New Phytologist

***New Phytologist* Supporting Information**

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

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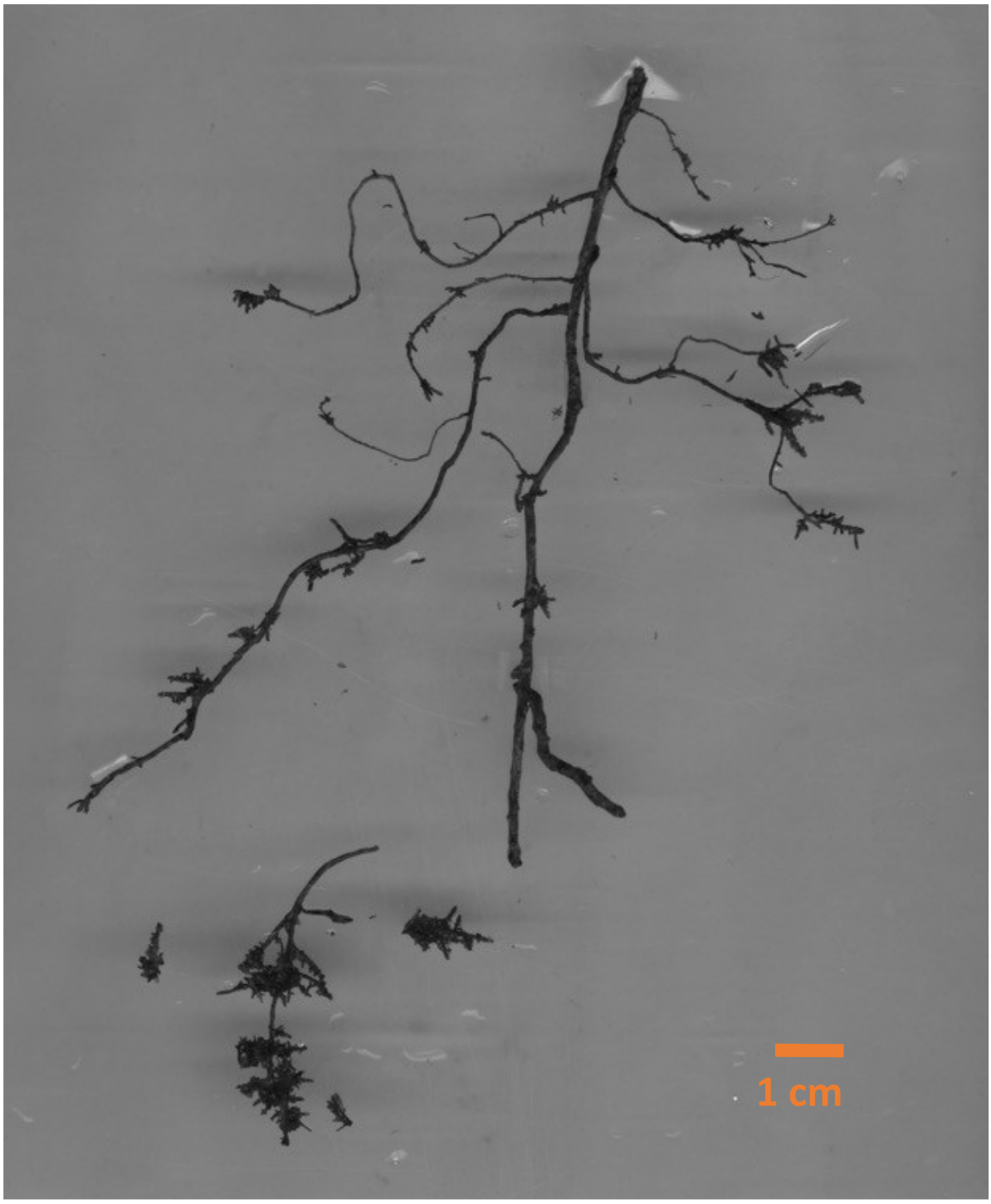

Figure S1: Example of a sampled root branch (*P. abies* in 30 cm soil depth), b/w scan (1,200 dpi; Epson Perfection 4990 Photo, SEIKO Epson CORPORATION, Tokyo, Japan).

