- Letter -

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 Siegwolf[6], Arthur Gessler[1,7,8#], Matthias Arend[1,9#*]

[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 Center for Environmental Health (GmbH), Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

[4] Research Institute of Forestry, Chinese Academy of Forestry, Xiangshan Road, 100091 Beijing, P.R., China

[5] Department of Forest Genetics, Dendrology and Botany, Faculty of Forestry, University of 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

* corresponding author (Email: matthias.arend@wsl.ch)

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 investigated the effects of drought and drought release on the C 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 C 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 C balances.

Forests play a crucial role in the global carbon (C) cycle because they hold a large fraction of the global C stock and act as a major sink for atmospheric CO_2[5]. However, drought reduces primary productivity, thereby turning forests from C sinks into C sources[6]. It has generally been assumed that plant and ecosystem C 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 comparable importance, but even less understood, are the mechanisms controlling plant and ecosystem C balances after drought release, though 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 C allocation in trees prevents us from predicting C balances of forests under future environmental conditions characterized by greater variability of precipitation and thus alternating drought and recovery periods.

Using two experimental setups, with beech growing either in model ecosystems in open-top chambers (Supplementary Figure 1) or in pots, we studied tree and ecosystem C 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 C dynamics and tracked assimilate transport through the plant-soil system. Based on a hypothetical framework (Figure 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 C balances. If source activity controls C 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 (Figure 1a). A comparable response would occur upon drought release, with an initial recovery of A_N and leaf carbohydrate concentrations followed by a delayed increase of R_s (Figure 1b). If, however, the C balance is sink-controlled, drought would directly reduce R_s, leading to an accumulation of carbohydrates in roots due to reduced C demand. In this case, A_N would acclimate to the reduced sink demand after a delay and leaf carbohydrate concentrations consequently would not change (Figure 1c). Upon drought release, R_s would increase and, with a delay, the increased belowground C demand would positively feed back on A_N (Figure 1d).

In our model ecosystems, A_N and R_s decreased by 44% and 28%, respectively, over the entire drought season (Figure 2a,b,c; Supplementary Figure 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 (Figure 3a,c,e,g). The reduction in $^{13}CO_2$ soil efflux was 83% and thus comparable 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 (approx. 50%), indicating that other C sources, either related to heterotrophic soil respiration or tree internal C storages [10], contributed to soil respiration but were less sensitive to drought. Under drought, the ^{13}C peak in continuously monitored soil CO_2

(Supplementary Figure 3a) was delayed by one day and mean residence times (*MRT*) 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 (NSC) 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 C 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 comparable to those in the model ecosystems (Figure 4a,b; Supplementary Figure 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. 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 NSC might serve as osmotic adjustment[15], which is not directly related to changes in C supply and demand. In our study, however, accumulation of NSC was only observed in roots, whilst 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 NSC 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 C flux to belowground sinks under drought (Figure 3c,e,g) was a consequence of decreased sink activity (Figure 1c). Due to the rather slow build-up of drought over time in our model ecosystems, a clear

79 order of the response of source (A_N) *vs.* sink (R_s) activities could not be derived directly, especially
80 since changes in R_s might have been only partially due to changes in autotrophic root-rhizosphere
81 respiration.

82 The recovery of plant and ecosystem C fluxes after prolonged drought was examined by re-watering
83 the model ecosystems. R_s responded rapidly, reaching control values within the first three days and
84 exceeding thereafter values in controls until the end of the growing season (Figure 2b,c;
85 Supplementary Table 3). This stimulation of R_s nearly compensated for the previous drought
86 reduction, with the flux integrated over the entire growing season amounting to 98% of that in
87 controls. A_N responded similarly but the recovery was delayed by approx. one week due to metabolic
88 limitation, as shown by impaired PSII photochemistry (Figure 2a,c; Supplementary Table 3). Further,
89 the stimulation of A_N occurred later and compensated for only 82% of the previous drought
90 reduction. A second $^{13}CO_2$-pulse label was applied to the tree canopies when R_s exceeded the values
91 in controls but A_N was still slightly below that of controls. The previous drought exposure increased
92 the translocation of recent assimilates to belowground sinks compared to controls, as shown by
93 higher ^{13}C signals in mycorrhizal roots and soil microbial biomass and by an enhanced $^{13}CO_2$ soil efflux
94 (Figure 3b,d,f,h). The latter signal was enhanced by 50% and thus increased in relation to
95 photosynthetic ^{13}C uptake, which was not affected. This increase represents exclusively autotrophic
96 respiration, and the comparable increase in R_s (68% R_s *vs.* 50% $^{13}CO_2$ soil efflux) shows that the plant-
97 driven C flux was primarily responsible for the observed stimulation of R_s. Heterotrophic soil
98 respiration was small in our model ecosystems containing low soil organic content and can therefore
99 be excluded as the cause of stimulated R_s because it only responds transiently to rewetting of dry
100 soils by the so-called "Birch Effect"[18,19]. Our results thus clearly show that a drought effect is
101 imprinted on plant source and sink tissues, supporting the concept of an ecological stress memory of
102 which the underlying mechanisms are still poorly understood[20].

