Supplementary Information

Rhizosphere activity in an old-growth forest reacts rapidly to changes in soil moisture and shapes whole-tree carbon allocation

Jobin Joseph, Decai Gao, Bernhard Backes, Corinne Bloch, Ivano Brunner, Gerd Gleixner, Matthias Haeni, Henrik Hartmann, Günter Hoch, Christian Hug, Ansgar Kahmen, Marco M. Lehmann, Maihe Li, Jörg Luster, Martina Peter, Christian Poll, Andreas Rigling, Kaisa A Rissanen, Nadine Ruehr, Matthias Saurer, Marcus Schaub, Leonie Schönbeck, Benjamin Stern, Frank M Thomas, Roland A Werner, Willy Werner, Thomas Wohlgemuth, Frank Hagedorn, Arthur Gessler

Supplementary Methods

Forest and tree characteristics

The experimental site covers 1.2 hectares and has about 800 trees, with a mean canopy height of 10.8 m, a mean diameter at breast height (DBH) of 21.9 cm, a basal area of 27.3 m^2 ha^{-1} and a stand density of approximately 730 stems ha^{-1}. The site is randomly divided into eight plots each 25 x 40 m^2, separated by a 5-m buffer zone. The plots are adjacent to a watering channel fed by the Rhone river, and water is pumped to sprinklers irrigating four of the plots during nighttime during the frost-free period of the year. The 10 trees used for pulse labelling were on average 11.0 ± 0.8 m tall and had a DBH of 20.4 ± 2.5 cm thus being representative of the stand.

Measurement details

Extraction of water-soluble compounds and starch for ^{13}C detection

In addition to bulk material, the ^{13}C fraction and C concentration were determined for the water-soluble compounds (WSC; mainly soluble carbohydrates, organic acids and amino acids) and starch of needles, branches, stems and coarse roots (**see SI Appendix Figs. S10, S11**). WSC and starch were extracted from the homogenized

plant material according to (1). Both the WSC and the starch extracts were transferred into tin capsules and the remaining solvents were evaporated at 60°C.

The isotope ratios and the C concentrations (%) of bulk samples as well as WSC and starch extracts were determined using a Thermo Flash 2000 elemental analyser coupled to a Thermo Finnigan Delta Plus XP IRMS via a Conflo IV interface (ThermoFinnigan, Bremen, Germany). The stable isotope values are given on the δ scale referenced to the international standard Vienna Pee Dee Belemnite (VPDB).

^{13}C mass balance calculation

To calculate the percent ^{13}C enrichment above the natural abundance (^{13}C excess; see (2)) for individual tree compartments, microbial biomass and respired CO_2, the δ values obtained from isotope laser spectrometer and IRMS measurements were converted to atom% and the excess was calculated as follows:

$$^{13}C\ excess = \frac{atom\%\ ^{t_0} - atom\%\ ^{t_i}}{100} \quad \text{(Eqn. 1)}$$

where $atom\%\ ^{t_0}$ is the natural abundance of ^{13}C (in atom %) in the samples (background ^{13}C) before pulse labelling and $atom\%\ ^{t_i}$ are the values determined at a given time point after pulse labelling.

The total ^{13}C enrichment in different tree compartments as a result of labelling ($^{13}C_{pool}^{tree}$; mg ^{13}C) was determined as follows;

$$^{13}C_{pool}^{tree} = {}^{13}C\ excess * C_{pool}[g] * 1000 \quad \text{(Eqn. 2)}$$

where C pool is the total C content in the different tree biomass pools (for the calculation see Materials and Methods in the main document)

Similarly, the total ^{13}C enrichment in soil microbial biomass as a result of labelling ($^{13}C_{MB}$; mg) was determined as follows;

$$^{13}C_{MB} = {}^{13}C\ excess * C_{MB}[g] * 1000 \quad \text{(Eqn. 3)}$$

where C_{MB} is the C pool of the microbial biomass in a circular soil area (0-10 cm depths) (see Materials and Methods in the main document). This area was thought to cover the whole rooting zone of the individual trees (3).

For the calculation of the ^{13}C distribution given in **Fig. 3** and **SI Appendix Tab. S2** the $^{13}C_{pool}$ values for day 30 after pulse labelling were calculated.

^{13}C enrichment in respiratory CO_2 fluxes ($^{13}C_{resp}$; mg ^{13}C h^{-1}) from the canopy (high-resolution automatic measurements), stem (high-resolution automatic measurements) and soil (from soil collars with a daily resolution) as a result of labelling were determined as follows

$$^{13}C_{resp} = {}^{13}C\ excess * CO_2Flux\ [mg\ C\ m^{-2}h^{-1}] *\ Area\ [m^2] \quad \text{(Eqn. 4)}$$

where the CO_2 flux was determined in chambers as described in the main document and area is the surface area of the needles, stem and soil, respectively (for surface area determination see Material and Methods in the main document).

Canopy and stem respiration fluxes and $\delta^{13}C$ were measured for the first 20 days after labelling (**SI Appendix Fig. S9**) and for that period $^{13}C_{resp}$ was determined. $^{13}C_{resp}$ for days 20 to 30 was extrapolated using exponential decline models fitted to the data from

the measurement period (**SI Appendix Tab. S4**). $^{13}C_{resp}$ for the canopy, stem and soil was plotted against time [h] and the integral below the curve (integrated flux $^{13}C_{resp}^{integrated}$; [mg]) from the start of the labelling until day 30 was calculated.

For the pulse labelling experiments before the rainfall event (n=3 for both irrigation treatment and dry control) we have determined all relevant C pools (bulk, WSC, SC) in leaves, branches, stems, roots and microbial biomass, as well as all fluxes (canopy, stem, soil). For these trees, we summed up the absolute ^{13}C enrichment of the different tree compartments ($^{13}C_{pool}^{tree}$) and the microbial biomass ($^{13}C_{pool}^{microbe}$) at day 30 after labelling and the 30-day integrated fluxes ($^{13}C_{resp}$) and we related the single pools and fluxes to this sum to calculate the relative ^{13}C (in %) and thus the allocation of recent assimilates. Moreover, we related the sum of all pools and fluxes to the total amount of ^{13}C taken up during the pulse labelling ($^{13}C_{total\ uptake}$) (**SI Appendix Tab. S1**) to calculate recovery rates. $^{13}C_{total\ uptake}$ was calculated based on the amount of 99%^{13}C-CO_2 supplied to the pulse labelling chamber from the gas cylinder.

