Supporting information

Article title: Dynamics of initial C allocation after drought release in mature Norway spruce - Increased belowground allocation of current photoassimilates covers only half of the C used for fine-root growth

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Table S1: Detailed data of the labeled four control and three recovering (previously drought-stressed) trees: Diameter at breast height (DBH), tree height, height of the crown base, the daily mean change of stable carbon isotope composition ($\delta^{13}C$) and CO_2 concentration in crown air during labeling, spatial contribution and area of each tree for the calculation of belowground C sink activity and allocation of newly assimilated C (C_{new}): in fine-root growth, ectomycorrhizae (ECM), root exudates, and soil CO_2 efflux (see Material and Methods). The changes are given in means ± SE.

Tree	DBH [cm]	Tree height [m]	Height of the crown base [m]	Change in crown air $\delta^{13}C$ [‰]	Change in CO_2 concentration [ppm]	Spatial contribution belowground [%]	Area [m^2]
Conrol_1	30.5	33.7	23.6	-6.7 ± 0.4	111 ± 8	21	11
Conrol_2	34.9	32.6	23.1	-6.7 ± 0.4	112 ± 8	23	13
Control_3	46.3	34.3	23.1	-7.2 ± 0.4	119 ± 8	31	17
Control_4	37.7	32.5	21.0	-8.8 ± 0.4	162 ± 10	25	14
Recovery_1	45.1	32.0	22.7	-5.0 ± 0.3	72 ± 5	41	26
Recovery_2	27.3	28.3	22.5	-7.3 ± 0.4	132 ± 8	25	16
Recovery_3	38.3	33.6	23.3	-2.9 ± 0.3	35 ± 5	36	22

Table S2: Days of samplings/assessments of each parameter (days marked in gray are the timing of samplings/assessments) and number of samples per treatment (i.e. control and recovery) and day. n.a., not assessed.

Days respective to watering/ start of labeling	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	after growing season	samples treatment^{-1} day^{-1}
Branch growth																																					■	3-4
Branch efflux																																						n.a.
Stem growth																																					■	4
Stem efflux						■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		3
Coarse-root growth																																					■	3-4
Fine-roots in mesh bags	■														■										■										■			8
Additional fine-roots										■	■	■	■	■		■	■	■	■	■	■	■	■			■		■		■			■					6-13
Ectomycorrhizae	■									■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■		■		■			■		■			5-12
Root exudates			■		■		■		■		■		■		■													■		■		■						3-5
Soil efflux		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		3

Table S3: Total length and surface area of branch/twig and stem estimated for each tree based on field data. Data of branches and twigs are separated into sun and shade crowns.

	Sun branch/twig length [m]	Sun branch/twig area [m^2]	Shade branch/twig length [m]	Shade branch/twig area [m^2]	Stem area (< 6.5 m) [m^2]	Stem area (> 6.5 m) [m^2]
Conrol_1	3359	40	1287	14	6	11
Conrol_2	3847	45	1474	18	7	12
Control_3	5103	60	1956	25	9	17
Control_4	4140	38	1590	21	7	12
Recovery_1	1883	21	1411	22	9	15
Recovery_2	1138	13	853	12	5	8
Recovery_3	1597	20	1198	14	7	14

Methods S1: Determination of proportional growth using dendrometer

To determine the proportional growth (in %) during the 28 days after watering (ratio of the radial growth during 28 days to the total annual growth), dendrometer data at 6 am was used and fitted with the following sigmoid curve:

$$X = \mathrm{d} + \frac{\mathrm{a} - \mathrm{d}}{1 + e^{\frac{DOY - c}{b}}} \quad (Eqn. S1),$$

where X is the output voltage (in mV) corresponding to the radial growth, DOY is the day of year, a is the starting value of X before the growing season, b the slope coefficient of the regression, c the inflection point of the curve, and d the end value of X after the growing season.

