specific root exudation rates of the mature beech trees in our study ($12\text{--}22 \mu\text{g C g}^{-1} \text{h}^{-1}$) were similar to rates reported in other studies for beech in moist soil ($10\text{--}23 \mu\text{g C g}^{-1} \text{h}^{-1}$, Liese et al. 2017; $16\text{--}65 \mu\text{g C g}^{-1} \text{h}^{-1}$, Meier et al. 2020) and corresponded also to values found in other tree species in the temperate zone (e.g., black locust: $10\text{--}22 \mu\text{g C g}^{-1} \text{h}^{-1}$, Uselman et al. 2000; loblolly pine: $12\text{--}26 \mu\text{g C g}^{-1} \text{h}^{-1}$, Meier et al. 2013). Our simple and multiple regression analyses indicate that the thermal conditions during and 7 days prior to sampling are the most important factor controlling exudation of beech across the studied elevational gradient. Exudation per root mass increased by around $2 \mu\text{g C g}^{-1} \text{h}^{-1}$ per 1°C temperature increase. In two Mediterranean tree species, Jakoby et al. (2020) found a linear increase in exudation rate per root surface area of about $0.15 \mu\text{g C cm}^{-2} \text{d}^{-1}$ per 1°C increase, and soil temperature together with soil moisture explained exudation dynamics best in one of the species. Uselman et al. (2000) found a 70% higher root exudation in *Robinia pseudoacacia* seedlings when temperature was increased from 26 to 30°C .

Several explanations of a positive temperature effect on exudation are possible, which relate to the 'push' and 'pull' hypotheses of the control of C acquisition by roots (Farrar et al. 2003). In the humid climate of our study region, higher summer temperatures are usually related to higher insolation and thus greater carbon assimilation, as beech photosynthesis is primarily limited by radiation at montane elevation (Schulze 1970). Consequently, under warmer conditions, more C should in principle be allocated to roots, which may enhance root exudation due to surplus C (Prescott et al. 2020) and a steeper concentration gradient of soluble organic compounds between root cells and the soil (Jones et al. 2004). In addition, the photosynthetic capacity of beech shows a pronounced seasonality with a peak in the warmest months (Schulze 1970), which might imprint on the seasonality of exudation, as photosynthetic capacity and root exudation have been found to be closely related (Sun et al. 2017). Another possible explanation focuses on the role of root exudation for the metabolic activity of rhizosphere biota and its stimulating effect on nutrient supply for root uptake (Jones et al. 2004). Warmer weather likely increases the plant demand for nitrogen and other nutrients as photosynthetic capacity is ramped up, which might trigger roots to stimulate

soil microbial activity through active secretion of labile C as an energy source for microbes (Pausch and Kuzyakov 2017). Such a mechanism would fit to the 'pull' hypothesis, as exudation then were primarily controlled by factors other than C supply from the canopy (Karst et al. 2017). It is possible that both 'push' and 'pull' mechanisms are underlying the observed increase in exudation with rising temperature.

The fact that exudation was responsive to increases in the prevailing temperature but showed no significant relation to mean summer season temperature (MST), i.e., the average thermal regime of the site, may partly be a consequence of the limited number of sites and the relatively short MST gradient studied by us. On the other hand, specific exudation rate was on average higher at the five sites ≤ 600 m a.s.l. than at higher elevation, and the dominant temperature effect in the multiple regression model reflects the influence of both short-term temperature fluctuation at the site and MST differences between sites. Lower exudation at the higher, cooler sites might well be counteracted by the higher FRB density in the organic layer of these sites (see Fig. 4b), which should result in elevated exudate concentration in the soil solution and thus enhanced priming of soil biological activity, despite reduced temperatures.

While our model does not consider all possible influencing factors (e.g., photosynthesis and mycorrhizal infection were not included), important abiotic factors such as the prevailing temperature, soil moisture and measures of soil fertility (C/N ratio, P_{resin} content) were considered. This allows some conclusions on the relative importance of possible drivers of exudation and shows the importance of temperature compared to other factors.

Since other processes of plant carbon turnover such as respiration and photosynthesis are subject to marked thermal acclimation (Atkin and Tjoelker 2003; Hikosaka et al. 2006) and exudation depends on photosynthetic C gain, we would expect that beech trees growing at lower elevation in a warmer climate acclimate their exudation rate to a certain degree. If this happened, mean exudation rates would become more similar across the elevational gradient. More data from field studies along temperature gradients are needed to test the hypothesis of exudation homeostasis.