An average recovery rate (Rr) of 75.8 ± 15.4 % of the total ^{13}C assimilated was determined for both irrigated and dry control trees (n = 6) pulse labelled before the precipitation event. For the trees labelled after the rainfall event (n = 4), aboveground ^{13}C fluxes were not determined. For these trees the ^{13}C in the above ground respiration fluxes integrated over 30 days (combined canopy and stem fluxes; $^{113}C_{flux\_est\_above}^{integrated}$; mg) was estimated by multiplying $^{13}C_{total\ uptake}$ for the specific trees (**SI Appendix Tab. S1**) with the Rr of 75.8% and then subtracting the sum of ^{13}C in the other tree pools ($\sum {}^{13}C_{pool}^{tree}$), in soil microbial biomass and in the 30-day integrated soil flux ($^{13}C_{resp\_soil}^{integrated}$)

$$^{13}C_{flux\_est\_above}^{integrated} = \left({}^{13}C_{total\_uptake} * Rr\right) - \left(\sum {}^{13}C_{pool}^{tree} + {}^{13}C_{pool}^{microbe} + {}^{13}C_{flux\_soil}^{integrated}\right) \quad \text{(Eqn. 5)}$$

The relative ^{13}C allocation to different pools and fluxes was then calculated as for the trees labelled before the rainfall event.

Supplementary figures and tables:

Supplementary figures

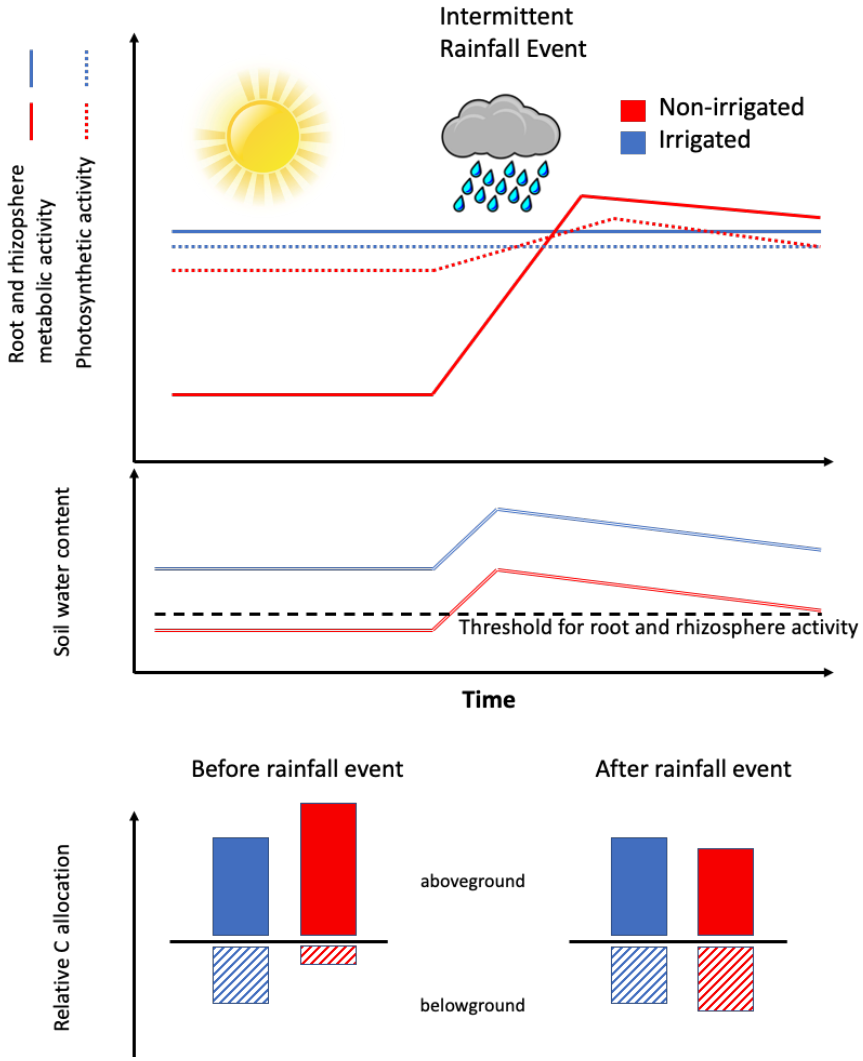

Figure S1. Hypothetical trajectories of belowground sink and photosynthetic activity and the consequences for the distribution of new assimilates in forest plots with different long- and short-term soil water availability. Trees exposed to chronic long-term drought show considerably reduced root (i.e. sink) activity compared with (irrigated) trees with no long-term water limitation, whereas photosynthetic source activity is less impacted by drought. Assuming sink control of carbon allocation, this unequal effect on source and sink activity leads to less carbon from new assimilates being transported belowground and more of carbon being stored, incorporated and respired in or close to the source tissues. An intermittent rainfall event that increases the soil water content in the uppermost soil layer above a certain threshold value in normally drought exposed trees may release the drought-induced repression of rhizosphere activity and result in an increased transport belowground to meet the increased C demand, thereby altering whole-tree C allocation patterns.