Using these curves, proportional growth was calculated by relating the growth during the 28 days to the total annual growth. Since only two labeled trees per treatment were assessed with the dendrometers, additional spruce trees in neighboring plots were included in the evaluation of the proportional growth (n = 9 for control and n = 6 for recovering trees).

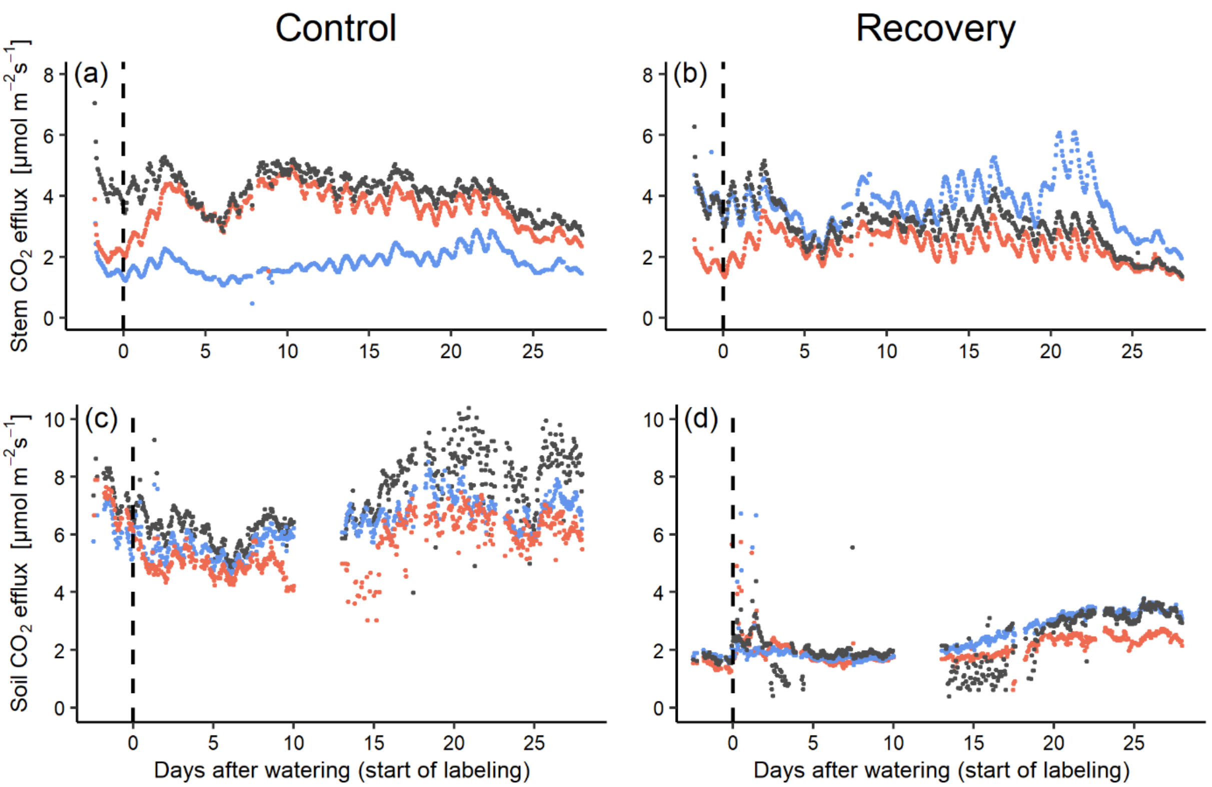

Fig. S1: Rates of stem (a, b) and soil CO_2 efflux (c, d) in control (left) and recovery (right) trees during the study period. Each color represents each measurement tree (n = 3).

