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# Recovery of trees from drought depends on belowground sink control

Frank Hagedorn[1†], Jobin Joseph[1,2†], Martina Peter[1], Jörg Luster[1], Karin Pritsch[3], Uwe Geppert[3], Rene Kerner[3], Virginie Molinier[1], Simon Egli[1], Marcus Schaub[1], Jian-Feng Liu[4], Maihe Li[1], Krunoslav Sever[5], Markus Weiler[2], Rolf T. W. Siegwolf[6], Arthur Gessler[1,7,8†] and Matthias Arend[1,9†*]

**Climate projections predict higher precipitation variability with more frequent dry extremes[1]. $CO_2$ assimilation of forests decreases during drought, either by stomatal closure[2] or by direct environmental control of sink tissue activities[3]. Ultimately, drought effects on forests depend on the ability of forests to recover, but the mechanisms controlling ecosystem resilience are uncertain[4]. Here, we have investigated the effects of drought and drought release on the carbon balances in beech trees by combining $CO_2$ flux measurements, metabolomics and $^{13}CO_2$ pulse labelling. During drought, net photosynthesis ($A_N$), soil respiration ($R_S$) and the allocation of recent assimilates below ground were reduced. Carbohydrates accumulated in metabolically resting roots but not in leaves, indicating sink control of the tree carbon balance. After drought release, $R_S$ recovered faster than $A_N$ and $CO_2$ fluxes exceeded those in continuously watered trees for months. This stimulation was related to greater assimilate allocation to and metabolization in the rhizosphere. These findings show that trees prioritize the investment of assimilates below ground, probably to regain root functions after drought. We propose that root restoration plays a key role in ecosystem resilience to drought, in that the increased sink activity controls the recovery of carbon balances.**

Forests play a crucial role in the global carbon cycle because they hold a large fraction of the global carbon stock and act as a major sink for atmospheric $CO_2$ (ref. 5). However, drought reduces primary productivity, thereby turning forests from carbon sinks into carbon sources[6]. It has generally been assumed that plant and ecosystem carbon balances under drought are controlled by restricted photosynthetic source activity rather than by changes in the sink activity of plant tissues[7,8]. Recently, direct environmental control of sink activity with feedbacks to $CO_2$ assimilation has been proposed[9], but no unequivocal evidence has been obtained yet. Of similar importance, but even less understood, are the mechanisms controlling plant and ecosystem carbon balances after drought release, although the ability of plants to restore $CO_2$ assimilation and other functions determines the resilience of trees and forest ecosystems. Further, limited knowledge on the principles that control carbon allocation in trees prevents us from predicting carbon balances of forests under future environmental conditions characterized by greater variability of precipitation and thus alternating drought and recovery periods.

Using two experimental setups, growing either in model ecosystems in open-top chambers (Supplementary Fig. 1) or in pots, we studied tree and ecosystem carbon fluxes during drought and after drought release. By combining measurements of net photosynthesis ($A_N$) and soil respiration ($R_S$) as indicators of source and sink activity, respectively, with $^{13}CO_2$ pulse labelling and metabolomic analyses, we followed seasonal carbon dynamics and tracked assimilate transport through the plant–soil system. Based on a hypothetical framework (Fig. 1), we aimed to test if changes in $A_N$ and $R_S$, as well as shifts in carbohydrate allocation, indicate source or sink control of carbon balances. If source activity controls carbon balances under drought, we expected an initial decrease in $A_N$ and leaf carbohydrate concentrations and a delayed depletion of carbohydrates in roots, leading to a reduction in $R_S$ (Fig. 1a). A similar response would occur on drought release, with an initial recovery of $A_N$ and leaf carbohydrate concentrations followed by a delayed increase of $R_S$ (Fig. 1b). If, however, the carbon balance is sink controlled, drought would directly reduce $R_S$, leading to an accumulation of carbohydrates in roots because of reduced carbon demand. In this case, $A_N$ would acclimate to the reduced sink demand after a delay and leaf carbohydrate concentrations consequently would not change (Fig. 1c). On drought release, $R_S$ would increase and, with a delay, the increased belowground carbon demand would positively feed back on $A_N$ (Fig. 1d).