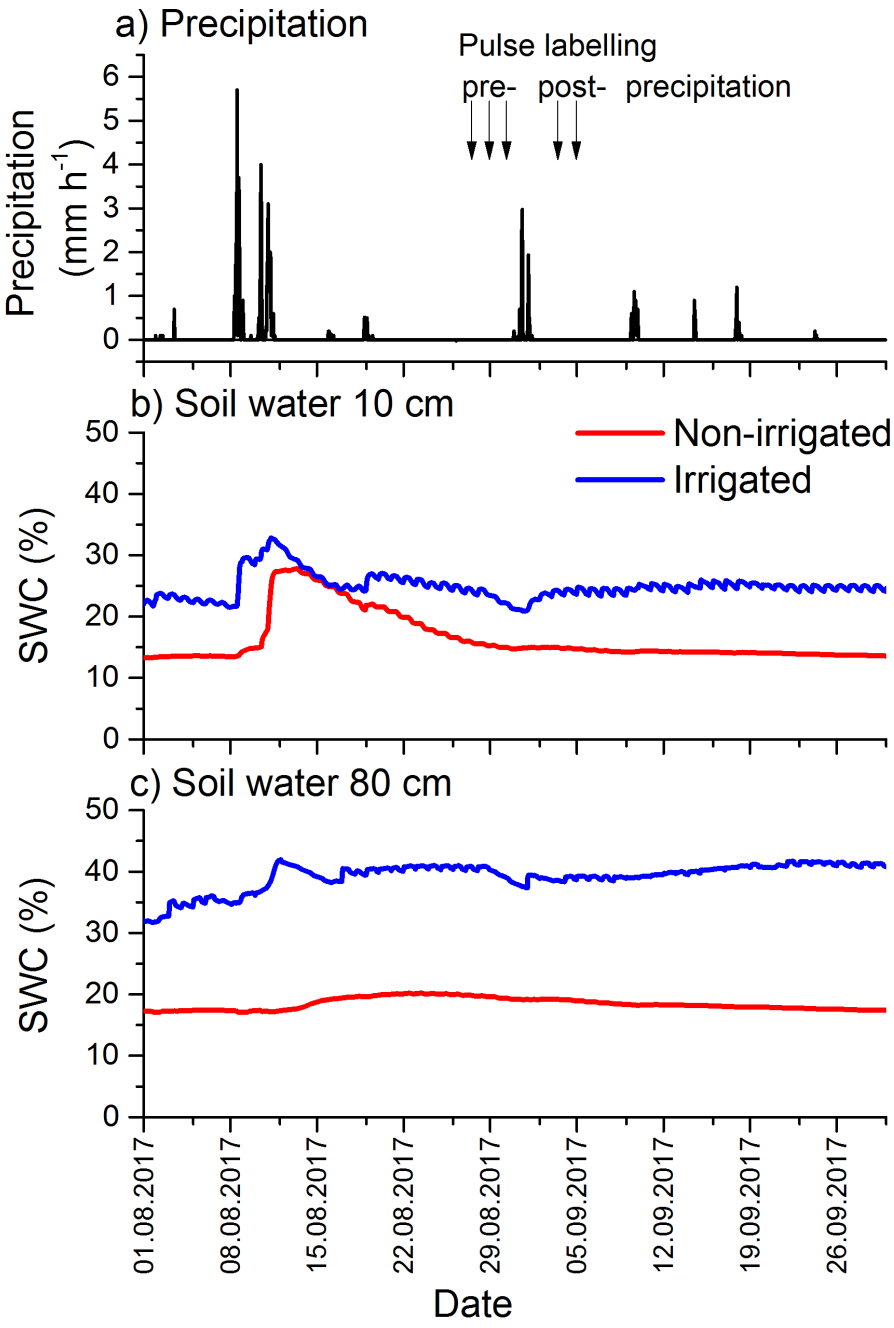

Figure S2: Precipitation and soil water content (SWC) data in the non-irrigated and irrigated plots before and after the rainfall event. (a) shows precipitation (mm h^{-1}) recorded in the experimental area during the field campaign. (b), and (c) show volumetric soil water content (SWC) measured at 10 and 80 cm soil depth, respectively (average values; n = 6). Arrows in (a) show the timing of pulse labelling conducted before and after the rainfall event.

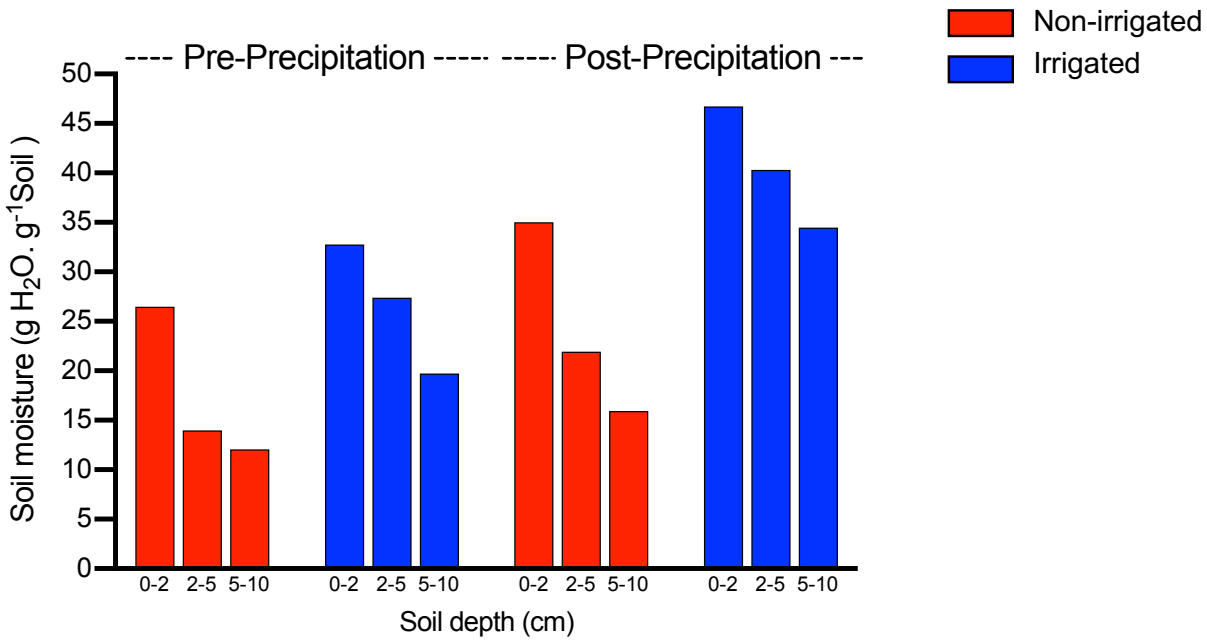

Figure S3: Soil moisture availability in the upper soil (0-2, 2-5, 5-10 cm) in the dry and irrigated plots in Pfynwald. Mean values are shown for soil water content at various depths (0–2, 2–5, 5–10 cm) in the upper soil layers before and after rainfall event (when the pulse labelling was performed). Both the irrigation treatment (non-irrigated vs. irrigated) and the rainfall event (pre-precipitation vs. post-precipitation) significantly affected soil water availability in the upper 0–5 cm (see **Tab. 1**).