103 The increased C demand of belowground sinks resulted in only a slight feedback on the velocity of C
104 transport. While the peak time of $^{13}CO_2$ soil efflux was comparable, 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 Figure 3b). However, the mass flow of assimilates to belowground sinks can additionally be increased if less C is unloaded from the transport pathway for storage or growth in aboveground tree organs[21]. Indeed, the C allocation to growth in twigs, stem and roots did not fully recover after re-watering, 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 belowground sinks after re-watering shows that trees give high priority to investing into their roots upon recovery from drought. The likely reason for this response is the metabolic need for root and mycorrhizal restoration in order 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 Figure 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 upon drought release feeds back on A_N, triggering the delayed recovery and stimulation of CO_2-assimilation (cf. Figure 1d). Furthermore, our findings support sink control of the C balance under previous drought conditions, as a drought-induced depletion of belowground C 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 C 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 C balances and resilience. Here, we show that tree C 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 upon drought release. The observed response has important consequences for ecosystem C cycling, as it increases the input of plant-derived labile C into soils, thereby fueling 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 C storage compartments. Taken together, our findings suggest a resilience mechanism that attenuates drought disturbances of seasonal tree C balances and needs to be considered when estimating the impact of climate change on the C balances of forest ecosystems.

MATERIAL AND 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 C content (Supplementary Figure 1; Supplementary Methods; 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 function (predawn water potentials below -2 MPa[27,28]), the lysimeters were intensely re-watered and afterwards regularly irrigated until the end of the vegetation season (Supplementary Figure 2a).

The pot experiment was carried out with beech saplings (*Fagus sylvatica* L.) in a greenhouse environment (Supplementary Methods). 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. Fine root and leaf samples were taken weekly.

Measurements of net-photosynthesis and soil respiration

Net-photosynthesis (A_N) was measured on 3-4 trees per lysimeter between 11:00 and 16:00 CET using a photosynthesis system (LI-COR 6400, Lincoln, USA) equipped with a broadleaf cuvette. The conditions inside the cuvette were kept constant at 400 ppm CO_2 and a photon flux of 1000 µmol m^{-2} s^{-1}. Metabolic constraints on A_N were tested by chlorophyll fluorescence analysis using the performance index PI_{total} of PSII[29]. Soil respiration (R_S) was measured with a custom-made static chamber[30] equipped with a diffusion-aspirated non-dispersive infrared analyser connected to a humidity/temperature sensor (GMP343 CO_2 probe, HMP75 rH/T probe; Vaisala, Vantaa, Finland). 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) two weeks after re-watering. 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 1500 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 3-4 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 3-4 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 C from non-fumigated and fumigated samples were determined by oxidizing extractable C to CO_2[31]. The ^{13}C of microbial biomass was calculated as described previously[32]. The ^{13}C signature of soil-respired CO_2 was determined by the closed chamber method[33]. 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 δ^{13}C values and the CO_2-concentration were analysed with a GasBench II coupled to a Delta V Plus mass spectrometer (ThermoFinnigan, Bremen, Germany). The ^{13}C signatures in solid samples were measured with an Elemental Analyzer (Euro EA, Eurovector, Milano, Italy) coupled to

189 the mass spectrometer. The $\delta^{13}C$ value of soil-respired CO_2 was calculated as a mixture of ambient
190 and soil-respired CO_2 sampled in the chamber[34]. The ^{13}C signal ($\Delta^{13}C$) in mycorrhizal roots was the
191 difference between $\delta^{13}C$ values during and before labelling. The amount of ^{13}C assimilated by plants,
192 in soil microbial biomass and in soil-respired CO_2 was estimated by first expressing the δ notations in
193 atom% and then calculating the excess ^{13}C values considering each pool and flux size[11]
194 (Supplementary Methods). The mean residence time (*MRT*) for the ^{13}C soil efflux was calculated as
195 described previously[11].