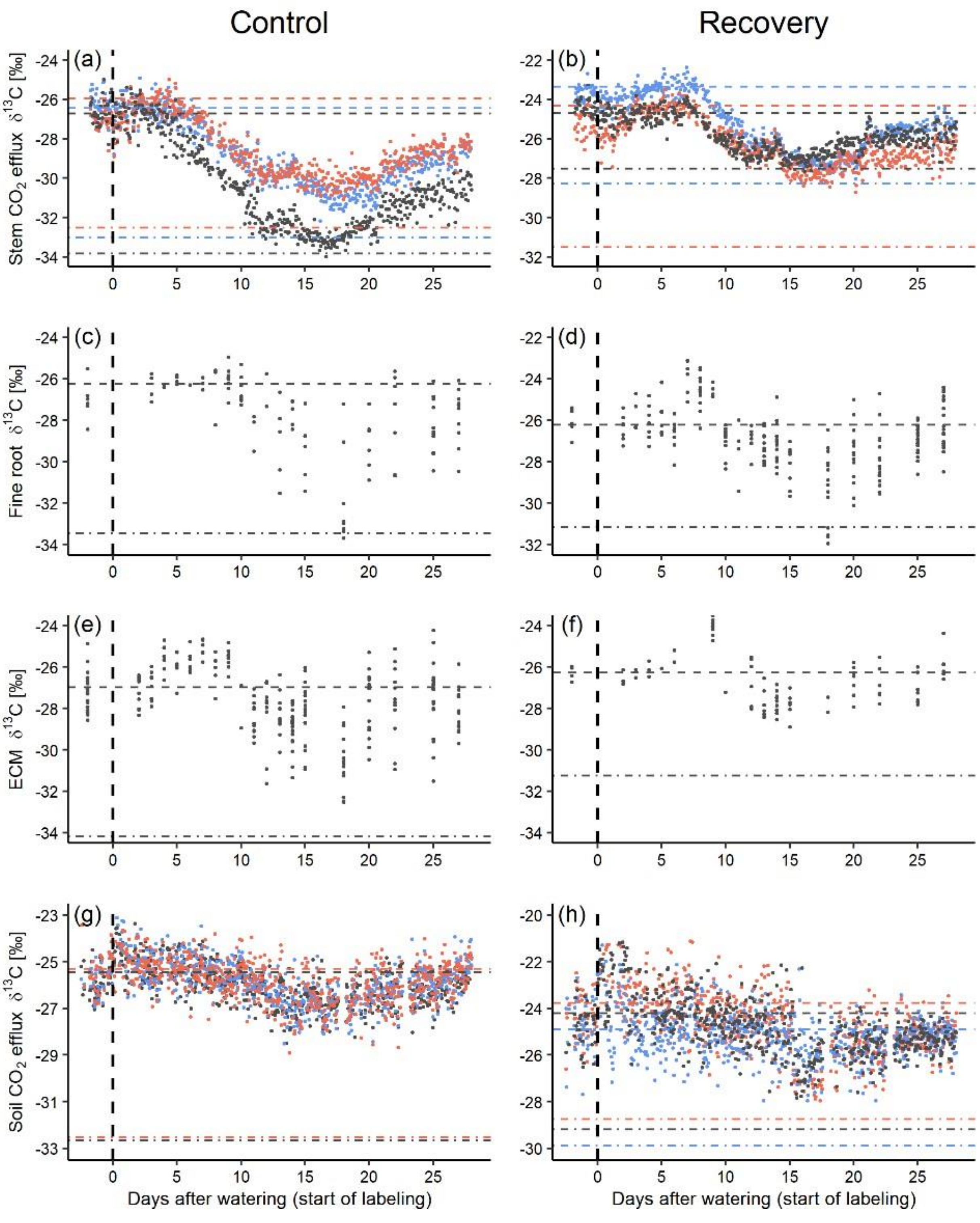

Fig. S2: Raw $\delta^{13}C$ data ($\delta^{13}C_{sample}$) used for Eqn. 10. (a,b) stem CO_2 efflux, (c,d) non-mycorrhized fine-root tips, (e, f) ectomycorrhizae (ECM), and (g,h) soil CO_2 efflux, separated in control trees (left) and recovering (previously drought-stressed, right) trees. Different colors represent each measurement tree (n = 3) for stem CO_2 efflux and soil CO_2 efflux. All measurements were pooled for non-mycorrhizal root tips and ECM. Horizontal dashed and dot-dash lines display $\delta^{13}C_{old}$ and $\delta^{13}C_{new}$ in Eqn. 10, respectively. $\delta^{13}C_{new}$ was calculated with Eqn. S2,S3 using $\delta^{13}C_{old}$ and the individual change in crown air $\delta^{13}C$ (Table S1).

Methods S2: Calculation of $\delta^{13}C_{new}$ for Eqn. 10

$\delta^{13}C_{new}$ for Eqn. 10 was calculated as described by Kuptz *et al.* (2011), following (Schnyder *et al.*, 2003):

$$\delta^{13}\mathrm{C}_{\mathrm{A-O}}\ (‰) = \left(\frac{1000\ +\ \delta^{13}\mathrm{C}_{Air-Unlabeled}}{1000\ +\delta^{13}\mathrm{C}_{old}} - 1\right) \times 1000\ (\mathrm{Eqn.}\,S2),$$

which gives the mean apparent ^{13}C discrimination ($\delta^{13}C_{A\text{–}O}$) between unlabeled crown air (reference air above canopy, $\delta^{13}C_{Air\text{-}Unlabeled}$) and $\delta^{13}C_{old}$.

$$\delta^{13}\mathrm{C}_{new}\ (‰) = 1000\ \times \frac{1000\ +\ \delta^{13}\mathrm{C}_{Air-Labeled}}{1000\ +\delta^{13}\mathrm{C}_{\mathrm{A-O}}} - 1000\ (\mathrm{Eqn.}\,S3),$$

where $\delta^{13}C_{Air\text{–}Labeled}$ is the mean $\delta^{13}C$ of crown air of each tree. For belowground sinks that were not assigned to specific trees, mean $\delta^{13}C_{Air\text{–}Labeled}$ of four (control) or three (recovering) trees was used.