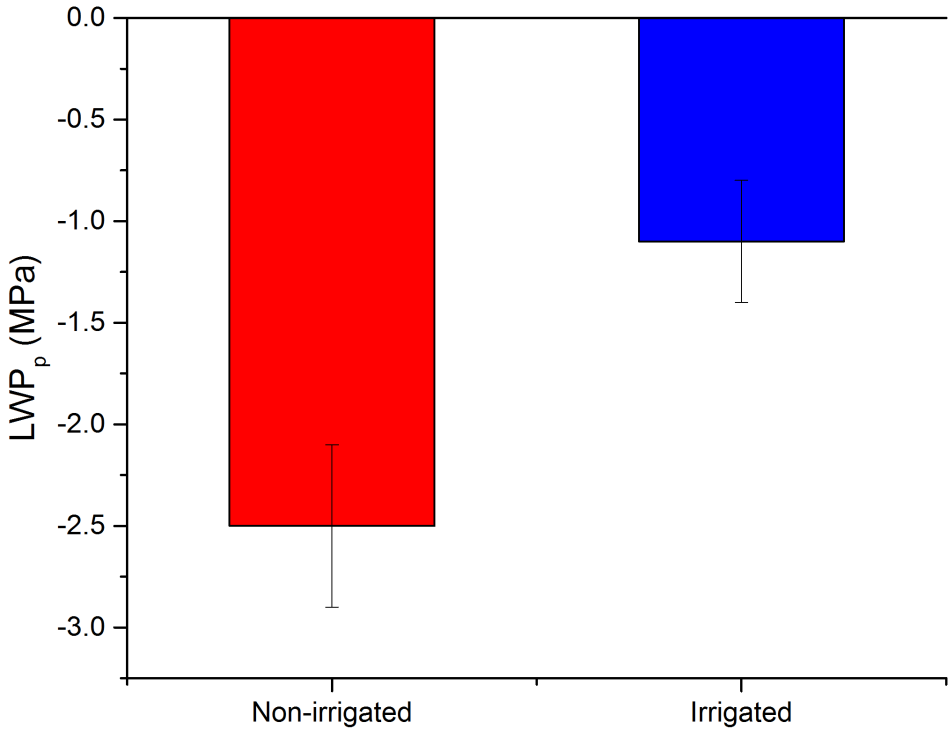

Figure S4: Predawn leaf water potential (LWP_p) of Scots pine trees in Pfynwald before the rainfall event on 29 August 2017. The blue bar represents the irrigated trees and the red bar represent non-irrigated trees. Error bars = ±1 SD (n = 6). The irrigation treatment significantly ($P < 0.01$) affected LWP_p (for detailed statistics see **Tab. 1**).

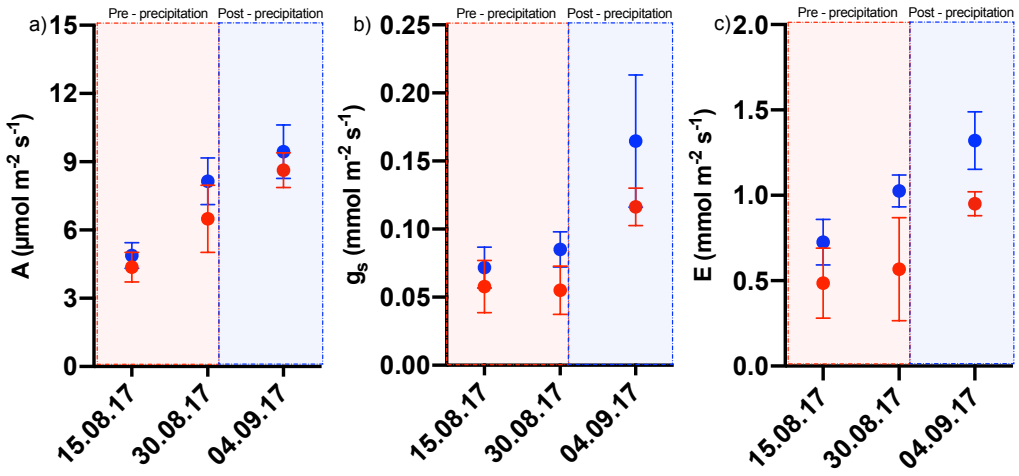

Figure S5: Gas exchange data for Scots pine trees in Pfynwald. Blue circles represent irrigated and red circles represent non-irrigated plots. (a) Rate of photosynthesis at light saturation (A), (b) stomatal conductance (g_s), and (c) rate of transpiration (E). Error bars = ±1 SD (n = 4). The light red rectangular areas show gas exchange data obtained during the pre-precipitation period and the light blue rectangular areas show data collected after the rainfall event. There was no significant effect of irrigation treatment or the rainfall event on A_{sat}, g_s or E (for detailed statistics see **Tab. 1**).

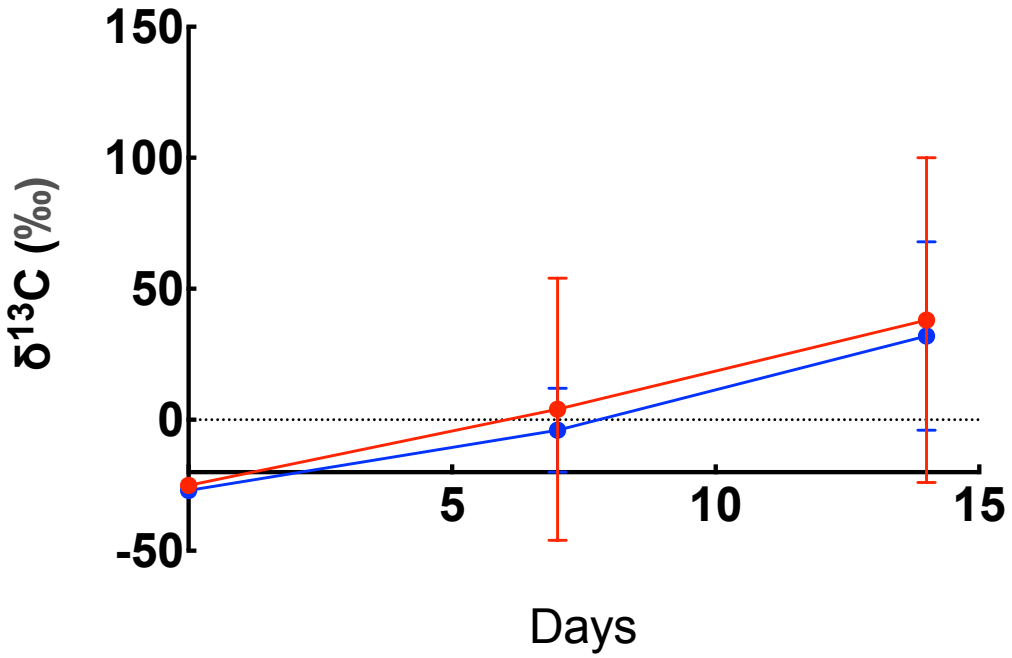

Figure S6: $\delta^{13}C$ values (‰) of ectomycorrhizal root tips in the non-irrigated (red) and irrigated plots (blue) pulse labelled before the rainfall event. The figure shows the increase in $\delta^{13}C$, and thus the incorporation of new labelled assimilates into ectomycorrhizal root tips, over time after pulse labelling. Error bars = ±1 SD.