In our model ecosystems, $A_N$ and $R_S$ decreased by 44% and 28%, respectively, throughout the entire drought season (Fig. 2a–c and Supplementary Fig. 2a). At the end of the drought, a $^{13}CO_2$ pulse label was applied to the canopies to trace the fate of recent assimilates in the plant–soil system. Under drought, the uptake of $^{13}CO_2$ decreased by 81% and assimilate translocation to belowground sinks was reduced, as shown by lower $^{13}C$ signals in mycorrhizal roots and soil microbial biomass and reduced $^{13}CO_2$ soil efflux (Fig. 3a,c,e,g). The reduction in $^{13}CO_2$ soil efflux was 83% and thus similar to that in $^{13}CO_2$ uptake. However, the $^{13}CO_2$ soil efflux showed a stronger reduction (83%) than that observed for $R_S$ (≈50%), indicating that other carbon sources, either related to heterotrophic soil respiration or tree internal carbon storages[10],

[1]Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Zürcherstrasse 111, 8903 Birmensdorf, Switzerland. [2]Chair of Hydrology, Faculty of Environment and Natural Resources, University of Freiburg, Fahnenbergplatz, 79098 Freiburg, Germany. [3]Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, German Research Centre for Environmental Health (GmbH), Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany. [4]Research Institute of Forestry, Chinese Academy of Forestry, Xiangshan Road, 100091 Beijing, China. [5]Department of Forest Genetics, Dendrology and Botany, Faculty of Forestry, University of Zagreb, Svetošimunska 25, HR-10000 Zagreb, Croatia. [6]Laboratory of Atmospheric Chemistry, Ecosystem Fluxes Group, Paul Scherrer Institute (PSI), 5232 Villigen, Switzerland. [7]Institute for Landscape Biogeochemistry, Leibnitz Centre for Agricultural Landscape Research (ZALF), Eberswalder Strasse 84, 15374 Müncheberg, Germany. [8]Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstrasse 6, 14195 Berlin, Germany. [9]School of Forest Science and Resource Management, Technical University of Munich, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany. †These authors contributed equally to the work. *e-mail: matthias.arend@wsl.ch



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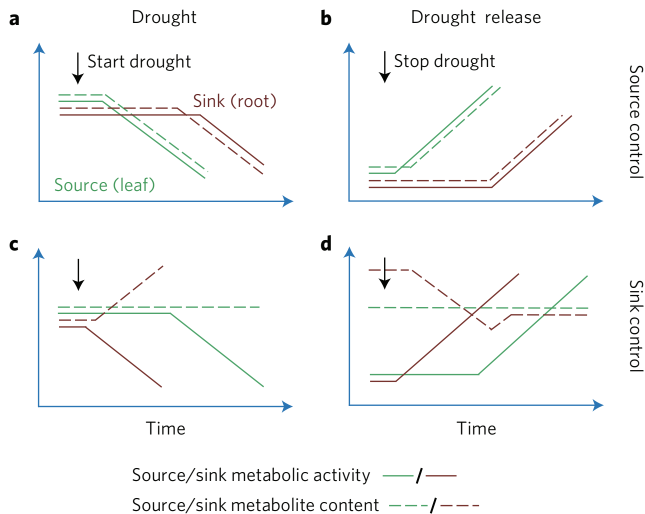