Soil moisture is another abiotic factor with a presumably large effect on exudation. Low to moderate drought seems to increase exudation (Jakoby et al. 2020; Liese et al. 2017; Preece et al. 2018), apparently as a stress response similar to the exudation increase observed upon soil cooling to 4°C (Karst et al. 2017). The seven beech forests in our study represent a precipitation and soil moisture gradient with annual precipitation in the study year 2015 decreasing to nearly a half (1050–570 mm) and mean soil moisture in summer to less than a half (ca. 45 to ca. 20 wt.%) from 800 to 310 m elevation. In fact, the highest exudation rates were measured at the driest sites and the negative relationship to mean soil water content was highly significant, which may offer an alternative explanation of the exudation pattern along the slope, apart from a positive temperature effect. Yet, mid and late-summer moisture contents in the topsoil of 15–25 wt.% likely have exposed the trees to only mild drought, and it thus remains unclear, how important water availability is for the explanation of the observed exudation patterns.

Soil nutrient availability is another abiotic factor with a possible influence on the exudation rate. For example, P deficiency results in enhanced root secretion of phenolic compounds in certain species (Neumann and Römheld, 1999), and exudation is higher in beech at acidic, N-poor sites than in more fertile soils (Meier et al. 2020), which may trigger increases in decomposition rate and thus N availability through a rhizosphere priming effect (Chen et al. 2014; Jones et al. 2004; Phillips et al. 2011). The negative correlation between topsoil N content and exudation rate in our sample suggests a stimulating effect of N deficiency on exudation. There are, however, also reports of no influence of soil nitrogen deficiency on exudation, or stimulation by N addition (Uselman et al. 2000; Yin et al. 2013b). Along the studied elevational gradient, organic layer mass on the forest floor doubled from 310 to 800 m a.s.l. with the consequence that topsoil C and N pools increased, whereas C/N ratio, P_{tot} and P_{resin} content as well as soil pH did not change, suggesting that plant-availability of N and P varied only little. We thus assume that variation in soil nutrient availability is only of minor importance for explaining the observed exudation patterns along the slope.

Influence of fine root system structure and morphology on exudation

Not much is known about the plasticity in morphology and functionality of the fine root system of European beech in response to changes in climate and edaphic conditions (Kirfel et al. 2019). Given the broad amplitude of growing conditions tolerated by beech (Leuschner et al. 2006), a high plasticity of fine root morphology and physiology and root system structure would be expected in this species. To enable sufficient nutrient supply under unfavorable soil chemical and physical conditions, trees may either invest into the enlargement of its root system ('extensive strategy') or modify root morphology to increase uptake per root mass or area ('intensive strategy'; Ostonen et al. 2007b). Adjusting root exudation would represent another option.

It appears that the beech trees along our transect are mainly pursuing an extensive strategy to acquire belowground resources, given the negligible adjustment in fine root morphology along the elevational gradient. Earlier studies in beech similarly reported only limited variation in fine root morphology along soil chemical gradients (Kirfel et al. 2019; Leuschner et al. 2004). Other than root morphology, FRB in the topsoil changed markedly from 310 m to 800 m a.s.l. FRB in the organic layer increased roughly tenfold in parallel with the increasing depth of this layer, while FRB in the mineral topsoil varied without a clear elevational trend. We interpret this pronounced shift of FRB to the surface layer as a consequence of decreasing litter decomposition rates toward higher elevations, prompting the beech trees to concentrate their fine root mass in the organic surface layers with highest mineralization rates. While specific exudation rate was somewhat lower, FRB was much larger in the topsoil of the high-elevation beech forests with the consequence that daily exudation rates per topsoil volume were significantly higher above 400 m elevations. This seems plausible, as lower mineralization rates in cooler soil should increase the necessity for the trees to conduct rhizosphere priming by stimulating soil microbial activity through the provision of labile C as an easily accessible energy source.

In contrast to the variation in topsoil FRB, none of the four studied root morphological traits changed directionally along the elevational gradient. We thus assume that the temperature decrease by 2.4°C along the slope is not an important determinant of root morphology, even though local edaphic conditions may cause considerable small-scale variation in fine root morphology. Nevertheless, we found exudation rate to be significantly positively influenced by SRL and SRA and negatively by TD, while RTF had no effect. This influence is reflected in our multiple regression models, which show a highly significant positive influence of SRA on the exudation rate (and of SRL on annual C flux) in our sample. This is in line with an increasing number of studies showing root morphology and root system architecture to be important determinants of the quantity and quality of root exudation (Badri and Vivanco 2009; Meier et al. 2020). Various studies have demonstrated that SRL and/or SRA and branching intensity are positively correlated with root exudation (Darwent 2003; Groleau-Renaud et al. 1998; Xu and Juma 1994; Yin et al. 2013a). Meier et al. (2020) found a ca. 5fold increase in exudation rate with a doubling of SRL in a comparison of beech stands along a soil fertility gradient. This supports the conclusion of Ostonen et al. (2007a) that SRL is a meaningful indicator of fine root functionality in trees.