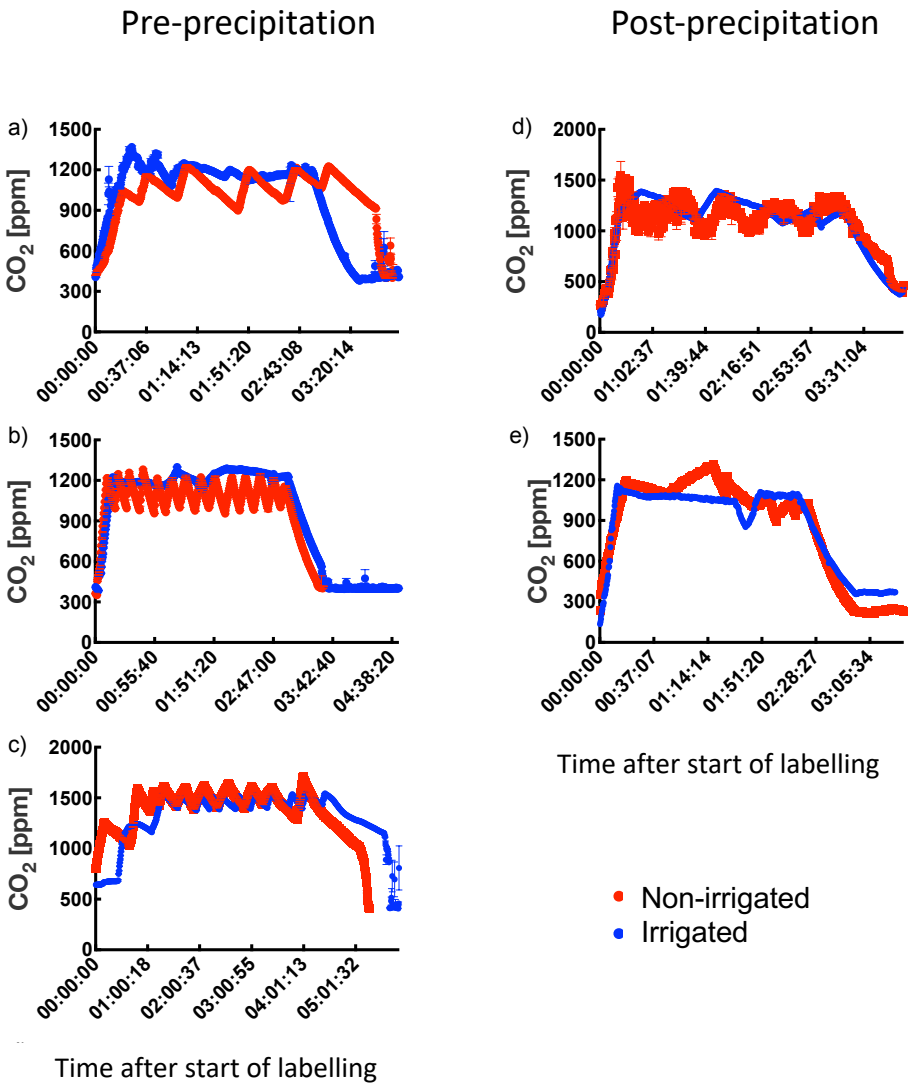

Figure S7: CO_2 concentration inside the pulse labelling chamber during 99(%) ^{13}C-CO_2 pulse labelling in the non-irrigated and irrigated plots. CO_2 concentrations measured inside the chamber during pulse labelling before (a–c) and after (d, e) the rainfall event are shown.

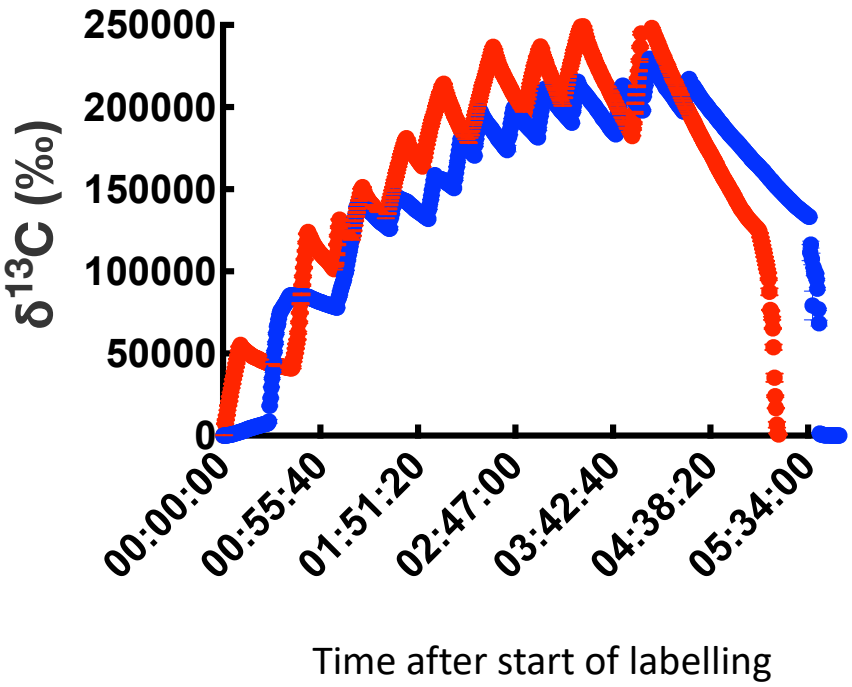

Figure S8: $\delta^{13}C$ (‰) values observed inside the pulse labelling chambers during ^{13}C-CO_2 pulse labelling in the non-irrigated (red) and irrigated plots (blue).