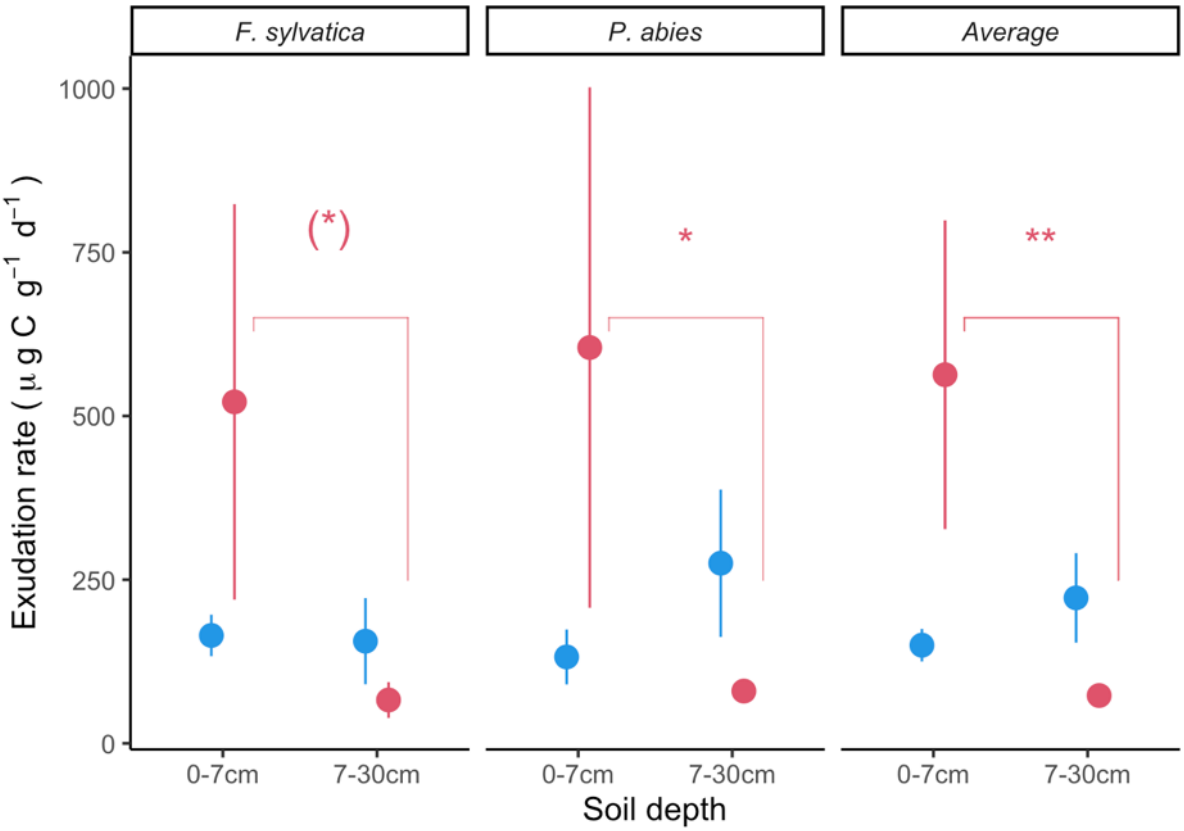

Figure S2: Fine-root exudation rates per dry-root biomass of sampled root branches of *F. sylvatica* and *P. abies* and average values over both species on control (blue) and drought plots (red) in the KROOF experiment. Significant differences between 0-7 cm and 7-30 cm soil depth for the drought plots are indicated with red asterisks, where (*) is $p = .1$, * is $p < .05$ and ** is $p < .01$. Symbols and whiskers indicate means ± standard errors for $n = 3$ plots per treatment.