196 **Analysis of metabolites**

197 Metabolites were analysed according to previous studies[35,36]. In brief, frozen tissue was homogenized
198 and extracted with 87% methanol. Phloem exudates were obtained as previously described[37], dried
199 and re-dissolved in 87% methanol. Aliquots were derivatized and injected into a GC-quadrupole MS
200 system (GC: 7890A; MS: 5975C; Agilent Technologies, Waldbronn, Germany). GC-MS data were then
201 deconvoluted, peak areas quantified and mass spectra identified according to[35]. Relative
202 concentration changes were calculated as $\log_{10}$ ratios between drought and control treatments.

203 **Statistical analysis**

204 Data were analysed by fitting linear mixed effects models using maximum likelihood (lme function;
205 nlme package, R version 3.1.2.)[38] (Supplementary Table 3). For the entire measurement period,
206 season (dry: 22 May to 1 Aug *vs.* wet: 2 Aug to 31 Oct), treatment (drought/post-drought *vs.* control)
207 and date of measurement were used as fixed effects and lysimeter and individual tree were included
208 as random effects. The corAR1 function was included in the model to account for repeated
209 measurements with a first-order autoregressive covariate structure. Treatment effects were
210 additionally analysed for dry and wet season. To account for the varying ^{13}C signal in the
211 consecutively labelled lysimeters, we included a co-variate as a fixed effect, thereby normalizing the
212 ^{13}C tree uptake in each lysimeter to the treatment mean of the wet and dry season, respectively,
213 which allowed us to consider the treatment-specific ^{13}C uptake by trees. In all final models, normality

214 and homoscedasticity of the residuals were verified with diagnostic plots and the dependent

215 variables were all log or square root transformed.

AUTHOR TO WHOM CORRESPONDENCE SHOULD BE ADDRESSED

Matthias Arend, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL),

Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

Email matthias.arend@wsl.ch

ACKNOWLEDGMENTS

We thank Peter Bleuler for technical assistance and for maintaining the model ecosystem facility. We also thank Barbara Meier, Raphael Appenzeller, Yves Bicker, Dominik Brödlin, Daniel Christen, Noureddine Hajjar, Roger Köchli, Florian Schreyer and Alois Zürcher for their help with sampling and measurements and Franz Buegger, Lola Schmidt and Alessandro Schlumpf for completing the isotope analysis. We are grateful to Melissa Dawes for critically reading and providing helpful comments on the manuscript.

REFERENCES

Main text

1 IPCC. *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation*. **582** (Cambridge University Press, 2012).

2 McDowell, N. *et al.* Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol*. **178,** 719-739 (2008).

3 Körner, C. Carbon limitation in trees. *J. Ecol.* **91,** 4-17 (2003).

4 Reichstein, M., et al. Climate extremes and the carbon cycle. *Nature* **500,** 287-295 (2013).

5 Pan, Y. *et al.* A large and persistent carbon sink in the world's forests. *Science* **333,** 988-993 (2011).

6 Ciais, P. *et al.* Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437,** 529-533 (2005).

7 Zha, T. S. *et al.* Gross and aboveground net primary production at Canadian forest carbon flux sites. *Agr. Forest Meteorol.* **174–175,** 54-64 (2013).

8 Guillemot, J. *et al*. The dynamic of the annual carbon allocation to wood in European tree species is consistent with a combined source–sink limitation of growth: implications for modelling. *Biogeosciences* **12,** 2773-2790 (2015).

9 Fatichi, S., Leuzinger, S. & Körner, C. Moving beyond photosynthesis: from carbon source to sink-driven vegetation modeling. *New Phytol.* **201,** 1086-1095 (2014).

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

11 Ruehr, N. *et al.* Effects of drought on allocation of recent carbon: From beech leaves to soil respiration. *New Phytol.* **184,** 950-961 (2009).

12 Arndt, S.K., Clifford S.C., Wanek W., Jones H.G., Popp M. Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. *Tree Physiol.* **21,** 705-715 (2001).

13 Galvez, D.A., Landhausser, S.M., Tyree, M.T. Root carbon reserve dynamics in aspen seedlings: does simulated drought induce reserve limitation? *Tree Physiol.* **31,** 250-257 (2011).