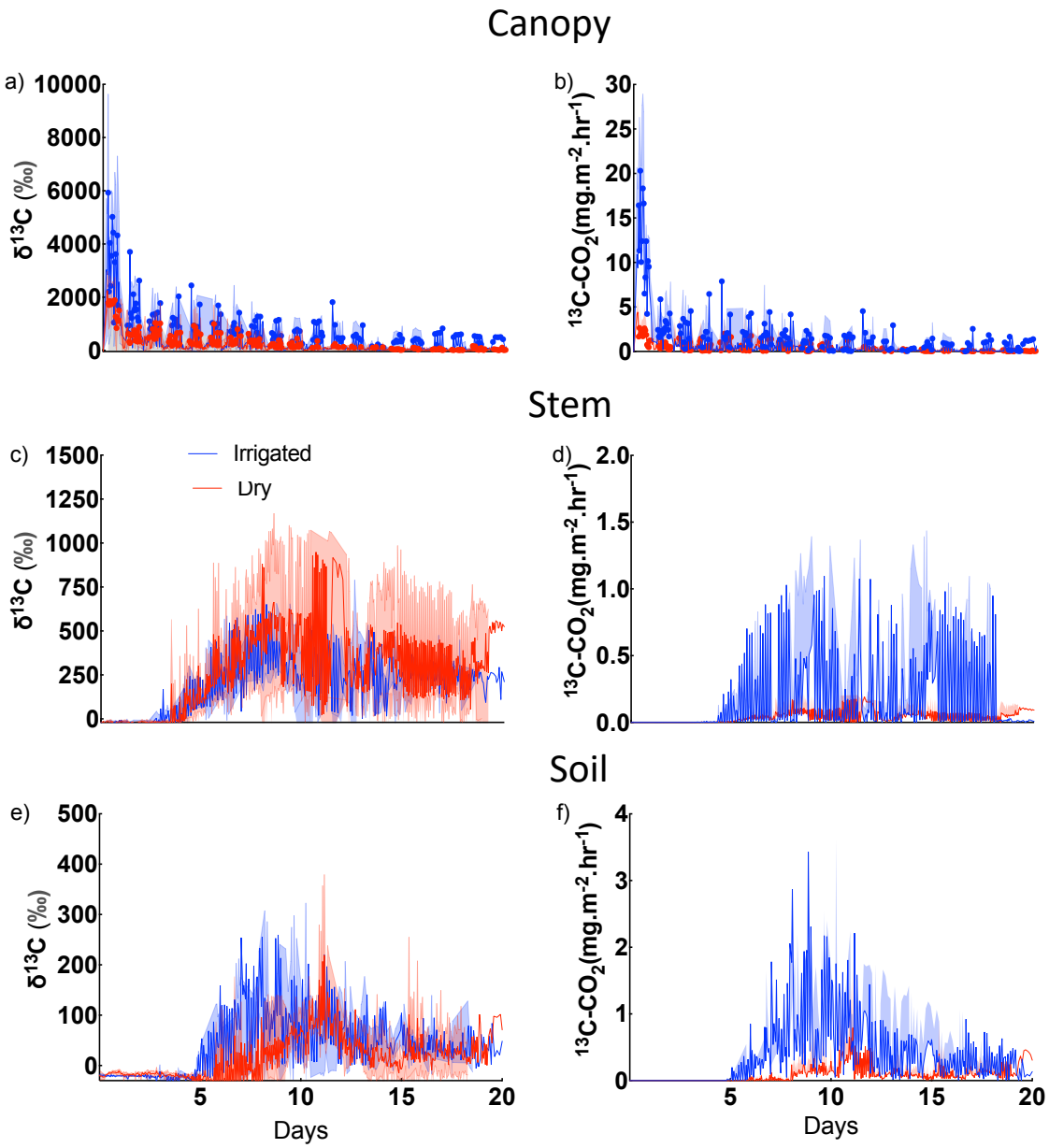

Figure S9: $\delta^{13}C$ (‰) of respired CO_2 and ^{13}C-CO_2 respiratory flux from the tree canopy and stem in the non-irrigated (red) and irrigated plots (blue) in the pulse labelling experiment before the rainfall event, measured using laser spectrometers. The time course after labelling is shown for the $\delta^{13}C$ (‰) of respired CO_2 measured in needles and branches (canopy; a), stems (c) and soil (e), and for ^{13}C-CO_2 fluxes in the canopy (b), stems (d) and soil (f). Lines represent average values (n = 3).