Conclusions

Our study of root exudation of mature beech trees in seven forests along an elevation (and associated temperature) gradient provides evidence that mass-specific exudation increases with temperature at the time of measurement, whereas the dependence on the site's average summer temperatures was weak. Although we fully recognize the limitations of our data set with an only short MST gradient and only three measuring campaigns at the seven sites, our results are convincing with respect to the prominent positive temperature effect on exudation, which was more important than other climatic, edaphic or root morphological factors. Moreover, with data on FRB at the sites and information on the length of the growing season, we were able to compare the seven forests with respect to exudation per ground area and to give rough estimates of cumulative exudation per growing season. Both a higher mass-specific exudation rate and a longer growing season period contribute to a generally higher cumulative exudation at the warmer sites. Future research should investigate whether higher exudation and thus more intense rhizosphere priming is a factor that contributes to the generally higher availability of N, P, and other nutrients in soil when temperature increases. In combination with earlier studies in European beech forests across edaphic and climatic gradients (Meier et al. 2020; Tückmantel et al. 2017), this study deepens our understanding of environmental controls of root exudation in this model tree species.

Declarations

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Declarations

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Authors declare no conflict of interest.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

Code availability: Not applicable

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References

1. Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends Plant Sci* 8:343–351
2. Badri DV, Vivanco JM (2009) Regulation and function of root exudates. *Plant Cell Environ* 32:666–681
3. Bai W, Wan S, Niu S, Liu W, Chen Q, Wang Q, Zhang W, Han X, Li L (2010) Increased temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: Implications for ecosystem C cycling. *Glob Change Biol* 16:1306–1316
4. Darwent MJ (2003) Biosensor reporting of root exudation from *Hordeum vulgare* in relation to shoot nitrate concentration. *J Exp Bot* 54:325–334
5. Farrar J, Hawes M, Jones D, Lindow S (2003) How roots control the flux of carbon to the rhizosphere. *Ecology* 84:827–837
6. Fransson PMA, Johansson EM (2010) Elevated CO₂ and nitrogen influence exudation of soluble organic compounds by ectomycorrhizal root systems. *FEMS Microb Ecol* 71:186–196
7. Freschet GT, Pagès L, Iversen CM, Comas LH, Rewald B, Roumet C, Klimešová J, Zadworny M, Poorter H, Postma JA, Adams TS et al (2021) A starting guide to root ecology: strengthening ecological concepts and standardizing root classification, sampling, processing and trait measurements. *New Phytol* 232:973–1122
8. Graham SL, Hunt JE, Millard P, McSeveny T, Tylianakis JM, Whitehead D (2014) Effects of soil warming and nitrogen addition on soil respiration in a New Zealand tussock grassland. *PLoS ONE* 9:91204
9. Groleau-Renaud V, Plantureux S, Guckert A (1998) Influence of plant morphology on root exudation of maize subjected to mechanical impedance in hydroponic conditions. *Plant Soil* 201:231–239
10. Hikosaka K, Ishikawa K, Brojigidai A, Muller O, Onoda Y (2006) Temperature acclimation of photosynthesis: mechanisms involved in the changes in temperature dependence of photosynthetic rate. *J Exp Bot* 57:313–347
11. Jakoby G, Rog I, Megidish S, Klein T (2020) Enhanced root exudation of mature broadleaf and conifer trees in a Mediterranean forest during the dry season. *Tree Physiol* 44:1595–1605
12. Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. *New Phytol* 163:459–480
13. Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant Soil* 321:5–33
14. Kannenberg SA, Philipps RP (2017) Plant responses to stress impacts: The C we do not see. *Tree Physiol* 37:151–153
15. Karst J, Gaster J, Wiley E, Landhäuser SM (2017) Stress differently causes roots of tree seedlings to exude carbon. *Tree Physiol* 37:154–164
16. Kaspar F, Mächel H, Jacob D, Kottmeier C (2017) Beobachtung von Klima und Klimawandel in Mitteleuropa und Deutschland. In: Basseur GDJ, Schuck-Zöller S (eds) *Klimawandel in Deutschland*. Springer Spektrum, Berlin, pp 17–26. https://doi.org/10.1007/978-3-662-50397-3_3
17. Kirfel K, Hertel D, Heinze S, Leuschner C (2019) Effects of bedrock type and soil chemistry on the fine roots of European beech – A study on the belowground plasticity of trees. *For Ecol Manage* 444:256–268
18. Kuzyakov Y (2002) Review: Factors effecting rhizosphere priming effects. *J Plant Nutr Soil Sci* 165:382–396
19. Leuschner C, Hertel D, Schmid I, Koch O, Muhs A, Hölscher D (2004) Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. *Plant Soil* 258:43–56
20. Leuschner C, Meier I, Hertel D (2006) On the niche breadth of *Fagus sylvatica*: soil nutrient status in 50 Central European beech stands on a broad range of bedrock types. *Ann For Sci* 63:355–368
21. Liang J, Xia J, Liu L, Wan S (2013) Global patterns of the responses of leaf-level photosynthesis and respiration in terrestrial plants to experimental warming. *J Plant Ecol* 6:437–447