14 Sala, A., Woodruff, D. R. & Meinzer, F. C. Carbon dynamics in trees: feast or famine? *Tree Physiol.* **32,** 764-775 (2012).

15 Hasibeder, R., Fuchslueger, L., Richter, A. & Bahn, M. Summer drought alters carbon allocation to roots and root respiration in mountain grassland. *New Phytol.* **205,** 1117-1127, (2015).

16 Ashraf, M. & Foolad, M. R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **59,** 206-216 (2007).

17 Wiley, E. & Helliker, B. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytol*. **195,** 285-289 (2012).

18 Unger, S., Máguas, C., Pereira, J. S., David, T. S. & Werner, C. The influence of precipitation pulses on soil respiration – Assessing the "Birch effect" by stable carbon isotopes. *Soil Biol. Biochem*. **42,** 1800-1810 (2010).

19 Birch, H. F. Mineralisation of plant nitrogen following alternate wet and dry conditions. *Plant Soil* **20,** 43-49 (1964).

20 Ogle K., *et al*. Quantifying ecological memory in plant and ecosystem processes. *Ecol. Lett.* **18,** 221-235 (2015).

21 Kuzyakov, Y. & Gavrichkova, O. Review: Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. *Glob. Change Biol.* **16,** 3386-3406 (2010).

22 Volkmann T, Haberer K, Gessler A, Weiler M. High-resolution isotope measurements resolve rapid ecohydrological dynamics at the soil-plant interface. *New Phytol.* http://doi.org/10.1111/nph.13868 (2016).

23 Lehto, T. & Zwiazek, J.J. Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza* **21,** 71-90 (2011)

24 Anderegg, W.R.L., *et al*. Pervasive drought legacies in forest ecosystems and their implications for carbon models. *Science* **349,** 528-532 (2015).

279 25 Hill, P.W., *et al*. Living roots magnify the response of soil organic carbon decomposition to
280 temperature in temperate grassland. *Glob. Change Biol.* **21,** 1368-1375 (2015).

281 26 van Bel, A. The phloem, a miracle of ingenuity. *Plant Cell Environ.* **26,** 125–149 (2003).

282 Material and Methods

283 27 Tognetti, R., Michelozzi, M., Borghetti, M. The response of European beech seedlings from two
284 Italian populations to drought and recovery. *Trees - Struct. Funct.* **9,** 348-354 (1995).

285 28 Gallé, A., Feller, U. Changes of photosynthetic traits in beech under severe drought stress and
286 during recovery. *Physiol.Plant*. **131,** 412-421 (2007).

287 29 Albert, K.R., Mikkelsen, T.N., Michelsen, A., Ro-Poulsen, H., van der Linden, L. Interactive effects
288 of drought, elevated CO_2 and warming on photosynthetic capacity and photosystem
289 performance in temperate heath plants. *J. Plant Physiol.* **168**, 1550-1561 (2011).

290 30 Bader, M.K.F., Körner, C., No overall stimulation of soil respiration under mature deciduous
291 forest trees after 7 years of CO_2 enrichment. *Glob. Change Biol.* **16,** 2830-2843 (2010).

292 31 Lang, S.Q., Bernasconi, S.M., Früh-Green, G.L. Stable isotope analysis of organic carbon in small
293 (µg C) samples and dissolved organic matter using a GasBench preparation device. *Rapid. Comm.*
294 *Mass Spectrom.* **26,** 9-16 (2012).

295 32 Högberg, P., *et al*. High temporal resolution tracing of photosynthate carbon from the tree
296 canopy to forest soil microorganisms. *New Phytol*. **177,** 220-228 (2008).

297 33 Ohlsson, K.E.A., *et al*. Uncertainties in static closed chamber measurements of the carbon
298 isotopic ratio of soil-respired CO_2. *Soil Biol. Biochem*. **37,** 2273-2276 (2005).

299 34 Subke, J.A., *et al*. Feedback interactions between needle litter decomposition and rhizosphere
300 activity. *Oecologia* **139,** 551-559 (2004).

301 35 Erxleben, A., Gessler, A., Vervliet-Scheebaum, M., & Reski, R. Metabolite profiling of the moss
302 *Physcomitrella patens* reveals evolutionary conservation of osmoprotective substances. *Plant Cell*
303 *Rep.* **31,** 427-436 (2012).