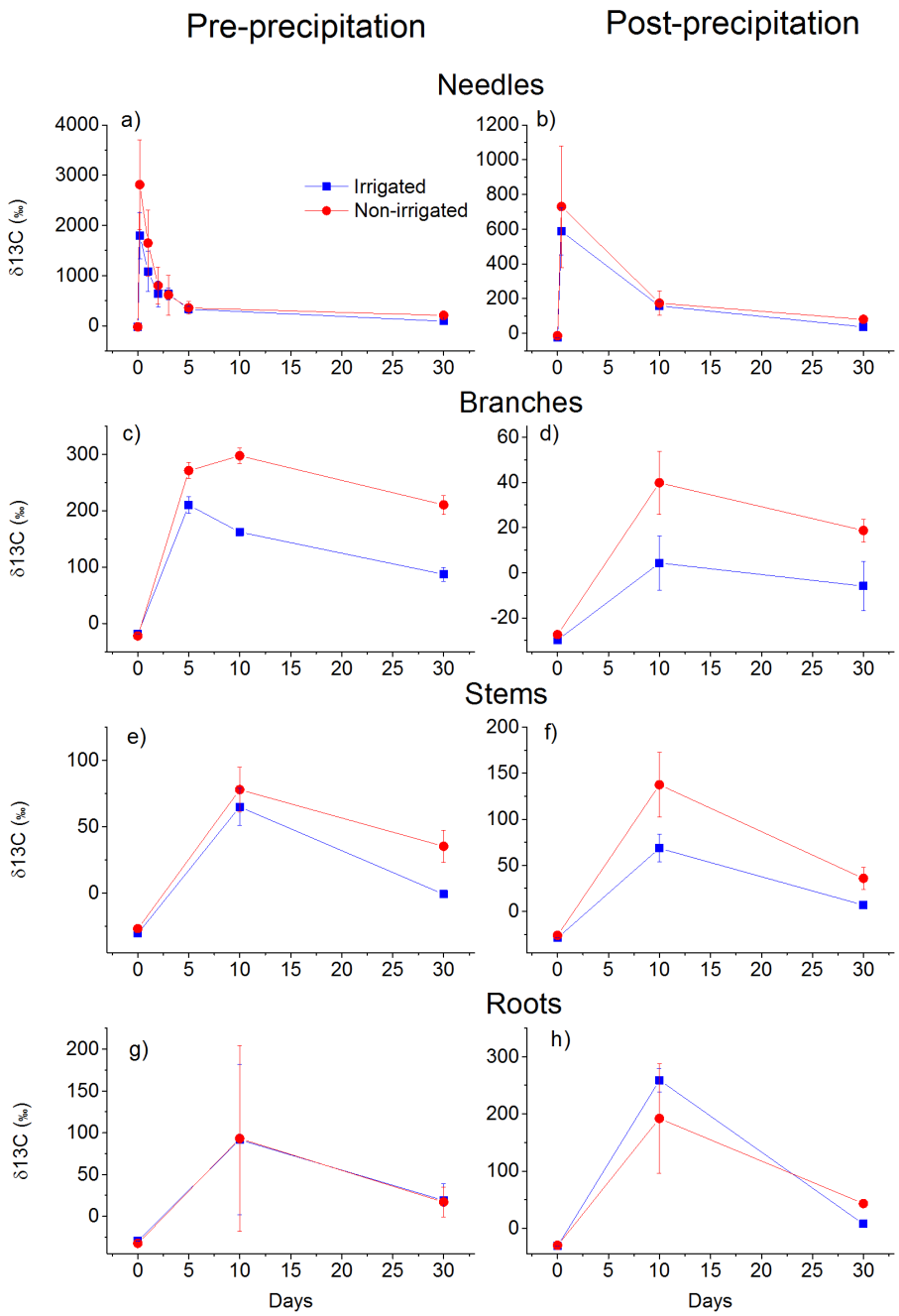

Figure S10: $\delta^{13}C$ (‰) of water-soluble carbohydrates (WSC) in various plant tissues in the non-irrigated and irrigated plots. WSC $\delta^{13}C$ (‰) values of needle (a), branch (c), stem (e) and root samples (g) from trees pulse labelled during the pre-rainfall period, and WSC $\delta^{13}C$ (‰) values of needle (b), branch (d), stem (f) and root samples (h) from trees pulse labelled after the rainfall. Values at day 0 depict the natural ^{13}C abundance before labelling.

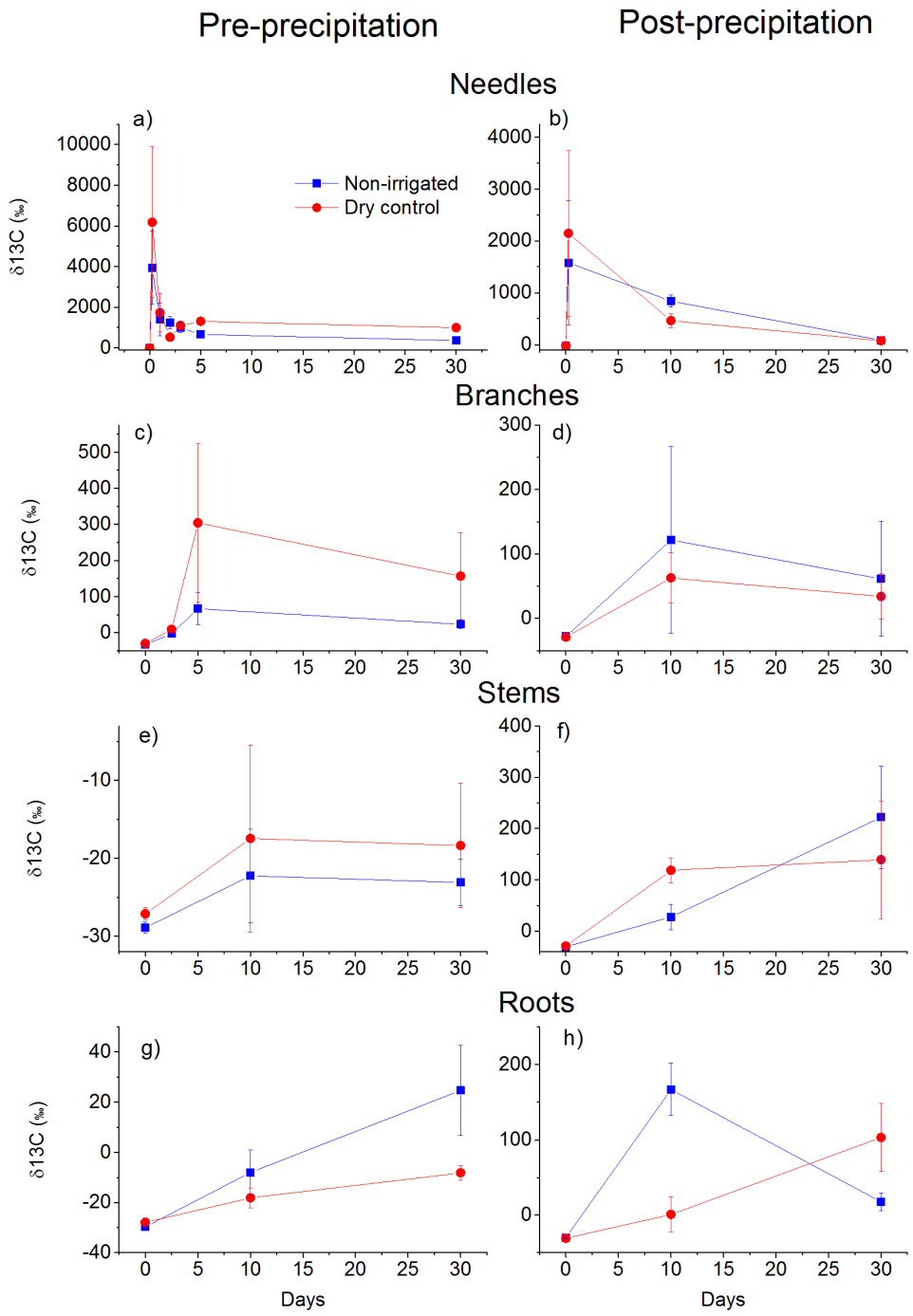

Figure S11: $\delta^{13}C$ (‰) of starch in various plant tissues in the non-irrigated and irrigated plots. Starch $\delta^{13}C$ (‰) values of needle (a), branch (c), stem (e) and root samples (g) from trees pulse labelled during the pre-rainfall period, and starch $\delta^{13}C$ (‰) values of needle (b), branch (d), stem (f) and root samples (h) from trees pulse labelled after the rainfall. Values at day 0 depict the natural ^{13}C abundance before labelling.

Supplementary tables

Total ^{13}C assimilation (g^{13} C per tree)			
1st pulse labelling (before rainfall)		**2nd pulse labelling (after rainfall)**	
Non-irrigated	**Irrigated**	**Non-irrigated**	**Irrigated**
7.9	9.6	11.0	13.4
6.7	11.7	12.6	13.3
10.0	8.5		

Table S1: Total ^{13}C assimilated ($^{13}C_{total\ uptake}$) by trees during pulse labelling conducted either before or after the rainfall event in Pfynwald. The rainfall event, but not the treatment (irrigated vs. non-irrigated), significantly affected ^{13}C uptake (for detailed statistics see **Tab. 1**).