22. Liese R, Lübke T, Albers NW, Meier IC (2018) The mycorrhizal type governs root exudation and nitrogen uptake of temperate tree species. *Tree Physiol* 38:83–95
23. Majdi H, Ohrvik J (2004) Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. *Glob Change Biol* 10:182–188
24. Meier IC, Avis PG, Phillips RP (2013) Fungal communities influence root exudation rates in pine seedlings. *FEMS Microbiol Ecol* 83:585–595
25. Meier IC, Tückmantel T, Heitkötter J, Müller K, Preusser S, Wrobel TJ, Kandeler E, Marschner B, Leuschner C (2020) Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. *New Phytol* 226:583–594
26. Murphy J, Righly JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
27. Nakayama M, Tateno R (2018) Solar radiation strongly influences the quantity of forest tree exudates. *Trees* 32:871–879
28. Neumann G, Römheld V (1999) Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant Soil* 211:121–130
29. Nguyen C (2003) Rhizodeposition of organic C by plants: Mechanisms and controls. *Agronomie* 23:375–396
30. Norby RJ, Rustad LE, Dukes JS, Ojima DS, Parton WJ, Del Grosso SJ, McMurtrie RE, Pepper DA (2007) Ecosystem responses to warming and interacting global change factors. In: Canadell JG, Pataki DE, Pitelka LF (eds) *Terrestrial Ecosystems in a Changing World*. Springer, Berlin, Heidelberg, New York, pp 23–36
31. Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lohmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007a) Specific root length as an indicator of environmental change. *Plant Biosystems* 141:426–442
32. Ostonen I, Lohmus K, Helmisaari H-S, Truu J, Meel S (2007b) Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. *Tree Physiol* 26:1627–1634
33. Pausch J, Kuzyakov Y (2017) Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. *Glob Change Biol* 24:1–12
34. Persson H (1978) Root dynamics in a young Scots pine stand in Central Sweden. *Oikos* 30:508–519
35. Pinton R, Varanini Z, Nannipieri P (2007) *The Rhizosphere Biochemistry and Organic Substances at the Soil-Plant Interface*. Taylor & Francis Group, New York
36. Preece C, Farré-Armengol G, Llusia J, Penuelas J (2018) Thirsty tree roots exude more carbon. *Tree Physiol* 38:690–695
37. Prescott CE, Grayston SJ, Helmisaari H-S, Kaštovská E, Körner C, Lambers H, Meier IC, Millard P, Ostonen I (2020) Surplus carbon drives allocation and plant–soil interactions. *Trends Ecol Evol* 35:1110–1118
38. Schulze E-D (1970) CO₂-Gaswechsel der Buche (*Fagus sylvatica* L.) in Abhängigkeit von den Klimafaktoren im Freiland. *Flora* 159:177–232
39. Sibbesen E (1977) A simple ion-exchange resin procedure for extracting plant-available elements from soil. *Plant Soil* 46:665–669
40. Sun L, Kominami Y, Yoshimura K, Kitayama K (2017) Root-exudate flux variations among four co-existing canopy species in a temperate forest, Japan. *Ecol Res* 32:331–339
41. Tückmantel T, Leuschner C, Preusser S, Kandeler E, Angst G, Mueller CW, Meier IC (2017) Root exudation patterns in a beech forest: Dependence on soil depth, root morphology and environment. *Soil Biol Biochem* 107:188–197
42. Uselman SM, Qualls RG, Thomas RB (2000) Effects of increased atmospheric CO₂, temperature, and soil N availability on root exudation of dissolved organic carbon by a N-fixing tree (*Robinia pseudoacacia* L.). *Plant Soil* 222:191–202
43. von Lütow M, Kögel-Knabner I (2009) Temperature sensitivity of soil organic matter decomposition—what do we know? *Biol Fertil Soils* 46:1–15
44. Wang Q, Chen L, Xu H, Ren K, Xu Z, Tang Y, Xiao J (2021) The effects of warming on root exudation and associated soil N transformation depend on soil nutrient availability. *Rhizosphere* 17:100263
45. Xu JG, Juma NG (1994) Relations of shoot C, root C and root length with root-released C of two barley cultivars and the decomposition of root-released C in soil. *Can J Soil Sci* 74:17–22
46. Yang L, Wang X, Mao Z, Jiang Z, Gao Y, Chen X, Aubrey DP (2020) Root exudation rates decrease with increasing latitude in some tree species. *Forests* 11:1045
47. Yin H, Xiao J, Li Y, Chen Z, Cheng X, Zhao C, Liu Q (2013a) Warming effects on root morphological and physiological traits: the potential consequences on soil C dynamics as altered root exudation. *Agric For Meteorol* 180:287–296
48. Yin H, Li Y, Xiao J, Xu Z, Cheng X, Liu Q (2013b) Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming. *Glob Change Biol* 19:2158–2167