**Figure 1 | Hypothetical trajectories of metabolic activity and metabolite concentration in leaves and roots as a consequence of drought onset and drought release. a–d**, For both drought (**a**,**c**) and drought release (**b**,**d**), the scenarios for full source (**a**,**b**) and full sink (**c**,**d**) control of the tree carbon balance after the change in soil moisture conditions are shown. Effects in source (green, leaves) and sink tissues (brown, root) are provided for each scenario. We refer to net photosynthesis ($A_N$) as a source metabolic activity and to soil respiration ($R_S$) as an integrator of sink metabolic activity in the roots. Metabolite content refers to the most abundant carbohydrates (the NSCs glucose, fructose, sucrose and starch; see also Fig. 4b). Under source control (**a**,**b**), source metabolic activity in leaves ($A_N$) reacts first to changing conditions and induces changes in assimilate (sugar) availability for sinks and thus affects sink metabolic activity in roots ($R_S$). Under sink control (**c**,**d**), sink metabolic activity is directly affected by the environmental conditions, leading to changes in sink metabolite levels. After a delay, source metabolic activity is impacted in response to the altered sink demand.

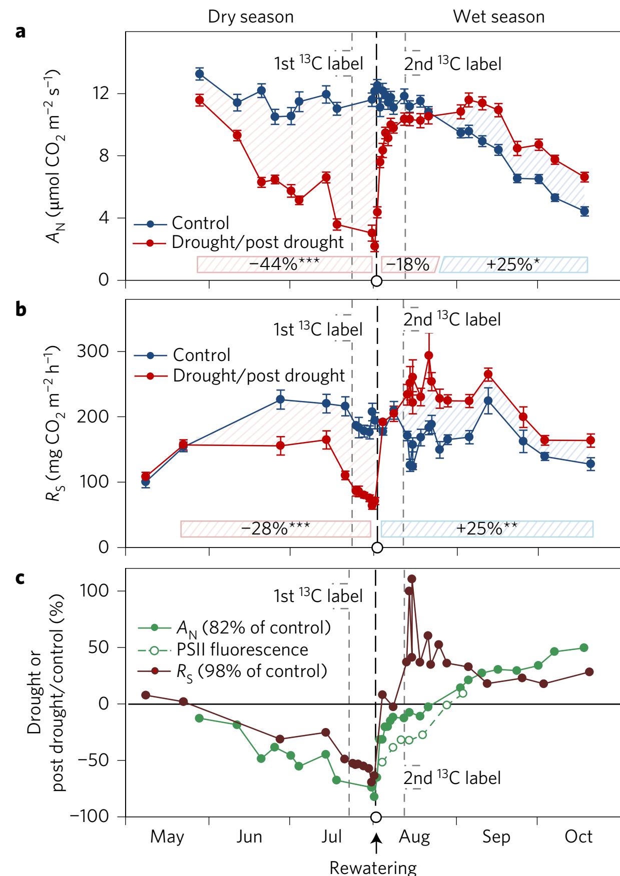


**Figure 2 | Reduction of net photosynthesis ($A_N$) and soil respiration ($R_S$) in the model ecosystem experiment during drought (dry season), and during recovery and stimulation after drought release (wet season).** **a**,**b**, Effects on $A_N$ and $R_S$, respectively, are shown. Numerical values provide quantitative measures of the drought limitation and the stimulation after full recovery (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; means ± s.e.m.; $A_N$, $n = 8$; $R_S$, $n = 3$–8). **c**, The development of drought and post-drought effects on $A_N$ (green) and $R_S$ (brown) are shown, together with the release of metabolic limitation of $A_N$ after rewatering (PSII chlorophyll fluorescence; $n = 8$). Responses are shown as relative deviations from control values.

contributed to soil respiration but were less sensitive to drought. Under drought, the $^{13}C$ peak in continuously monitored soil $CO_2$ (Supplementary Fig. 3a) was delayed by one day and mean residence times (MRTs) of assimilated $^{13}C$ in the plant–soil system increased (drought 76 h, control 30 h), indicating slower assimilate transport to belowground sinks, as previously reported[11]. The reduced and delayed assimilate transport might have been the result of either source limitation or sink control. However, non-structural carbohydrates (NSCs) were not depleted in source leaves in drought-treated model ecosystems (Supplementary Table 1) and thus source limitation was unlikely, as recently proposed[9].