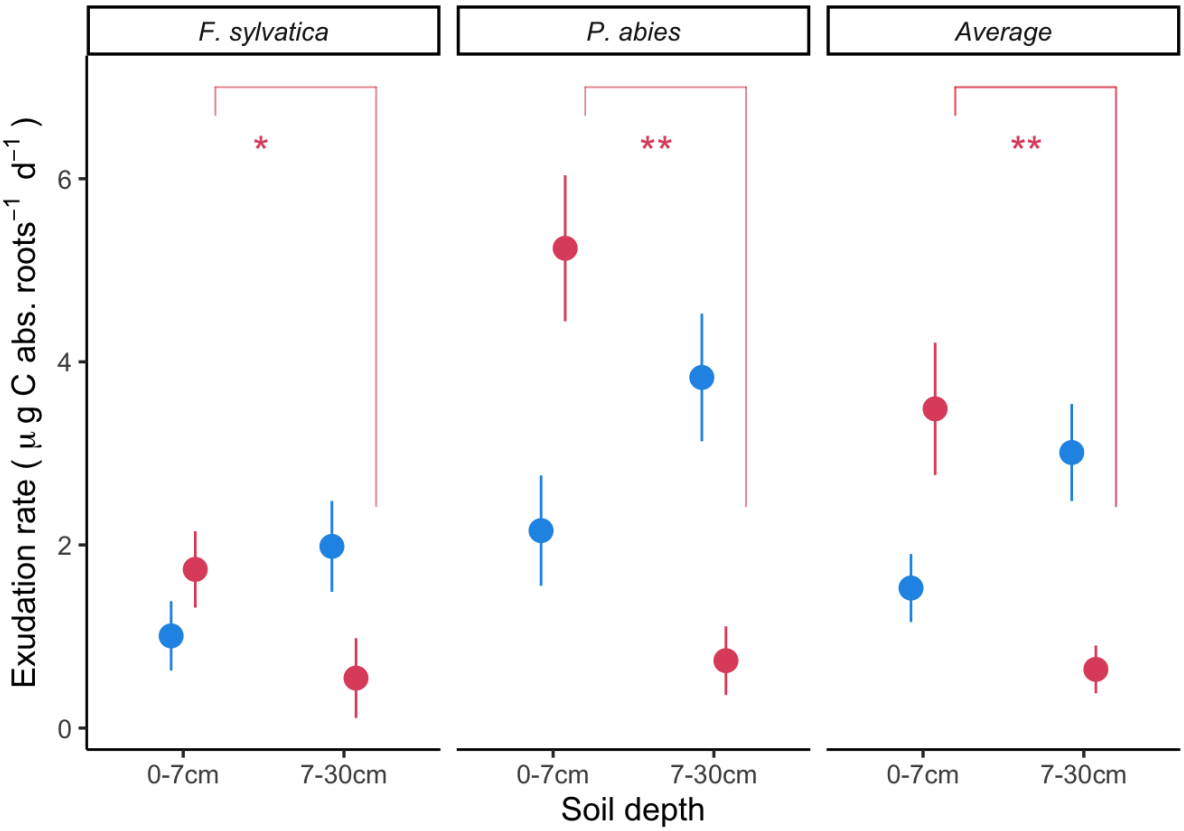

Figure S3: Fine-root exudation rates per density of absorptive roots (estimated as number of tips per surface area of sampled root branches) of *F. sylvatica* and *P. abies* and average values over both species on control (blue) and drought plots (red) in the KROOF experiment. Significant differences between 0-7 cm and 7-30 cm soil depth for the drought plots are indicated with red asterisks, where * is $p < .05$ and ** is $p < .01$. Symbols and whiskers indicate means ± standard errors for $n = 3$ plots per treatment.

