## nature plants

# SUPPLEMENTARY INFORMATION

**[DOI: 10.1038/NPLANTS](http://dx.doi.org/10.1038/nplants.2016.111).2016.111**



**Supplementary Figure 1: Field-based open top chamber/lysimeter with beech model ecosystems during the <sup>13</sup>C pulse labelling.** The chambers have a height of 3.5 m and a plantable area of 3 m<sup>2</sup> for each of two lysimeters inside the chamber (only one lysimeter used for the experiments). The beech trees were up to 2.5 m tall at the time of the experiments.



**Supplementary Figure 2: Drought development in the model ecosystem and in the pot experiment. a,** soil moisture in the model ecosystems measured volumetrically with soil moisture probes (5TM, Decagon, USA) at 10 cm soil depth in drought and re-watered ecosystems compared with the regularly watered controls (means ± SE, n = 8). Soil moisture data are taken from<sup>39</sup>. **b**, soil moisture measured gravimetrically in the pot experiment (means  $\pm$  SE, n = 5).

39 Arend, M., Sever, K., Pflug, E., Gessler, A. & Schaub, M. Seasonal photosynthetic response of European beech to severe summer drought: Limitation, recovery and post-drought stimulation. *Agr. Forest Meteorol.* **220,** 83-89 (2016).



**Supplementary Figure 3: High resolution measurements of soil**  $^{13}CO_2$ **. The soil CO<sub>2</sub>**  $\delta^{13}C$  **signature at** 10 cm soil depth was measured continuously *in situ* at a frequency of 1Hz CO<sub>2</sub> in soil air using an Off-Axis Integrated Cavity Output Spectrometer OA-ICOS (LGR-CCIA 36-d, LosGatos Research Ltd). Data were recorded during the two  $^{13}CO_2$ -pulse labelling experiments at **a**, the end of the drought period and **b**, after drought release (means  $\pm$  SE, n = 3 for dry season and n = 4 for wet season). Soil gas was drawn into the OA-ICOS from gas permeable and hydrophobic membrane tubes (Accurel® tubings, 8mm OD and 40 cm length) placed horizontally in the soil at 10 cm depth. The mean residence time of  $^{13}$ C was calculated as previously described<sup>11</sup>. The high resolution measurements show that the sealing of the labelling tent against the soil with plastic foil was effective, as no or only very small initial  $^{13}$ CO<sub>2</sub> peaks, which would indicate diffusion of  $^{13}$ CO<sub>2</sub> directly into the soil air, were observed.



weeks before/after re-watering

**Supplementary Figure 4: Quantity/quality of fine roots before and after re-watering.** Fine roots were collected from soil cores taken in the upper soil layer (0-10 cm) of drought/re-watered and control model ecosystems. **a,** the mass of fine roots per soil volume; **b,** the frequency of vital mycorrhizal root tips per soil volume; and **c,** the number of vital mycorrhizal root tips per fine root mass. Data analysis yielded no statistically significant differences between treatments (means ± SE, n = 4-8 lysimeters).

**Supplementary Table 1: Concentrations of main carbohydrates in leaves under drought and after drought release.** Sugars, starch and non-structural carbohydrates (sum of soluble sugars and starch) in beech leaves were analysed as described in<sup>40</sup> (% leaf dry weight; mean  $\pm$  SE, n = 8).



40 Li, M.-H., *et al.* Responses of leaf nitrogen and mobile carbohydrates in different *Quercus* species/provenances to moderate climate changes. *Plant Biol.* **15**, 177-184 (2013).

**Supplementary Table 2: Physical and chemical characteristics of the acidic soil type.** Data are taken from a previous study<sup>38</sup> (with permission of the publisher Wiley-Blackwell).

41 Kuster, T.M., Arend, M., Bleuler, P., Günthardt-Goerg, M.S., Schulin, R. Water regime and growth of young oak stands subjected to air warming and drought on two different forest soils in a model ecosystem experiment. *Plant Biol.* **15**, 138-147 (2013).



**Supplementary Table 3: Statistical analysis of seasonal flux and 13C pulse labelling data.** The applied linear mixed effects models were adapted to the experimental design and data structure of each measurement.



(a) repeated measurements, all data from dry season (drought *vs*. control) and/or wet season (postdrought *vs*. control) after full recovery

- (b) initial plant uptake after  $^{13}$ C pulse labelling
- (c) repeated measurements during  $^{13}$ C pulse labelling
- (d) total fluxes integrated during  $^{13}$ C pulse labelling
- (e) time period after re-watering with significant differences between control and treatment (Student's t-test with *P* < 0.05 after FDR correction for multiple comparisons)

**Supplementary Table 4: Allocation of C to tree growth in the drought-treated and control model ecosystems.** The amount of carbon allocated to tree growth (g C per tree) was derived from measurements of the stem diameter (10 cm above ground) before the start of the dry season, end of the dry season and end of the wet season (means  $\pm$  SE, n = 8). The corresponding tree biomass was estimated using a biomass/diameter correlation with the equation *y = -0.036x<sup>3</sup> +2.695x<sup>2</sup> -* 28.114x+102.61 and then converted to C using the molar ratio of 12/30 for C/CH<sub>2</sub>OH<sup>10</sup>.