To explore the mechanisms leading to reduced carbon fluxes to belowground sinks, we studied the dynamics of metabolites with progressing drought in a pot experiment. Reductions in soil moisture and $A_N$ in drought-treated pots were similar to those in the model ecosystems (Fig. 4a and Supplementary Fig. 2b). In roots, the NSCs fructose, glucose, sucrose and starch, as well as the osmoprotectant proline, increased under drought, and sucrose accumulated in the release phloem (Fig. 4b). The increase in the concentration of NSCs by up to 700% was very strong but still in the range reported in previous studies with trees[12,13]. In leaves, no NSC increase was observed except for a delayed accumulation of starch and proline as drought progressed. Although NSCs accumulated in roots, sink control of such an increase can only be inferred when the size of carbohydrate pools depends directly on the balance between supply through photosynthesis and demand for growth and respiration[14]. Alternatively, accumulation of NSCs might serve as osmotic adjustment[15], which is not directly related to changes in carbon supply and demand. In our study, however, accumulation of NSCs was only observed in roots, and proline, an indicator of osmotic regulation[16], was enriched in both roots and leaves. It is thus unlikely that osmotic adjustment via an increase in NSC content was achieved only in roots and not in leaves. Instead, active storage of carbohydrates in roots at the expense of metabolic processes might have occurred, as previously suggested[17]. Thus, the strong accumulation of NSCs in roots and the lack thereof in leaves reflect metabolic activity in sink and source tissues. Transferring this information to our model ecosystems indicates that the reduced carbon flux to belowground sinks under drought (Fig. 3c,e,g) was a consequence of decreased sink activity (Fig. 1c). Owing to the rather slow build up of drought over time in our model ecosystems, a clear order of the response of source ($A_N$) versus sink ($R_S$) activities could not be derived directly, especially since changes in $R_S$ might have been only partially because of changes in autotrophic root–rhizosphere respiration.

The recovery of plant and ecosystem carbon fluxes after prolonged drought was examined by rewatering the model ecosystems. $R_S$ responded rapidly, reaching control values within the first 3 days and exceeding thereafter values in controls until the end of the growing season (Fig. 2b,c and Supplementary Table 3). This stimulation of $R_S$ nearly compensated for the previous drought reduction, with the flux integrated over the entire growing season amounting to 98% of that in controls. $A_N$ responded similarly but the recovery



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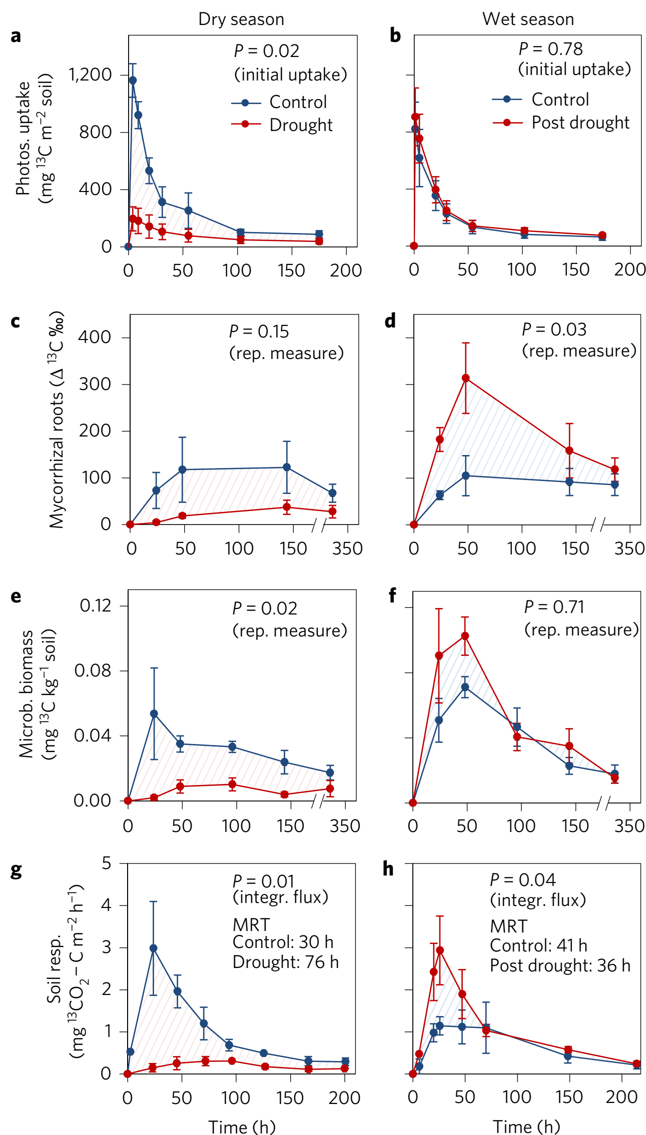