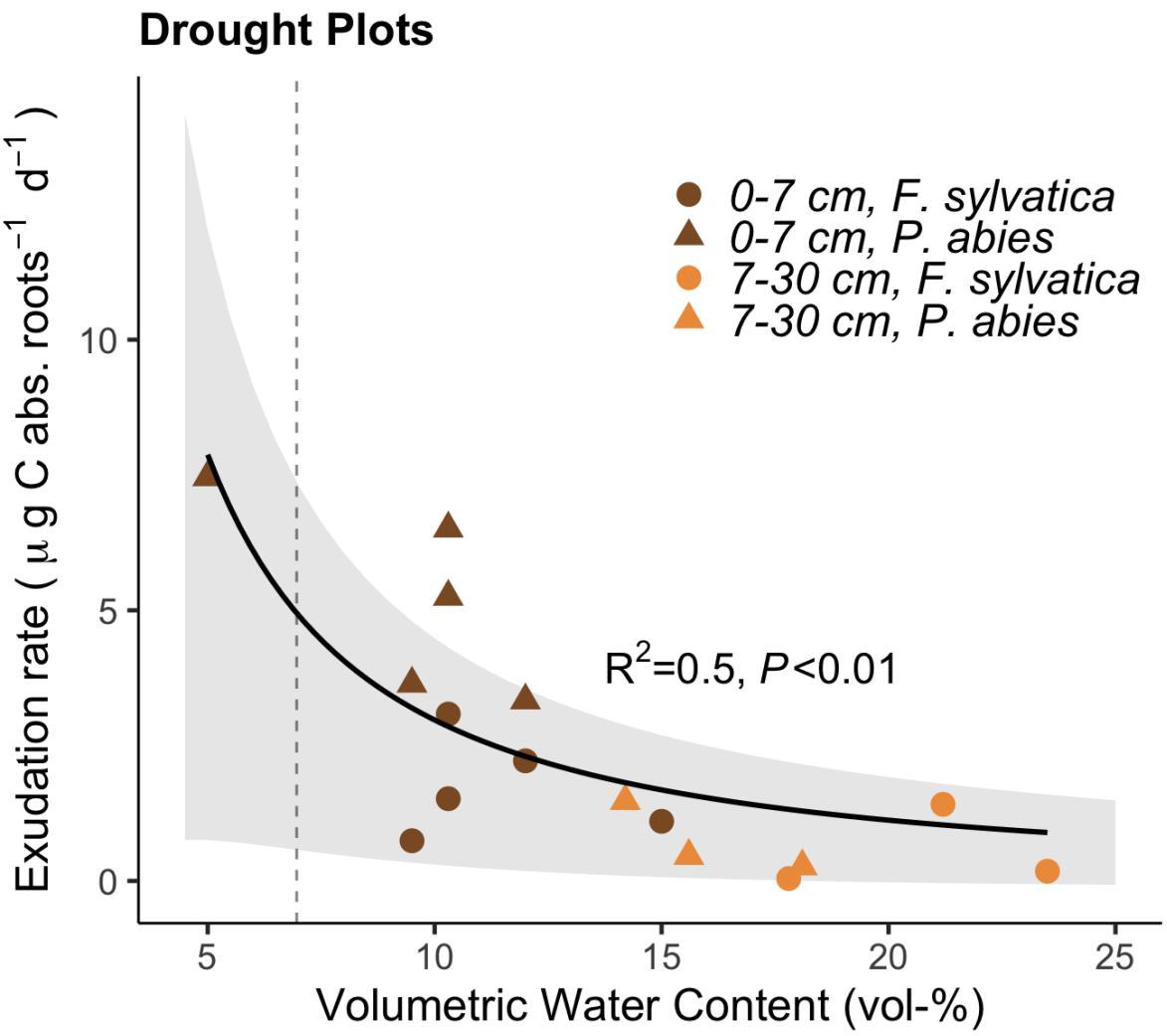

Figure S4: Relationships between exudation rate per density of absorptive roots (estimated as number of tips per surface area of sampled root branches) and volumetric soil water content (SWC) for the drought treatment over both studied species, *F. sylvatica* (circles) and *P. abies* (triangles). Dark brown symbols indicate 0-7 cm soil depth and light brown symbols indicate 7-30 cm soil depth. The regression line (with grey shading indicating 1 SE) is integrating the relationship over both species. The dashed grey line marks the maximum curvature of the drought regression at 7.0 vol-% SWC, indicating increased exudation below this SWC. There was no significant correlation for the control treatment with high water abundance. R^2 and *p*-value for the regression were calculated from power transformation and linear regression of the data. The same trend can be seen as in Figure 2 in the main manuscript (increased exudation below a threshold in SWC close to the wilting point of the plots), where exudation rates are related to the surface area of studied root branches.