Figures

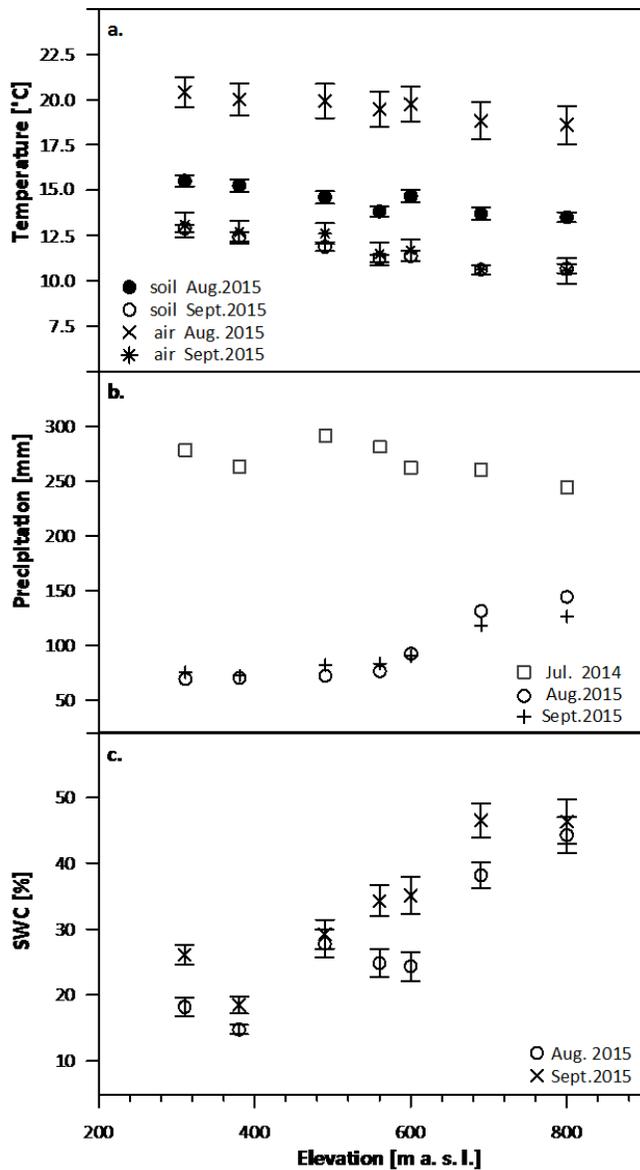


Figure 1

Soil and air temperature (a), precipitation (b) and soil water content (SWC; wt.%) (c) measured in seven forests at two sampling dates in summer/autumn 2015 and one sampling date in summer 2014 (precipitation only) (means \pm SE). Temperatures are means of seven-day periods prior to exudate sampling of each 5 sensors per stand, precipitation data are interpolated from gridded weather station data of the German Weather Service (DWD) corrected for elevation, and SWC data are gravimetric samples taken in the organic layer and 0-10 cm mineral soil at the date of sampling (n = 5).