**Figure 3 | Suppressed uptake and allocation of $^{13}$C assimilates in the model ecosystem experiment under drought (dry season) and increased transfer to and metabolization in the belowground compartment after drought release (wet season). a,b**, Leaf photosynthetic uptake of $^{13}$C. **c,d**, Incorporation of $^{13}$C into mycorrhizal root tips. **e,f**, Transfer of $^{13}$C to soil microbial biomass. **g,h**, Respiratory $^{13}$C release from the soil including mean residence times of recent assimilates calculated from the $\delta^{13}$C of continuously measured soil $CO_2$ (Supplementary Fig. 3). $P$ <0.05 indicates statistically significant treatment effects (means ± s.e.m.; $n$ = 3 for dry season and $n$ = 4 for wet season).

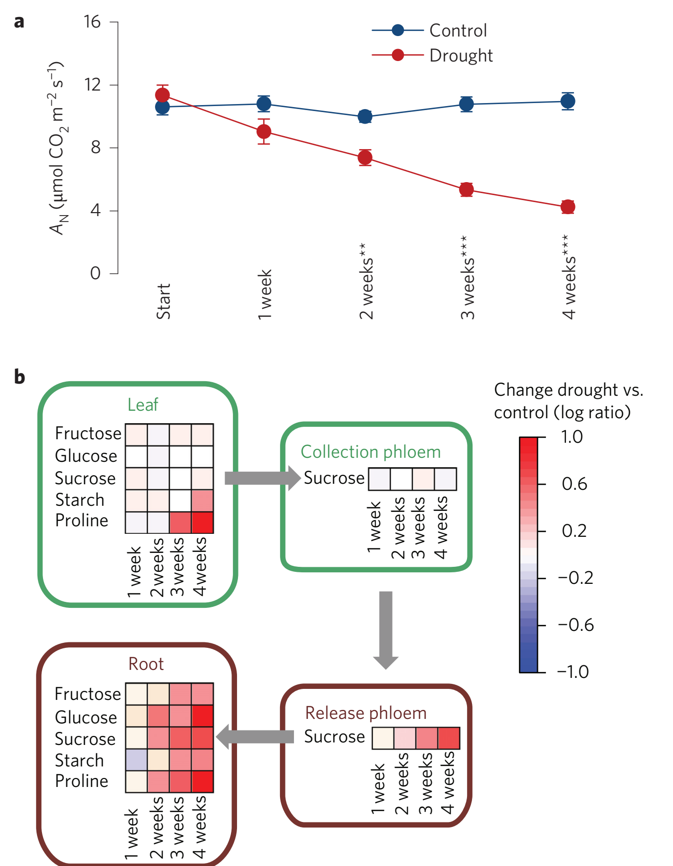


**Figure 4 | Decreased net photosynthesis ($A_N$) in the pot experiment during drought but unchanged metabolite concentrations in leaves and increased concentrations in roots. a**, Changes in $A_N$ during the course of drought development (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001; means ± s.e.m.; $n$ = 5) **b**, Changes in metabolite concentrations. Effects on metabolites are shown as $\log_{10}$ ratio of the drought treatment to the control treatment 1, 2, 3 and 4 weeks after the onset of drought. Analysed metabolites comprise the most abundant carbon compounds, as well as proline as an osmoprotectant, occurring in leaves and roots, and sucrose as the main transport sugar in the collection and release phloem[38]. Data shown are means of five replicates.