36 Gessler, A., *et al*. On the metabolic origin of the carbon isotope composition of CO_2 evolved from darkened light-acclimated leaves in *Ricinus communis*. *New Phytol.* **181,** 374-386 (2009).

37 Schneider, S., *et al*. Soluble N compounds in trees exposed to high loads of N: a comparison of spruce (*Picea abies*) and beech (*Fagus sylvatica*) grown under field conditions. *New Phytol.* **134,** 103-114 (1996).

38 Pinheiro, J., Bates, D., Debroy, S., Sarkar, D., the R Core Team. Nlme: linear and nonlinear mixed effects models. R package version 3.1-118. Available at: http://CRAN.R project.org/package=nlme. (2014).

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.

COMPETING INTERESTS

The authors declare no competing financial interests.

FIGURE LEGENDS

Figure 1: Hypothetical trajectories of metabolic activity and metabolite concentration in leaves and roots as a consequence of drought onset and drought release. 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 (leaves/green) and sink tissues (root/brown) 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 (i.e. the non-structural carbohydrates (NSC) glucose, fructose, sucrose and starch; see also Figure 4b). Under source control (**a**, **b**), source metabolic activity in leaves (A_N) reacts first to changing conditions and induces changes in assimilate (i.e. 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, and **b,** show effects on A_N and R_S, respectively. Numerical values provide quantitative measures of the drought limitation and the stimulation after full recovery ($P < 0.05$*, 0.01**, 0.001***; means ± SE; A_N: n = 8, R_S: n = 3-8). In **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 re-watering (PS II chlorophyll fluorescence; n = 8). Responses are shown as relative deviations from control values.

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, and **b,** leaf photosynthetic uptake of ^{13}C. **c,** and **d,** incorporation of ^{13}C into mycorrhizal root tips. **e,** and **f,** transfer of ^{13}C to soil microbial biomass. **g,** and **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 Figure 3). *P* values < 0.05 indicate statistically significant treatment effects (means ± SE, 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*, 0.01**, 0.001***; means ± SE, 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[26]. Data shown are means of 5 replicates.

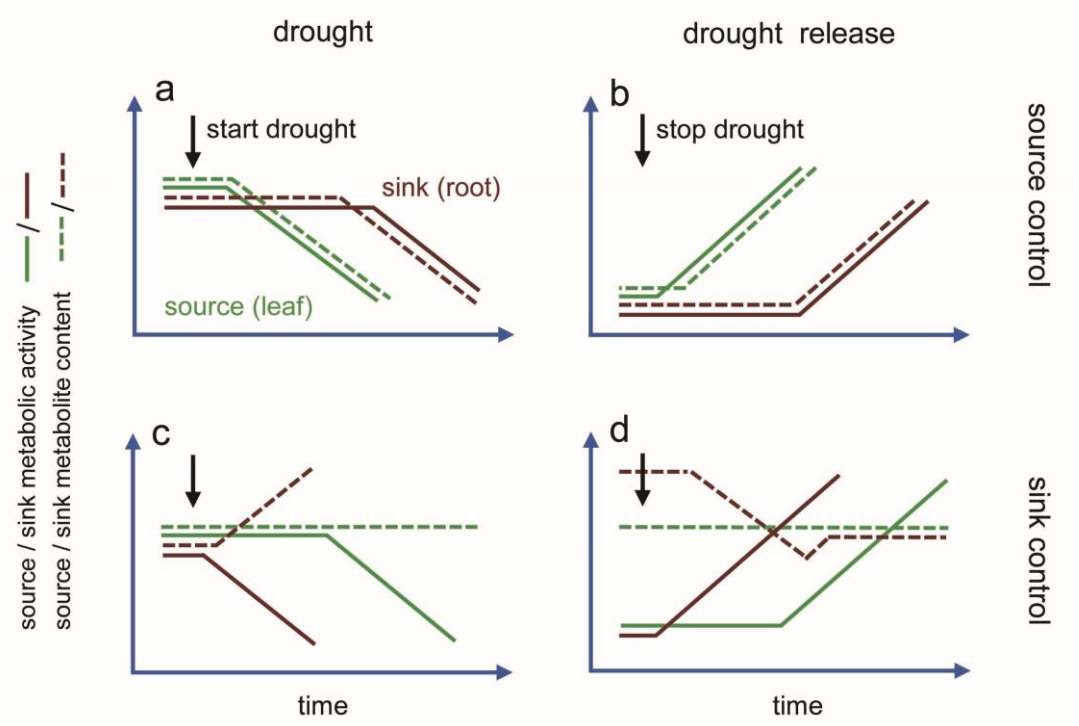
drought
drought release
a
start drought
sink (root)
source (leaf)
b
stop drought
source control
c
d
sink control
time
time
source / sink metabolic activity
source / sink metabolite content