Pool /Flux	Total ^{13}C allocation (%)					
	Pre-precipitation		Post-precipitation		Significance	
	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Irrigation	Irr x Precip
<u>Aboveground</u>						
AG respiration	55.3 ± 8.4	49.2±13.1	38.8 ± 1.0	46.3 ± 3.6	n.s.	n.s.
Canopy biomass (NSC + SC)	8.4 ± 3.3	7.0 ± 1.6	3.0 ± 0.3	2.3 ± 1.2	n.s.	n.s.
NSC	*0.6 ± 0.3*	*0.6 ± 0.1*	*0.3 ± 0.1*	*0.3 ± 0.1*	n.s.	n.s.
SC	*7.8 ± 3.3*	*6.4 ± 1.6*	*2.7 ± 0.3*	*2.0 ± 1.2*	n.s.	n.s.
Stem biomass (NSC+ SC)	19.1± 4.4	7.3 ± 1.8	13.8 ± 1.2	14.9 ± 0.4	n.s.	n.s.
NSC	*4.0 ± 1.1*	*1.0 ± 0.4*	*4.1 ± 0.7*	*4.3 ± 0.2*	n.s.	n.s.
SC	*15.1 ± 4.2*	*6.3 ± 1.8*	*9.7 ± 1.1*	*10.6 ± 0.3*	n.s.	n.s.
<u>Belowground</u>						
Soil respiration	10.7 ± 1.2	25.9 ± 5.4	16.2 ± 0.4	19.1 ± 0.9	0.009	0.04
Root biomass (NSC + SC)	5.6 ± 1.8	9.3 ± 1.6	25.6 ± 2.3	16.7 ± 5.2	n.s.	n.s.
NSC	*0.5 ± 0.2*	*3.7 ± 0.9*	*5.2 ± 0.5*	*5.1 ± 1.3*	n.s.	n.s.
SC	*5.1 ± 1.8*	*5.6 ± 1.3*	*21.4 ± 2.2*	*11.6 ± 5.0*	n.s.	n.s.
Microbial biomass	0.9 ± 0.3	1.2 ± 0.4	2.7 ± 0.2	0.6 ± 0.3	n.s.	n.s.
Total belowground	*17.2 ± 2.2*	*36.4 ± 10.8*	*42.9 ± 2.3*	*36.4 ± 5.3*	0.01	0.006

Table S2. ^{13}C allocation budget in the plant–soil continuum before and after the rainfall event. ^{13}C allocation (%) to different carbon pools and fluxes in the tree and the soil 30 days after $^{13}CO_2$ pulse labelling. The data shown are from trees in non-irrigated and irrigated plots exposed to $^{13}CO_2$ pulse labelling either before or after a rainfall event. (AG = aboveground, Canopy = needles + branches, NSC = non-structural carbohydrates, SC = structural carbohydrates). NSC and SC are in italics and sum up to canopy, stem and root biomass values in bold. For the pulse labelling experiment after the rainfall event, canopy and stem respiration were not measured and therefore the AG respiration was estimated as described in the Supplementary Methods section. P values from linear mixed effect models are shown in the last two columns. Allocation to soil respiration and to the total belowground pools and fluxes was significantly affected by the irrigation treatment and the interaction between the irrigation treatment and the rainfall event (Irr x Precip).

	Non-irrigated			Irrigated		
	Canopy	Stem	Soil	Canopy	Stem	Soil
a0	1488	509	76	2740	350	109
K1	0.26	0.08	0.18	1.36	0.05	0.11
a2	57	5	0	837	1	0
K2	0.104	0.004	0.002	0.104	0.008	0.005
MRT1 (days)	3.8	13.1	5.6	0.7	18.9	9.1
MRT2 (days)	9.6	250	500	9.6	125	200
Coefficient of determination (r^2)	0.991	0.873	0.723	0.991	0.698	0.980

Table S3: Parameter values of the exponential two-pool model used for calculating mean residence time (MRT) of recently assimilated ^{13}C in the canopy, stem and soil in non-irrigated and irrigated plots. The exponential model (equation: ^{13}C enrichment = (a0*(exp(-K1*t))) + (a1*(exp(-K2*t))) was fitted to the temporal course (t: time in days) of carbon isotope enrichment in respired CO_2 after reaching peak values. We assumed that the ^{13}C was incorporated into two labile C pools with different MRTs, i.e. a pool dominated by soluble sugars and a pool dominated by starch. a0 and a1 are the initial quantities of ^{13}C in the respective pools; K1 and K2 are the decay constants; MRT was calculated as 1/K.

Treatment	Flux source	a0	K	**Coefficient of determination (r^2)**
Non-irrigated	Canopy (Tree 1)	921.98	6.22	0.92
	Canopy (Tree 2)	2797.52	0.26	0.99
	Canopy (Tree 3)	343.88	1.60	0.97
	Stem (Tree 1)	6.37	6.64	0.94
	Stem (Tree 2)	3.48	3.90	0.93
	Stem (Tree 3)	96.60	8.30	0.99
Irrigated	Canopy (Tree 4)	467.16	4.59	0.92
	Canopy (Tree 5)	536.14	8.91	0.89
	Canopy (Tree 6)	243.73	0.65	0.97
	Stem (Tree 4)	26.99	27.89	0.91
	Stem (Tree 5)	1.39	6.25	0.92
	Stem (Tree 6)	4.47	14.15	0.92

Table S4: Parameter values of the exponential model used to extrapolate canopy- and stem-level ^{13}C-CO_2 respiration fluxes (Fig. S9 b, d) in non-irrigated and irrigated plots before the rainfall event for ^{13}C mass balance estimation. Equation used: $^{13}C_{respired}$ = a0*(exp(-(time)/K)). Trees 1 and 4, 2 and 5, and 3 and 6 were the pairs labelled on the same day.

Supplementary References

1. Lehmann MM*, et al.* (2015) Malate as a key carbon source of leaf dark-respired CO2 across different environmental conditions in potato plants. *Journal Of Experimental Botany* 66(19):5769-5781.
2. Ruehr NK*, et al.* (2009) Drought effects on allocation of recent carbon: from beech leaves to soil CO2 efflux. *New Phytol* 184(4):950-961.
3. Cermák J, Riguzzi F, & Ceulemans R (1998) Scaling up from the individual tree to the stand level in Scots pine. I. Needle distribution, overall crown and root geometry. *Ann. For. Sci.* 55(1-2):63-88.