was delayed by approx. one week because of metabolic limitation, as shown by impaired PSII photochemistry (Fig. 2a,c and Supplementary Table 3). Further, the stimulation of $A_N$ occurred later and compensated for only 82% of the previous drought reduction. A second $^{13}CO_2$ pulse label was applied to the tree canopies when $R_S$ exceeded the values in controls but $A_N$ was still slightly below that of controls. The previous drought exposure increased the translocation of recent assimilates to belowground sinks compared to controls, as shown by higher $^{13}$C signals in mycorrhizal roots and soil microbial biomass and by an enhanced $^{13}CO_2$ soil efflux (Fig. 3b,d,f,h). The latter signal was enhanced by 50% and thus increased in relation to photosynthetic $^{13}$C uptake, which was not affected. This increase represents exclusively autotrophic respiration, and the comparable increase in $R_S$ (68% $R_S$ vs. 50% $^{13}CO_2$ soil efflux) shows that the plant-driven carbon flux was primarily responsible for the observed stimulation of $R_S$. Heterotrophic soil respiration was small in our model ecosystems containing low soil organic content and can therefore be excluded as the cause of stimulated $R_S$ because it only responds transiently to rewetting of dry soils by the so-called 'Birch Effect'[18,19]. Our results thus clearly show that a drought effect is imprinted on plant source and sink tissues, supporting the concept of an ecological stress memory of which the underlying mechanisms are still poorly understood[20].

The increased carbon demand of belowground sinks resulted in only a slight feedback on the velocity of carbon transport. Whereas the peak time of $^{13}CO_2$ soil efflux was similar, the MRT of $^{13}$C in the plant–soil system was somewhat lower for the previously drought-exposed trees, as calculated from continuously monitored soil $CO_2$ (post drought 36 h, control 41 h; Supplementary Fig. 3b). However, the mass flow of assimilates to belowground sinks can additionally be increased if less carbon is unloaded from the transport pathway for storage or growth in aboveground tree organs[21]. Indeed, the carbon allocation to growth in twigs, stem and roots did not fully recover after rewatering, indicating that growth-related sink activity along the transport pathway was still reduced (Supplementary Table 4). The greater allocation and use of recent assimilates in below ground sinks after rewatering shows



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that trees give high priority to investing into their roots for recovery from drought. The likely reason for this response is the metabolic need for root and mycorrhizal restoration to restore trees' capability to acquire water and nutrients after an extended drought[22,23]. Effects on root growth can be excluded, as demographic root characteristics were not affected during or after drought (Supplementary Fig. 4a–c). Thus, root and mycorrhizal restoration relied mainly on increased metabolic activity, which explains the fast recovery and stimulation of $R_S$. Since $A_N$ showed a delayed recovery and later stimulation than $R_S$, the latter was clearly not source driven and instead reflects the metabolic need for root and mycorrhizal restoration. On the contrary, we postulate that increased belowground sink activity on drought release feeds back on $A_N$, triggering the delayed recovery and stimulation of $CO_2$ assimilation (cf. Fig. 1d). Furthermore, our findings support sink control of the carbon balance under previous drought conditions, as a drought-induced depletion of belowground carbon reserves should delay the recovery of $R_S$ compared to $A_N$ if the recovery is source controlled.