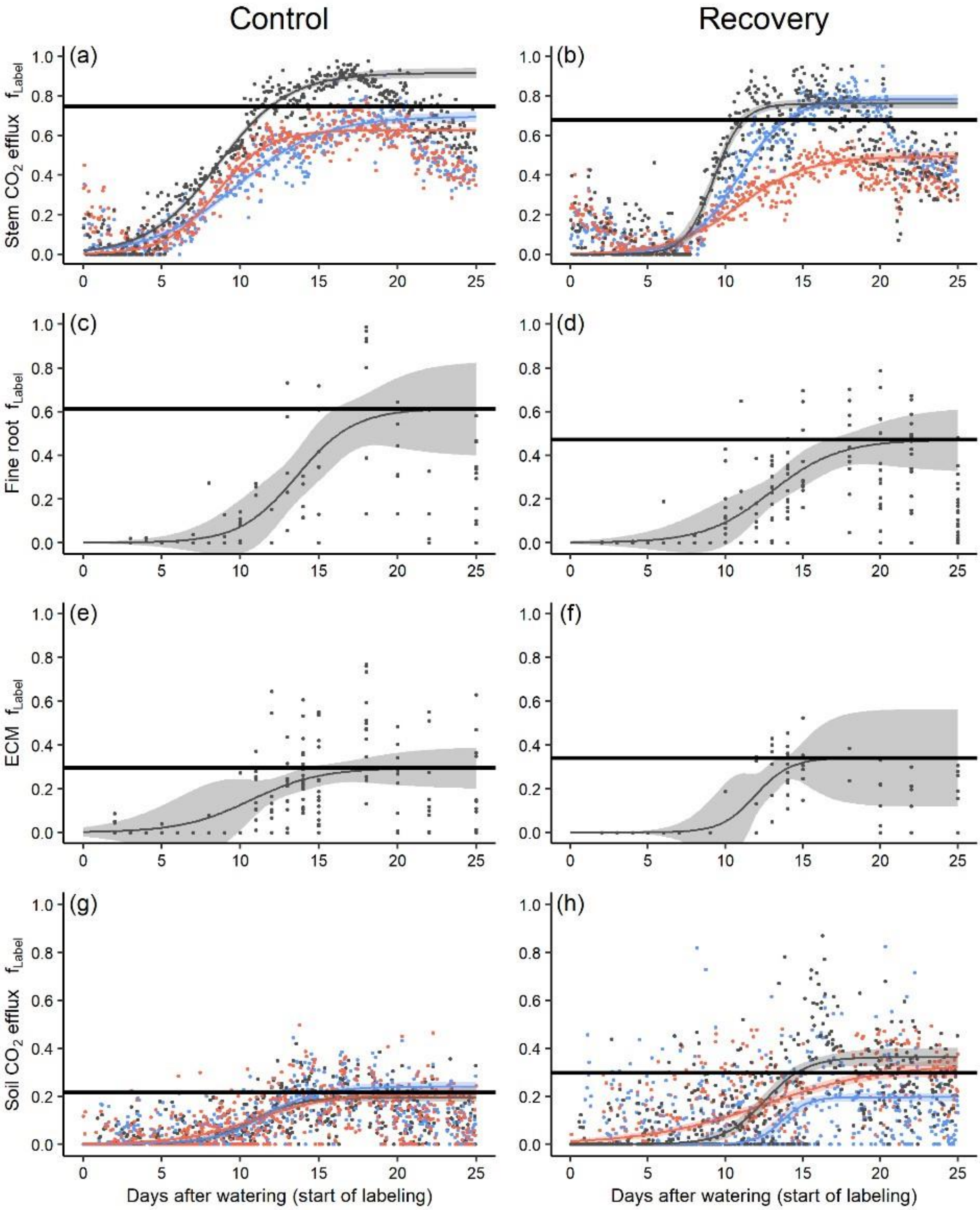

Fig. S3: f_{Label} (fraction of labeled C) and sigmoid curves with 95% confidence intervals to calculate the $contC_{new}$ (contribution of C_{new} to C sink activity) to stem CO_2 efflux (a,b), non-mycorrhized fine-root tips (c,d), ectomycorrhizae (ECM, e,f), and soil CO_2 efflux (g,h), separated in control trees (left) and recovering (previously drought-stressed, right) trees. Different colors represent each measurement tree (n = 3) for stem CO_2 efflux and soil CO_2 efflux. All measurements were pooled for non-mycorrhizal root tips and ECM. Only f_{Label} before reaching the maximum were used for the fitting, since f_{Label} decreased again after the end of labeling. Black horizontal lines display the mean $contC_{new}$.