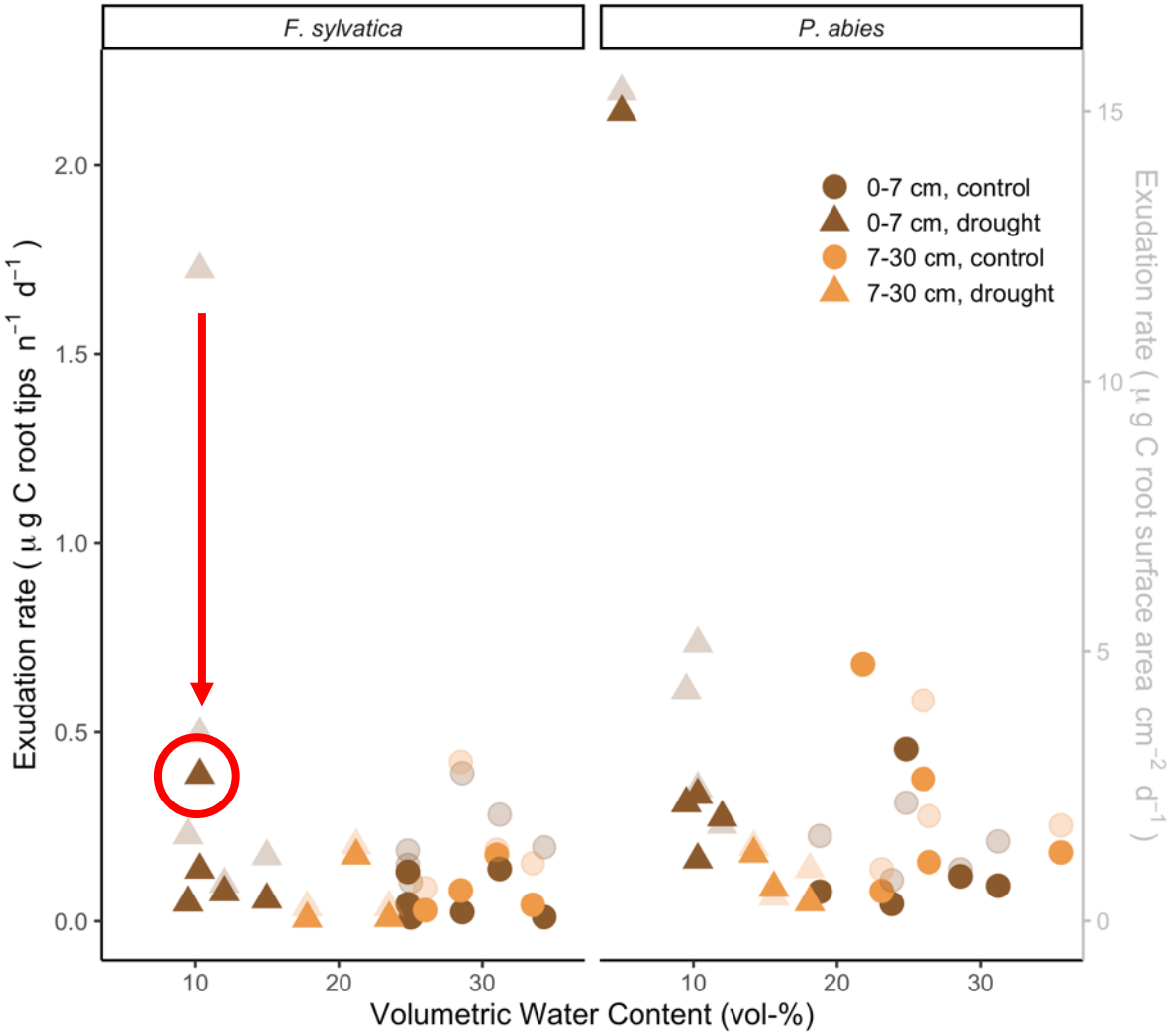

Figure S5 Fine-root exudation rates per number of root tips of sampled root branches related to volumetric soil water content. Circles indicate control plots and drought plots are shown as triangles. Dark brown symbols indicate 0-7 cm soils and light brown symbols indicate 7-30 cm soils. Highlighted is one root branch of *F. sylvatica* that showed the highest exudation per root surface area (light symbols on second y-axis; note different scaling between the two y-axes). When expressed per number of root tips, the exudation rate is closer to the average values of the other sampled *F. sylvatica* root branches.

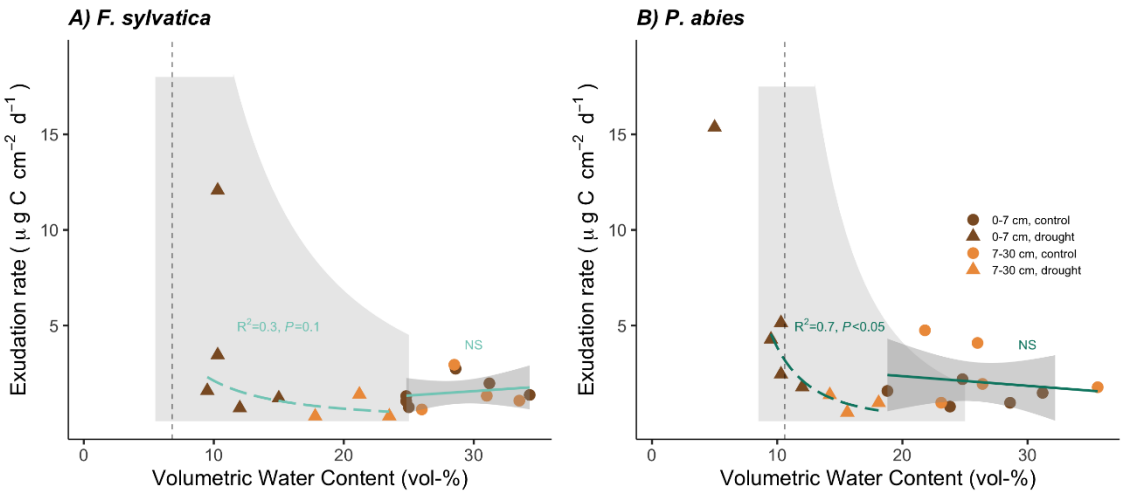

Figure S6: Relationships between exudation rate per fine-root surface area and volumetric soil water content (SWC) separated for drought (triangles) and control (circles) treatments for **A)** root branches of *F. sylvatica* and **B)** *P. abies*. Dark brown symbols indicate 0-7 cm soil depth and light brown symbols indicate 7-30 cm soil depth. Regression lines (with grey shading indicating 1 SE) are given for both species (note that the SE area exceeds the frame of the graph and correlations are not significant for control treatments). The dashed grey line marks the maximum curvature of the drought regression at 6.8 vol-% (*F. sylvatica*) and 10.6 vol-% SWC (*P. abies*), 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. Two statistical outliers (> 10 µg C cm^{-2} d^{-1}) were excluded for the analysis. The same trend can be seen as in Figure 2 in the main manuscript (increased exudation below a threshold in SWC close to the wilting point of the plots).