There is increasing evidence that drought not only influences ecosystem carbon balances concurrently but also triggers delayed responses that involve multiple mechanisms operating at different scales of time, plant function and ecosystem organization[4,24]. To date, such mechanisms are poorly understood and thus constitute a large uncertainty in projections of ecosystem carbon balances and resilience. Here, we show that tree carbon fluxes not only recover but even increase after drought to compensate for previous stress impacts. This compensation is sink driven, leading to a greater belowground allocation of recent assimilates on drought release. The observed response has important consequences for ecosystem carbon cycling, as it increases the input of plant-derived labile carbon into soils, thereby fuelling soil microbial communities[25]. We suggest that the ability of trees to reactivate root metabolism is vital for ecosystem resilience to drought. However, the extent of this effect very likely depends on the severity and duration of drought and may vary with tree age, as adult trees have larger carbon storage compartments. Taken together, our findings suggest a resilience mechanism that attenuates drought disturbances of seasonal tree carbon balances and needs to be considered when estimating the impact of climate change on the carbon balances of forest ecosystems.

## Methods

**Plant material and growth conditions.** The model ecosystem experiment was conducted in 16 field-based open-top chambers. In each chamber, a model ecosystem was established with young beech trees (*Fagus sylvatica* L.) growing on lysimeters filled with forest soil of low soil organic carbon content (Supplementary Fig. 1; Supplementary Methods and Supplementary Table 2). A summer drought was simulated by reducing the water supply from 22 May to 1 August by 78%. After the trees had developed the critical water deficit for leaf physiological functioning (predawn water potentials below −2 MPa[26,27]), the lysimeters were intensely rewatered and afterwards regularly irrigated until the end of the vegetation season (Supplementary Fig. 2a).

The pot experiment was carried out with beech saplings (*F. sylvatica* L.) in a greenhouse environment (Supplementary Methods). During the drought treatment lasting 4 weeks, the control pots were watered to field capacity whereas pots with the drought treatment received no water at all. Fine root, phloem and leaf samples were taken weekly.

**Measurements of net photosynthesis and soil respiration.** Net photosynthesis ($A_N$) was measured on three or four trees per lysimeter between 11:00 and 16:00 central European time using a photosynthesis system (LI-COR 6400) equipped with a broadleaf cuvette. The conditions inside the cuvette were kept constant at 400 ppm $CO_2$ and a photon flux of 1,000 μmol $m^{-2}$ $s^{-1}$. Metabolic constraints on $A_N$ were tested by chlorophyll fluorescence analysis using the performance index $PI_{total}$ of PSII[28]. Soil respiration ($R_S$) was measured with a custom-made static chamber[29] equipped with a diffusion-aspirated non-dispersive infrared analyser connected to a humidity/temperature sensor (GMP343 $CO_2$ probe, HMP75 rH/T probe; Vaisala). The increase in $CO_2$ concentrations in the chambers was measured in permanently installed PVC collars (5 cm height, two per lysimeter).

**$^{13}C$ pulse labelling.** Allocation of assimilates was followed by $^{13}C$ pulse labelling in six randomly selected lysimeters ($n = 3$ per treatment) at the end of the drought and in eight lysimeters ($n = 4$ per treatment) 2 weeks after rewatering. Before labelling, the soil was covered with plastic foil to minimize diffusion of $^{13}CO_2$ into the soil. All trees in a given lysimeter were covered with a tall tent made of transparent plastic foil. The $CO_2$ concentration inside was reduced to 200 ppm by flushing the tent with $CO_2$-free air. The labelling lasted 2 h, during which time we added 100% $CO_2$ with a 50:50 ratio of $^{13}CO_2$ and $^{12}CO_2$. The $CO_2$ concentration was kept constant at about 1,500 ppm, which is above the saturation point for $CO_2$ uptake.