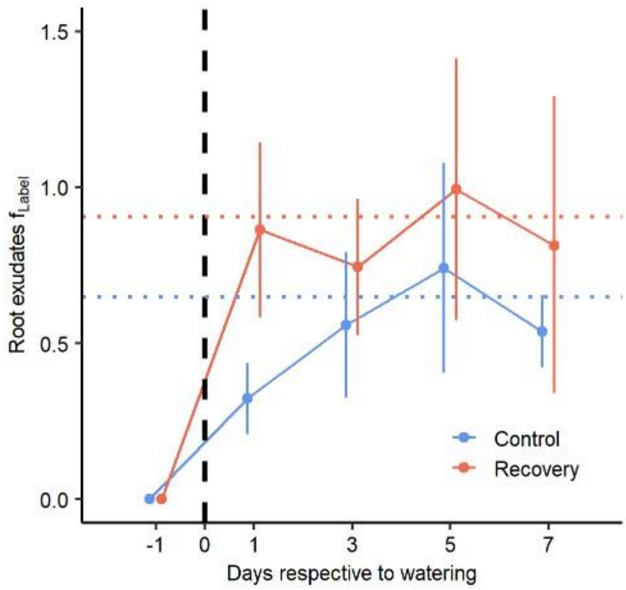

Fig. S4: f_{Label} (fraction of labeled C) and contC_{new} (contribution of C_{new} to C sink activity) to root exudates in control (blue) and recovering trees (previously drought-stressed, red). The calculated contC_{new} is shown in horizontal dotted lines for control (blue) and recovering trees (red), respectively. The data are displayed in mean ± SE.

Methods S3: Detailed descriptions for the calculation of fraction of labeled C (f_{Label}) and contribution of C_{new} to each C sink activity (contC_{new}) for branch, stem, and coarse-root growth

For branch, stem, and coarse-root growth, $\delta^{13}C_{old}$ and $\delta^{13}C_{sample}$ (for Eqn. 10) were determined by fitting the $\delta^{13}C$ of tree ring slices with a piecewise function (Hikino *et al.*, 2022). The applied labeling with ^{13}C-depleted CO_2 caused a sudden and steep decrease of $\delta^{13}C$, after the ^{13}C-depleted tracer was incorporated in the tree ring and was thus defined as tracer arrival. To determine this point, linear segments before and after the start of the steep decrease (e.g. slices 1 - 19 for the sample in Fig. S5a) were extracted from the course of the $\delta^{13}C$ data. Then, these linear segments were fitted by linear regression ("lm" function, R package "stats", version: 3.6.1). Subsequently, the "segmented" function (R package "segmented", version: 1.3-0) was used to determine the point where the linear relationship (slope and intercept) changes, giving the intersection between the two green lines as exemplified in Fig. S5. The $\delta^{13}C$ value at this point (marked by the green horizontal dashed lines in Fig. S5a,b) was then defined as $\delta^{13}C_{old}$.

After the steep decrease, $\delta^{13}C$ started to increase again as unlabeled C arrived after the end of labeling. We determined this minimum value of $\delta^{13}C$ by fitting with the piecewise function using the same method (intersection between the purple linear segments fitted to the data in Fig. S5). The $\delta^{13}C$ value at this point (purple horizontal dashed lines) was then defined as $\delta^{13}C_{sample}$. In addition to the labeled trees, we also determined the natural shifts of $\delta^{13}C$ of non-labeled control trees for each treatment (n = 3). These shifts without the effect of the labeling were subtracted from the $\delta^{13}C_{sample}$ determined above to correct for the effect of watering, weather fluctuation, and seasonal changes (Helle & Schleser, 2004). Finally, using $\delta^{13}C_{old}$, corrected $\delta^{13}C_{sample}$, and Eqn. 10, f_{Label} was calculated. Since we could not apply the sigmoid curve (Eqn. 11) to determine $contC_{new}$, the calculated f_{Label} was defined as $contC_{new}$. Thus, we could not consider that the new isotopic equilibrium was not completely reached by the labeling. However, since c. 98% of the calculated $contC_{new}$ to stem CO_2 efflux was reached with the recorded f_{Label} data (Fig. S3a,b), the underestimation of $contC_{new}$ to the branch, stem, and the coarse-root growth is likely negligible.