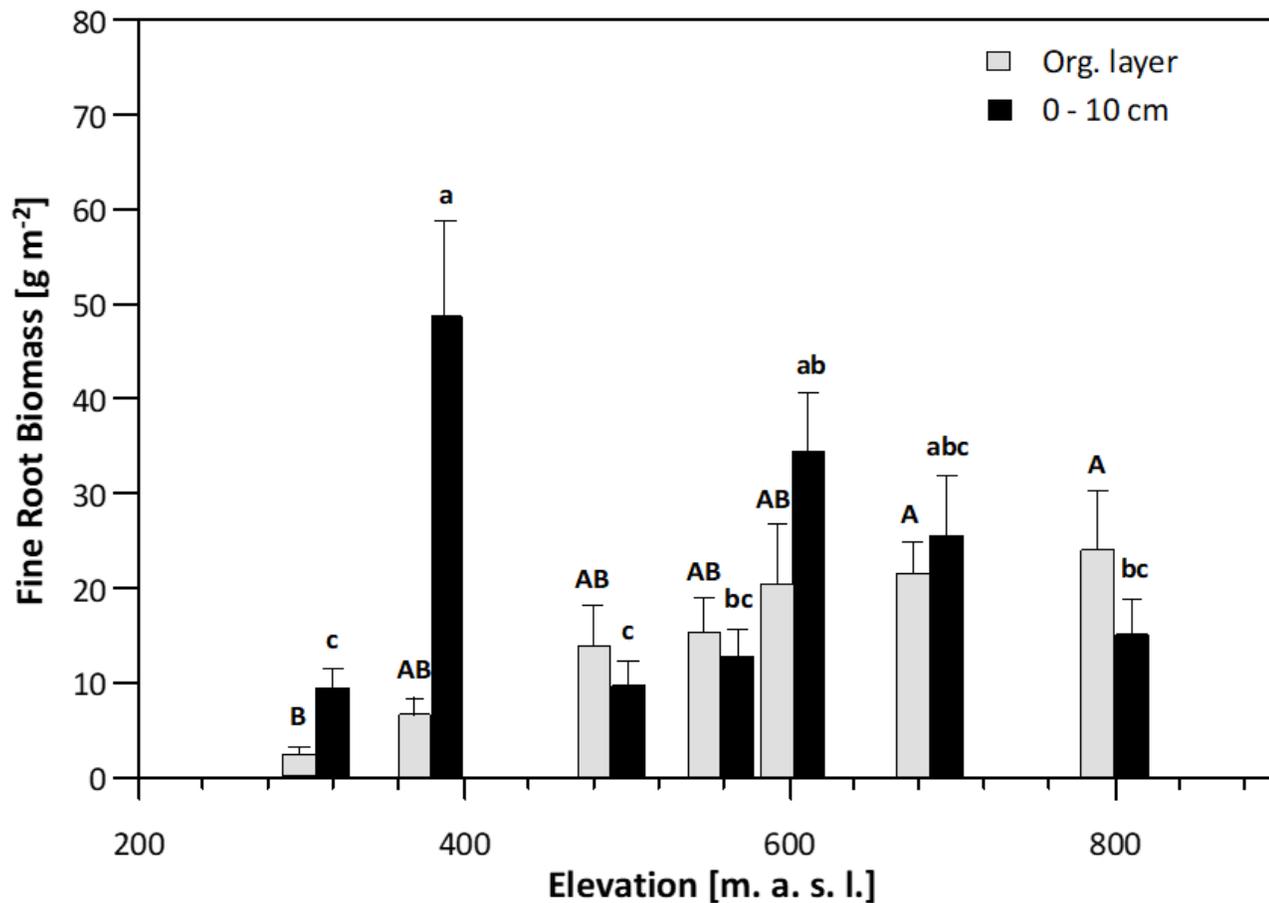


Figure 2

Fine root biomass of beech in the organic layer (grey bars) and the mineral topsoil (0-10 cm) of the seven beech forests in November 2018 (means \pm SE of 12 samples per layer). Different letters indicate significantly different means of organic layer samples (capital letters) and mineral soil samples (small letters).

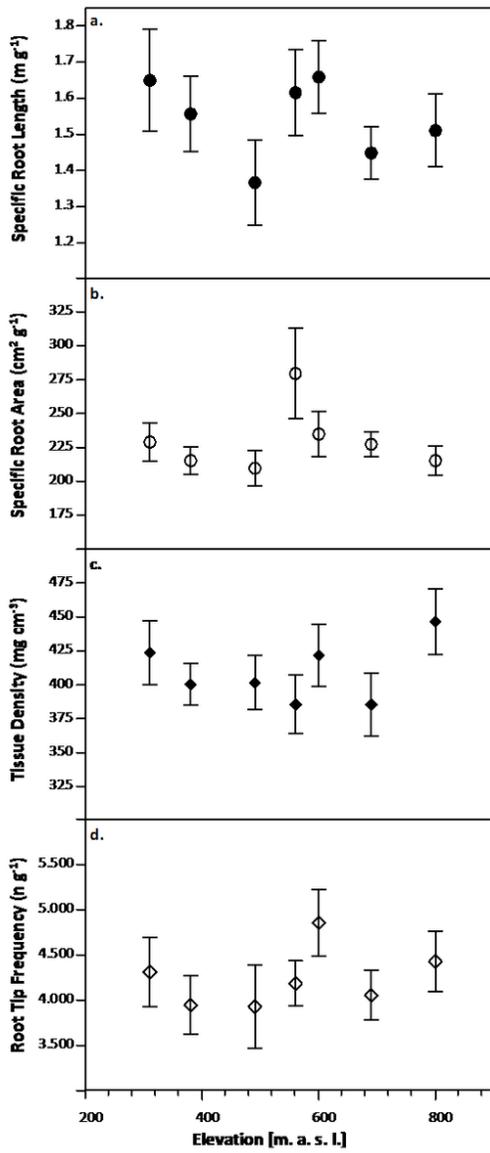


Figure 3

Specific root length, specific root area, root tissue density and root tip frequency of the fine root biomass samples used for exudation collection in the seven forests. Shown are means and standard errors of $n = 9$ samples per site and sampling date with averaging over the three sampling dates.