Methods S1: Scaling approach

To estimate species' C partitioning into root exudates under drought, we combined C-flux assessments with leaf, stem, and root surface area measurements. We defined exudation scaled to the entire rooting zone as root-system level exudation. The proportion of net-C assimilation partitioned to exudation was defined as tree-level exudation. Net-C assimilation was calculated as net-leaf assimilation minus stem respiration and root respiration.

Carbon assimilation measurements were taken immediately after our exudate collection between 09 June and 21 June 2019 from a canopy crane between 8 am and 3 pm on sunny days to ensure stable conditions. For each tree, we randomly selected 3-4 intact sun-exposed leaves for *F. sylvatica*, and one shoot with one-year-old needles for *P. abies*. Leaf areas were separated into sun- and shade crown, i.e. 28.8 % sun- and 71.2 % shade crown for *F. sylvatica* and 52.6 % sun- and 47.7 % shade crown for *P. abies* (Patzner, 2004). For *P. abies*, conversion factors (3.25 for sun leaves and 2.32 for shade leaves; Patzner (2004)) were used to calculate the total needle area from the projected area. During measurements of the light-saturated gas exchange rates at 400 ppm CO_2 (A_{sat}), we set the light intensity to 1500 µmol m^{-2} s^{-1} and kept the leaf temperature at 25 °C. The relative humidity was set at 60-65%. Leaves in the shade crown for both species were used to estimate A_{sat}. A ratio between $A_{sat,shade}$ to $A_{sat,sun}$ was calculated from typical A_{sat} values of both species (Table S4) (Larcher, 2001; Matyssek, 2010) and multiplied with measured A_{sat} values of the sun leaves. For *F. sylvatica*, we set $A_{sat,shade}$ as 36 % of $A_{sat,sun}$, while for needles in the shade crown of *P. abies* we estimated 63 % of $A_{sat,sun}$. For *P. abies*, needle age had to be considered. Trees contained *c.* six needle classes and the fraction of each from total foliage was assessed for one branch and assumed to be consistent for the whole tree. A_{sat} was assumed to decrease with increasing needle age estimated after Matyssek (2010) by *c.* 10 % per year, with 0 and 1-year needles having the same, maximum A_{sat} (Table S5). Typical light saturation and compensation points for both species in their respective sun and shade crown were taken from the literature (Larcher, 2001; Matyssek, 2010) (Table S4).

In drought plots, photosynthesis rates per unit leaf area at light saturation (A_{sat}) were significantly lower than in control plots in *F. sylvatica*. By contrast, in *P. abies* there was no significant difference in A_{sat} between treatments and values were significantly lower compared to *F. sylvatica* (Table S4).

Stem area was multiplied by the daily mean stem-respiration rate to obtain daily stem respiration per tree. Stem-respiration rates were estimated for the other trees in each plot using the average measured respiration rates per species and plot and scaling respiration to the tree level based on the diameter and height of each individual tree. To obtain stem respiration per m^2 plot area and day, daily stem respiration was summed for all trees per species and plot and divided by plot size (Grams *et al.*, 2021), assuming each species occupied 50% of the plot. Stem respiration per stem area did not differ

significantly between treatments (Table S6) but was lower for *F. sylvatica* (0.9 ± 0.1 µmol m^{-2} s^{-1}) than *P. abies* trees (3.7 ± 0.4 µmol m^{-2} s^{-1}) in drought plots.

Soil respiration measurements lasted between 2 - 5 min, depending on the CO_2 efflux rate, with a 25-s dead-band, a 30-s pre-purge period and a 45-s post-purge period. Soil respiration measurements started immediately after the exudate sampling (04 June) and paired drought and control plots were measured for seven consecutive days before the next plot pair was measured. Soil and root respiration rates per soil surface area tended to be lower in *P. abies* than in *F. sylvatica* and showed trends towards reduced rates in drought plots (Table S6).