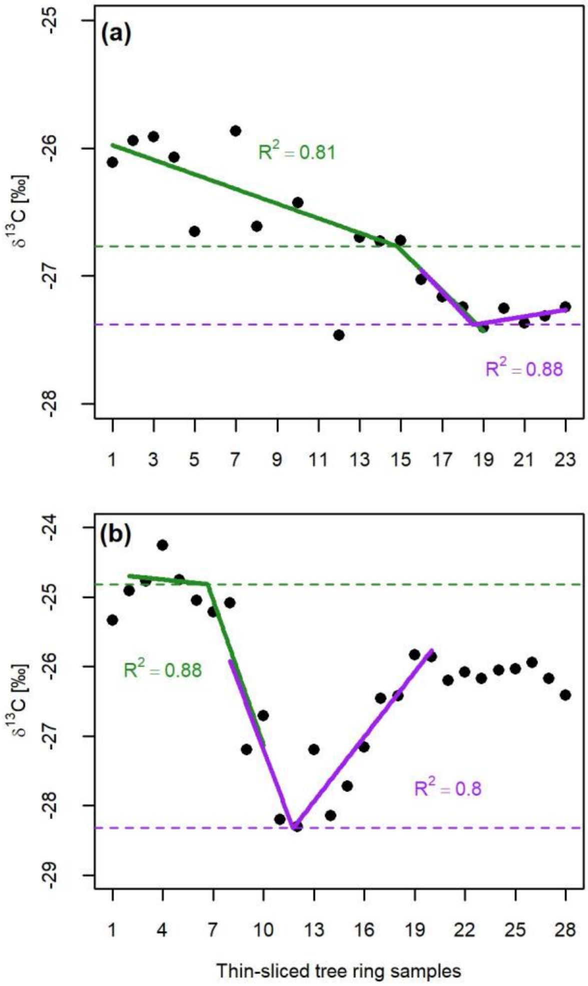

Fig. S5: Two examples for the calculation of $contC_{new}$ (contribution of C_{new} to C sink activity) to stem and coarse-root growth, using piecewise functions. X-axis is each tree ring sample thin-sectioned in radial direction (c. 5 µm thick). The green and purple line segments fitted to the data show the results of the piecewise functions for the arrival of ^{13}C-depleted tracer (green) and minimum $\delta^{13}C$ (purple), respectively. $\delta^{13}C$ at the intersections of two line segments of the respective color, marked with horizontal dashed lines, are the calculated $\delta^{13}C_{old}$ and $\delta^{13}C_{sample}$, respectively. These values were then used for the calculation of $contC_{new}$ (see main text and Methods S3).

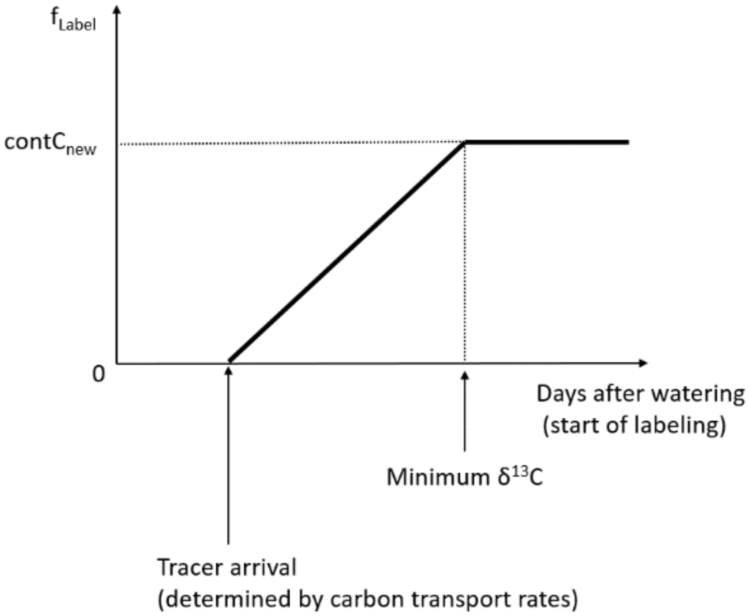

Fig. S6: Estimation of the course of f_{Label} (fraction of labeled C) in branch, stem, and coarse-root growth, using $contC_{new}$ (contribution of C_{new} to C sink activity) and the arrival time of the ^{13}C-depleted tracer. f_{Label} between tracer arrival and the minimum $\delta^{13}C$ was assumed to increase linearly.

References

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