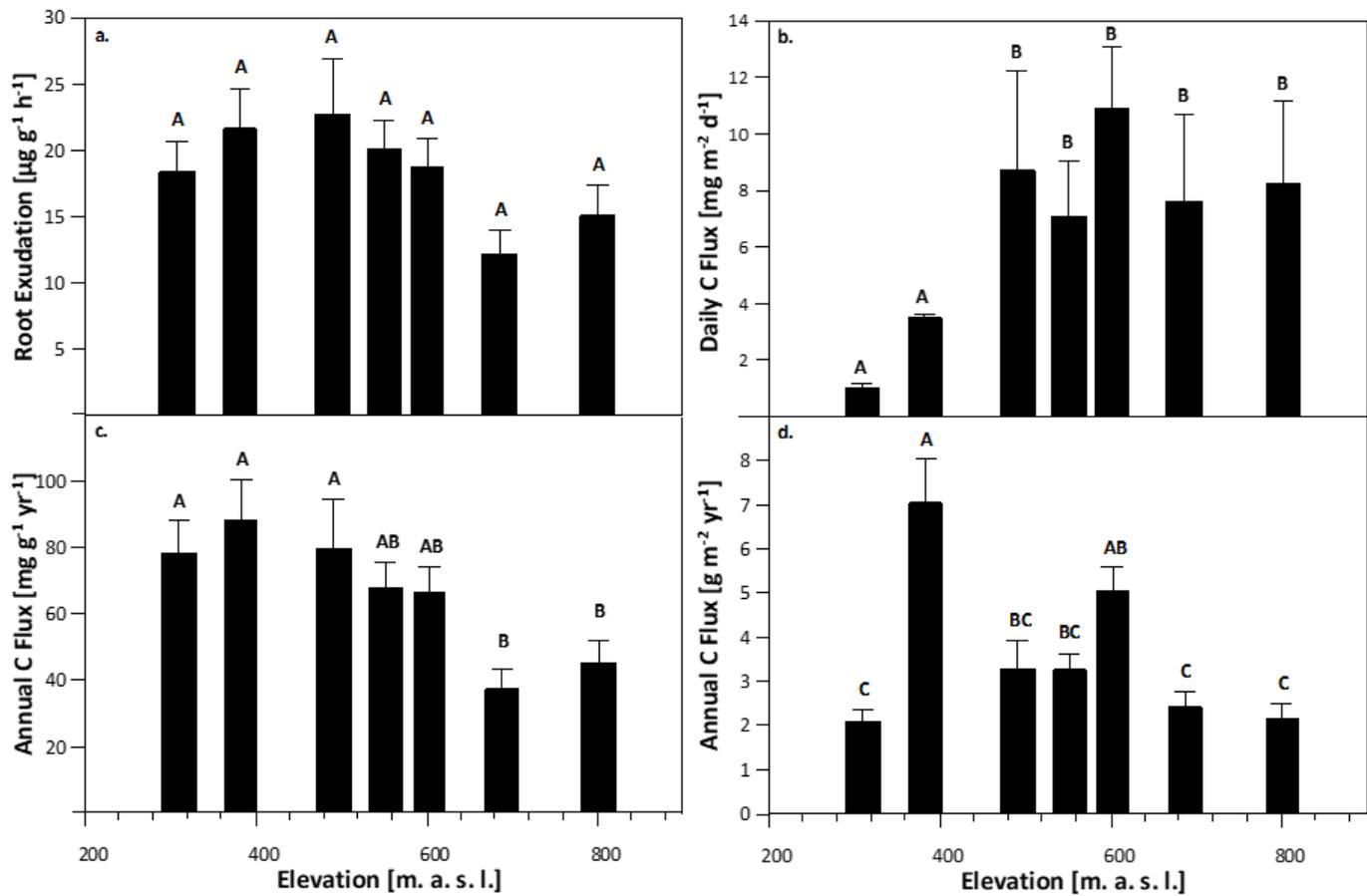


Figure 4

Means (and SE) of (a) measured average mass-specific root exudation rate, (b) daily exudation for the fine root mass in the organic layer expressed per m^2 ground area, (c) extrapolated annual mass-specific carbon flux with exudation, and (d) estimated annual C flux with exudation per m^2 ground area in the topsoil of the seven beech forests along the elevation gradient (averaged over $n = 9$ samples taken per date and $n = 3$ sampling dates in 2014 and 2015). Annual exudation was estimated from measured average flux and the specific length of the vegetation period at the sites (number of days with mean temperature $\geq 10^\circ\text{C}$). The exudation flux per ground area was extrapolated with the fine root biomass data from the organic layer (b) or the organic layer and the mineral topsoil (0-10 cm) (d). Different capital letters denote significantly different means at $p < 0.05$ with p -values adjusted by the Benjamini-Hochberg procedure for multiple comparisons (Kruskal-Wallis test).