**$^{13}C$ analysis in leaves, mycorrhizal root tips, soil microbial biomass and soil-respired $CO_2$.** Leaves from three or four trees per lysimeter were oven dried at 60 °C, milled and weighed into tin capsules for $^{13}C$ analyses. Mycorrhizal root tips and soil microbial biomass were randomly sampled in each lysimeter in the upper 10 cm soil depth by taking three soil cores with a diameter of 2 cm. Additional roots were taken directly from three or four trees. Vital mycorrhizal root tips were immediately collected under a stereomicroscope and kept at −70 °C until processing. They were pooled per lysimeter, oven dried at 80 °C, milled and weighed into tin capsules for $^{13}C$ analyses. Soil microbial biomass was determined using the chloroform fumigation extraction method, whereby the concentration and isotopic signature of extracted organic carbon from non-fumigated and fumigated samples were determined by oxidizing extractable carbon to $CO_2$[31] (ref. 30). The $^{13}C$ of microbial biomass was calculated as described previously[31]. The $^{13}C$ signature of soil-respired $CO_2$ was determined by the closed chamber method[32]. For each sample, the collars were closed with 7 cm tall PVC lids with cellular rubber and gas samples were taken after 15 min. In addition, ambient air close to the soil surface was collected at each sampling occasion.

In gas samples, the $\delta^{13}C$ values and the $CO_2$ concentration were analysed with a GasBench II coupled to a Delta V Plus mass spectrometer (ThermoFinnigan). The $^{13}C$ signatures in solid samples were measured with an Elemental Analyser (Euro EA, Eurovector) coupled to the mass spectrometer. The $\delta^{13}C$ value of soil-respired $CO_2$ was calculated as a mixture of ambient and soil-respired $CO_2$ sampled in the chamber[33]. The $^{13}C$ signal ($\Delta^{13}C$) in mycorrhizal roots was the difference between $\delta^{13}C$ values during and before labelling. The amount of $^{13}C$ assimilated by plants, in soil microbial biomass and in soil-respired $CO_2$ was estimated by first expressing the δ notations in atom% and then calculating the excess $^{13}C$ values considering each pool and flux size[11] (Supplementary Methods). The MRT for the $^{13}C$ soil efflux was calculated as described previously[11].

**Analysis of metabolites.** Metabolites were analysed according to previous studies[34,35]. In brief, frozen tissue was homogenized and extracted with 87% methanol. Phloem exudates were obtained as previously described[36], dried and redissolved in 87% methanol. Aliquots were derivatized and injected into a gas chromatography (GC)-quadrupole mass spectrometry (MS) system (GC, 7890A; MS, 5975C; Agilent Technologies). GCMS data were then deconvoluted, peak areas quantified and mass spectra identified according to ref. 35. Relative concentration changes were calculated as $\log_{10}$ ratios between drought and control treatments.

**Statistical analysis.** Data were analysed by fitting linear mixed effects models using maximum likelihood (lme function; nlme package, R version 3.1.2.)[37] (Supplementary Table 3). For the entire measurement period, season (dry, 22 May to 1 Aug, vs. wet, 2 Aug to 31 Oct), treatment (drought/post drought vs. control) and date of measurement were used as fixed effects and lysimeter and individual tree were included as random effects. The corAR1 function was included in the model to account for repeated measurements with a first-order autoregressive covariate structure. Treatment effects were additionally analysed for dry and wet season. To account for the varying $^{13}C$ signal in the consecutively labelled lysimeters, we included a co-variate as a fixed effect, thereby normalizing the $^{13}C$ tree uptake in each lysimeter to the treatment mean of the wet and dry season, respectively, which allowed us to consider the treatment-specific $^{13}C$ uptake by trees. In all final models, normality and homoscedasticity of the residuals were verified with diagnostic plots and the dependent variables were all log or square root transformed.

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## Author contributions

M.A., R.S., F.H., M.S. and A.G. designed the experiments; R.S., F.H., J.J. and M.A. performed the $^{13}C$ pulse labelling; F.H., K.S. and M.A. measured seasonal $CO_2$ fluxes and chlorophyll fluorescence; M.A., M.P., K.P., U.G., R.K., V.M., S.E. J.L., J.J., M.W., R.S. and F.H. analysed $^{13}C$ allocation patterns; A.G., J-F.L. and M.L. analysed metabolites; J.J. and F.H. performed statistical analysis; A.G., J.J., F.H. and M.A. wrote the manuscript.

## Additional information

Supplementary information is available online. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.A.

## Competing interests

The authors declare no competing financial interests.



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