10 Klein, T. & Hoch, G. Tree carbon allocation dynamics determined using a carbon mass balance approach. *New Phytol*. **205,** 147-159 (2015).



#### **Supplementary Methods:**

#### **Design of the model ecosystem and pot experiment**

The model ecosystem experiment was conducted in the model ecosystem facility of the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). The facility consists of 16 large model ecosystems in field-based open top chambers, each with a height of 3.5 m. The systems are equipped with automated irrigation systems and sliding roofs closing automatically at the onset of rainfall. Below ground, each system is split into two lysimeters with an area of 3  $m<sup>2</sup>$  and a depth of 150 cm, one filled with an acidic soil and one filled with a calcareous forest soil (calcareous soil not considered for this experiment). In spring 2011, 24 saplings with a height of about 20 cm were transplanted to each lysimeter. From May to October, natural precipitation was excluded and the systems were irrigated every second or third day with 67 l  $m<sup>2</sup>$  deionized water enriched with nutrients to simulate the average composition of ambient rainfall. During hot summer periods, the irrigation frequency was increased to counterbalance higher rates of evapotranspiration and hold the soil moisture at 10 cm soil depth above 20%. In 2014, when the trees had reached a height of up to 2.5 m, a summer drought was implemented in half of the systems by withholding irrigation from 22 May to 1 August. As evapotranspirational water loss was particularly high on hot days, a few intermediate irrigation pulses were applied to prevent the soil from drying too rapidly or intensely and to avoid irreversible drought damage of the trees. The water supply during the drought period was reduced by 78% compared to controls. After the first saplings reached predawn water potentials below -2.0 MPa, the systems were re-watered for 1 day with 200  $\text{I}$  m<sup>-2</sup> and afterwards regularly irrigated as described above.

The pot experiment was carried out with 2-year-old beech saplings in 5.5 l pots (one plant per pot). The trees were on average 56 cm tall and average total dry weight at the time of harvest was 25 g. The seeds originated from the Black Forest growth region in SW-Germany, which is close to the origin of the beech trees in the model ecosystem experiment. Plants were grown in a greenhouse with temperatures of 20°C/17°C (day/night). The photosynthetic photon flux density was kept at 600 µmol  $m^2$  s<sup>-1</sup> or greater at the upper level of the canopy by supplemental illumination and the light period was adjusted to 16 h. During the drought treatment lasting 4 weeks, the control pots were watered to field capacity while pots with the drought treatment received no water at all. Five plants per treatment were harvested before drought and 1, 2, 3 and 4 weeks after the onset of drought. Fine root, leaf and phloem samples were taken.

### **Calculations of 13C fluxes with the closed chamber method**

The  $\delta^{13}$ C values of soil respired CO<sub>2</sub> ( $\delta^{13}C_{resuired}$ ) were calculated as a mixture of ambient and soilrespired  $CO<sub>2</sub>$  sampled in the chamber:

$$
\delta^{13}C_{respired} = \frac{(\delta^{13}C_{chamber} \times CO_{2-chamber} - \delta^{13}C_{ambient} \times CO_{2-ambient})}{(CO_{2-chamber} - CO_{2-ambient})}
$$
(1)

where  $\delta^{32}C_{chamber}$  and  $\delta^{13}C_{ambient}$  are the measured isotopic ratios of CO<sub>2</sub> in the soil chamber and in ambient air, respectively, and *CO<sub>2-chamber*</sub> and *CO<sub>2-ambient* are the corresponding CO<sub>2</sub>-concentrations.</sub>

The amount of  $^{13}$ C assimilated by plants, in soil microbial biomass and in soil-respired CO<sub>2</sub> during pulse labelling was estimated by first expressing the  $\delta$  notations in atom% as follows:

$$
atom\% = \frac{100 \cdot 0.0111802 \cdot \left(\frac{\delta}{1000} + 1\right)}{1 + 0.0111802 \cdot \left(\frac{\delta}{1000} + 1\right)}\tag{2}
$$

(0.0111802 is the standard value for the isotope ratio of the Vienna Pee Dee Belemnite, V-PDB).

Finally, we calculated excess  $^{13}$ C values using the equation:

$$
\text{excess}^{13}\text{C} = \frac{(\text{atom}\%_{tx} - \text{atom}\%_{t0})}{100} \cdot \text{B}
$$
 (3)

with excess <sup>13</sup>C being the total amount of <sup>13</sup>C in each plant compartment, in microbial biomass (in mg ) or in soil CO<sub>2</sub> efflux (mg <sup>13</sup>CO<sub>2</sub>-C m<sup>-2</sup>h<sup>-1</sup>) originating from the pulse-labelling; atom%<sub>tx</sub> is the atom% of the sample taken at time x; atom%t0 is the atom% in each chamber before the labelling; B is the pool size (g C m<sup>-2</sup>) or the CO<sub>2</sub> efflux (mg CO<sub>2</sub>-C m<sup>-2</sup>h<sup>-1</sup>).