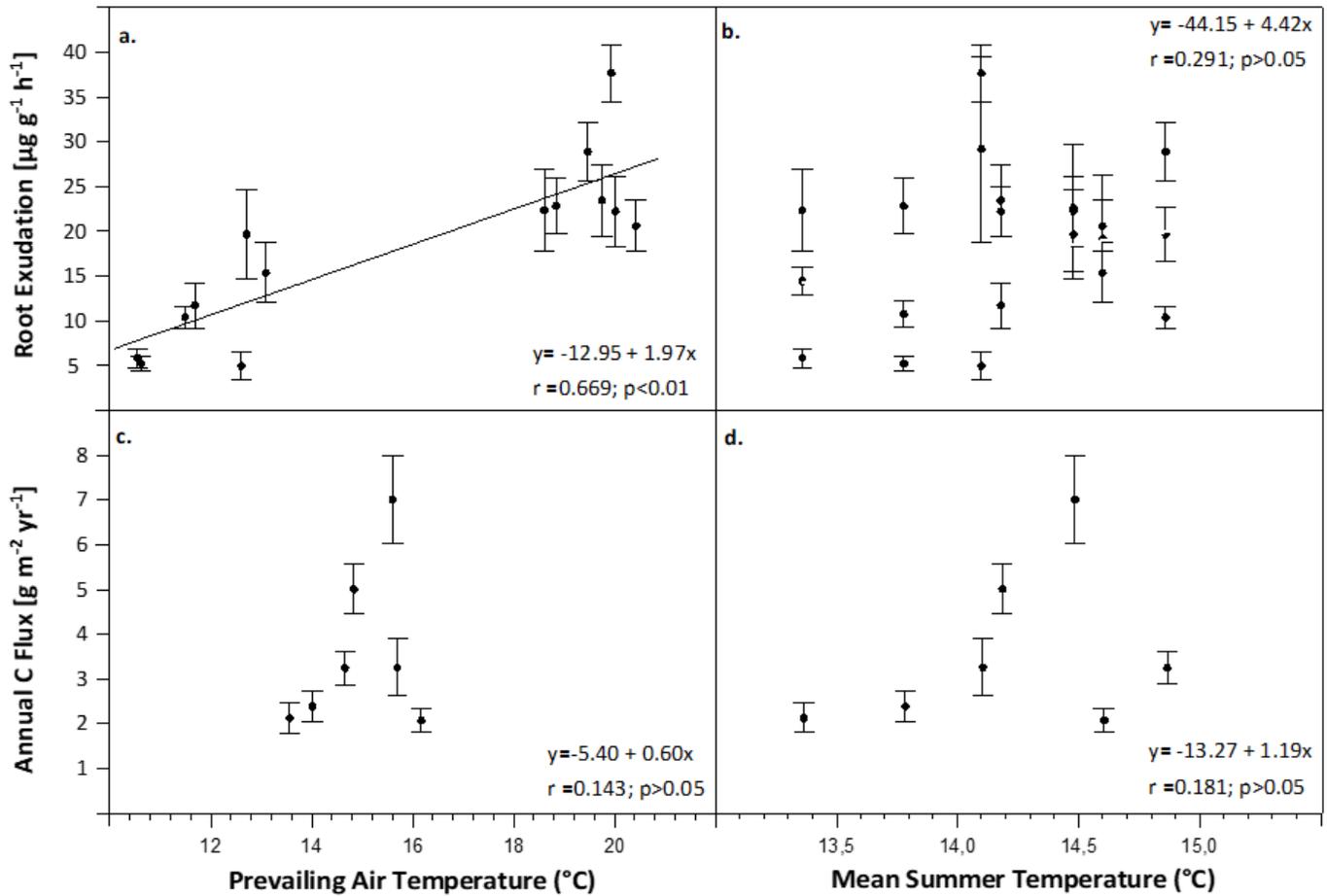


Figure 5

Relationships between actual air temperatures (mean of the seven days prior to exudate measurement; left panels) or mean summer air temperature (right panels) and (a) and (d) mass-specific root exudation rates, (b) and (e) cumulated annual C exudation per root mass, and (c) and (f) cumulated annual C exudation per ground area in the seven beech forests along the elevation gradient in summer 2015 (averaged over $n = 9$ samples per date and $n = 2$ dates per site; only 2015 data considered). Temperature data were measured with I-Buttons at the sampling sites.

Annual rates take the variable length of the vegetation period (no. of days $\geq 10^{\circ}\text{C}$) at the sites into account.