To **scale** exudation to the root-system level, we used data from soil coring in which fine-root biomass, surface area and number of tips were assessed. As we measured the total root biomass of our exudate-root branch samples (including woody transport roots with diameter > 2 mm), we could not use exudates expressed per unit total root biomass for scaling. As fine-root abundance can vary during the growing season, we checked for differences in minirhizotron fine-root tip abundance taken in October 2018 to sessions taken during the fine-root exudation sampling period (i.e. three sessions in May and early June 2019). No differences were found between the sessions, suggesting that fine-root area assessments, taken with soil cores in October 2018, were representative of the period of exudation measurements. Although 90% of roots grow at depths between 0-50 cm (Häberle *et al.*, 2012), potentially active roots at greater depth were excluded from the scaling and may underestimate C partitioned to exudates. We combined two very effective methods, i.e. destructive soil coring and the minirhizotron technique to increase the reliability of root-abundance assessment used for scaling. Fine roots seem to be the primary source of root exudation in woody species (Sun *et al.*, 2021) and therefore, capturing exudates from fine roots appears valid for assessment of root-system and tree-level exudation which we also tested for other measures of normalization. Root respiration was based on an estimation and any vertical differences in respiratory losses from roots were not accounted for. Further, only the exuded soluble fraction of C was integrated in our calculations. The inclusion of volatile organic compounds (VOCs) may increase the fraction that is allocated belowground which, however, would probably not change the evidence of our data.

Leaf- and stem surface area did not differ between treatments for *F. sylvatica* trees (Table S7). In *P. abies*, leaf- and stem surface area tended to be lower in drought compared to control plots, albeit not significantly so (Table S7).

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Table S1: Edaphic characteristics and their interspecific (*F. sylvatica* and *P. abies*) changes with soil depths (0-5, 5-15, 15-30 cm) and treatment (control and drought).

Species	Treatment	Soil depth (cm)	C:N ratio	pH
F. sylvatica	Control	0-5	14.1 ± 0.2	4.3
		5-15	13.1 ± 0.4	4.6
		15-30	10.7 ± 0.8	4.4
	Drought	0-5	15.1 ± 0.6	4.5
		5-15	12.4 ± 0.3	4.3
		15-30	9.8 ± 1.0	4.6
P. abies	Control	0-5	17.5 ± 0.7	3.8
		5-15	13.9 ± 0.5	4.2
		15-30	10.3 ± 1.1	4.3
	Drought	0-5	17.6 ± 1.3	3.9
		5-15	15 ± 1.1	4.1
		15-30	11.9 ± 1.4	4.3

Values are mean ± 1 SE, n = 5

Table S2: Root characteristics of root branches of *F. sylvatica* and *P. abies* trees, sampled for exudate collection in two different soil depths under drought and control conditions in the KROOF drought experiment.

Species	Treatment	Soil depth	Root mass (g)	Root-surface area (cm^2)	Fine-root surface area (cm^2) | % of total surface area	Tips	Absorptive root density (tips cm^{-2})
F. sylvatica	Control	0-7cm	0.20 (0.06)	18.3 (1.4)	17.4 (1.5) | 95 (1)	1089 (375)	60.4 (20.9)
	Control	7-30cm	0.37 (0.13)	35.0 (9.8)	33.0 (9.5) | 95 (2)	845 (287)	22.5 (5.9)
	Drought	0-7cm	0.14 (0.05)	16.6 (3.7)	16.1 (3.3) | 98 (1)	334 (49)	23.9 (4.3)
	Drought	7-30cm	0.10 (0.03)	12.6 (5.1)	12.5 (5.1) | 99 (1)	363 (201)	27.1 (9.8)
P. abies	Control	0-7cm	0.21 (0.06)	19.6 (5.8)	18.1 (5.5) | 93 (4)	270 (101)	13.1 (2.9)
	Control	7-30cm	0.19 (0.06)	17.3 (3.7)	15.4 (2.9) | 91 (3)	167 (36)	10.4 (1.0)
	Drought	0-7cm	0.23 (0.06)	15.8 (4.8)	15.3 (4.8) | 97 (1)	205 (83)	11.6 (2.0)
	Drought	7-30cm	0.07 (0.02)	6.3 (1.0)	6.2 (0.9) | 99 (1)	65 (23)	10.7 (4.4)

Fine-root surface area was estimated as surface area of all root diameters ≤ 2mm and used for normalization of root-exudation quantities at the root system and the tree level. Almost the entire sampled root branches consisted of root diameters ≤ 2mm (91 – 99 %). Absorptive-root density was estimated as number of root tips divided by total root-surface area. Integrated over treatment and soil depths, *F. sylvatica* had more tips ($p < .01$) and a higher absorptive-root density ($p < .001$) than *P. abies.* There were no significant differences within the different parameters between species, treatments, or soil depths. Values are given as means with standard errors for $n = 3$ plots per treatment.

Table S3 Number (*n*) of exudate samples taken in two soil depths from roots of *F. sylvatica* and *P. abies* in control and drought plots in the KROOF drought experiment in May/June 2019.

Species	Treatment	0-7 cm depth	7-30 cm depth
F. sylvatica	Control	6	4
	Drought	5	3
P. abies	Control	5	5
	Drought	5	3

Table S4: Typical rates of light-saturated gas exchange (A_{sat}), and PAR light intensity (in µmol m^{-2} s^{-1}) at light saturation and light compensation for sun and shade leaves of *F. sylvatica* and *P. abies* taken from Larcher (2001) and Matyssek (2010).

Species	Crown	A_{sat} (SE)	Light saturation	Light compensation
			(µmol m^{-2} s^{-1})	
F. sylvatica	Sun	12.5 (2.5)	700	35
	Shade	4.5 (1.5)	350	12.5
P. abies	Sun	9.5 (2.5)	900	35
	Shade	6.0 (2.0)	175	6

A_{sat} values were used to estimate the fraction of assimilation in shade leaves compared to sun leaves. Fractions were 36 % in *F. sylvatica* and 63 % in *P. abies*. At and above light saturation, we assumed measured A_{sat} values as actual assimilation rates, while assimilation rates linearly decreased below light saturation, using a linear descent between light saturation and light compensation.

Table S5: Estimated decrease of light-saturated gas exchange (A_{sat}) in *P. abies* trees with needle age and abundance of needles of each needle age in trees on control and drought plots.

Needle Age	A_{sat}	Control Area Fraction (SE)	Drought Area Fraction (SE)
		%	
Age 0 (2019)	100	20 (2)	34 (6)
Age 1 (2018)	100	21 (2)	31 (4)
Age 2 (2017)	85	26 (3)	22 (3)
Age 3 (2016)	77.5	20 (2)	11 (4)
Age 4 (2015)	65	8 (4)	3 (2)
Age 5 (2014)	48.8	6 (4)	0 (0)

Current and one-year-old needles were assumed to assimilate at full capacity (100%). Older ages were assumed to decrease in their A_{sat} according to Matyssek (2010). Abundance of needles of each needle age was counted for one branch per tree for control and drought-stressed trees. The counted abundance was assumed to be representative of the entire tree.

Table S6: Rates of photosynthesis under light saturation (A_{sat}), stem, soil- and root respiration of *F. sylvatica* and *P. abies* trees on control and drought plots in the KROOF drought experiment.

Species	Treatment	A_{sat} (µmol m^{-2} s^{-1})	Stem resp (µmol m^{-2} s^{-1})	Soil resp (µmol m^{-2} s^{-1})	Root resp (µmol m^{-2} s^{-1})
F. sylvatica	Control	13.3 (0.8)^{a}	2.4 (0.3)^{ab}	7.3 (2.2)^{a}	3.6 (1.1)^{a}
	Drought	8.3 (1.0)^{b}	0.9 (0.1)^{a}	2.7 (1.0)^{a}	1.3 (0.5)^{a}
P. abies	Control	2.7 (0.2)^{c}	3.9 (1.1)^{ab}	5.5 (1.8)^{a}	2.8 (0.9)^{a}
	Drought	2.1 (0.3)^{c}	3.7 (0.4)^{b}	1.7 (0.4)^{a}	0.7 (0.1)^{a}

Stem respiration (Stem resp) and A_{sat} are given per unit area of stem or leaf, respectively, whereas soil respiration (Soil resp) and root respiration (Root resp) are given per unit plot area. Root respiration was estimated from soil respiration following Nikolova (2007). Letters indicate significant differences ($p < .05$) between treatments and species. Values are given as means with standard errors for $n = 3$ plots per treatment.

Table S7: Leaf- and stem area used as parameters for scaling carbon fluxes of *F. sylvatica* and *P. abies* trees on control and drought plots in the KROOF drought experiment.

Species	Treatment	Leaf area	Stem area
		m^2	m^2
F. sylvatica	Control	89.5 (15.7)	15.0 (1.5)
	Drought	94.1 (18.9)	14.7 (1.8)
P. abies	Control	154.4 (14.6)	19.7 (1.3)
	Drought	136.5 (13.2)	17.9 (1.2)

Leaf area was estimated with allometric equations, while stem area was scaled from measured tree heights and diameters at breast height, assuming a conical shape of the trees. There were no significant differences between treatments. Values are given as means with standard errors for $n = 3$ plots per treatment.