Figure 1

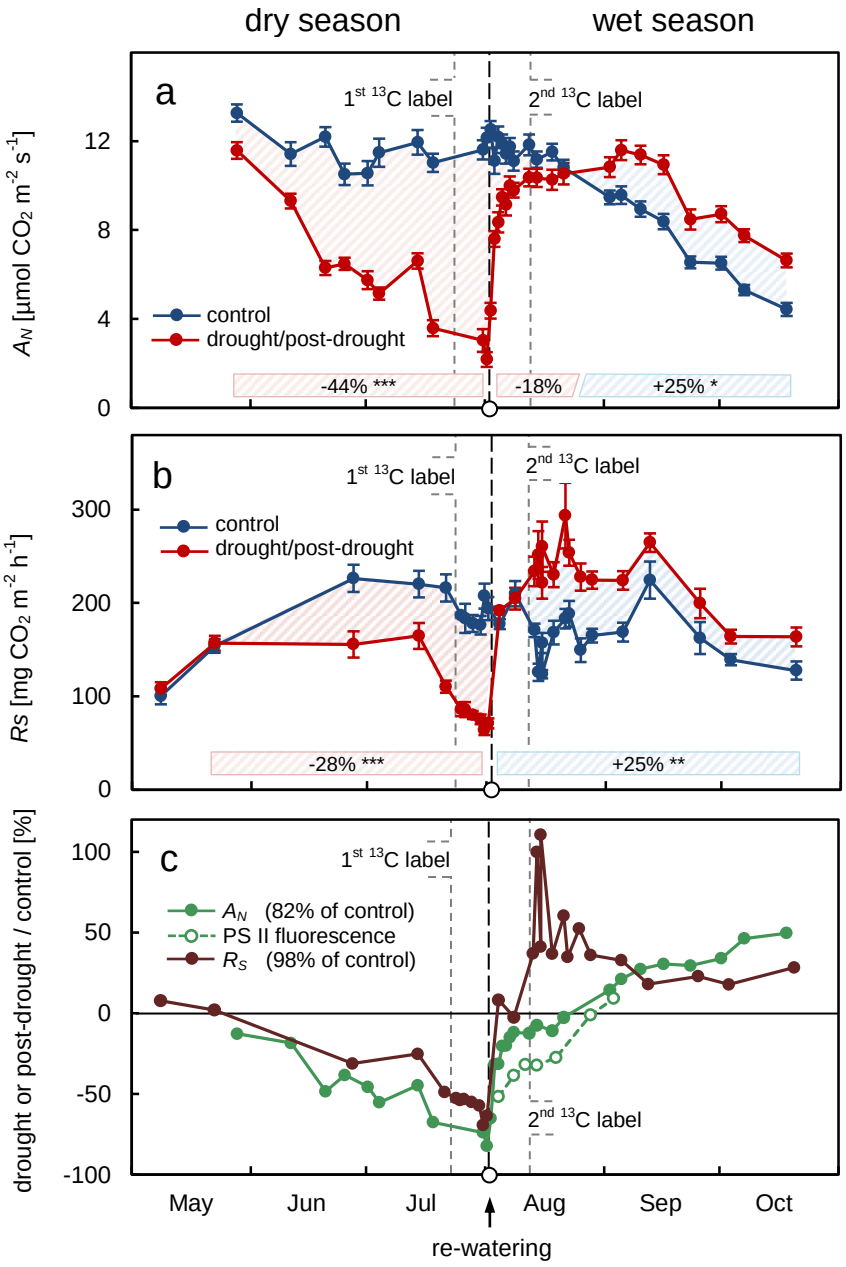
dry season
wet season
a
1st 13C label
2nd 13C label
control
drought/post-drought
-44% ***
-18%
+25% *
AN [µmol CO2 m-2 s-1]
b
-28% ***
+25% **
Rs [mg CO2 m-2 h-1]
c
AN (82% of control)
PS II fluorescence
RS (98% of control)
drought or post-drought / control [%]
May
Jun
Jul
Aug
Sep
Oct
re-watering

Figure 2

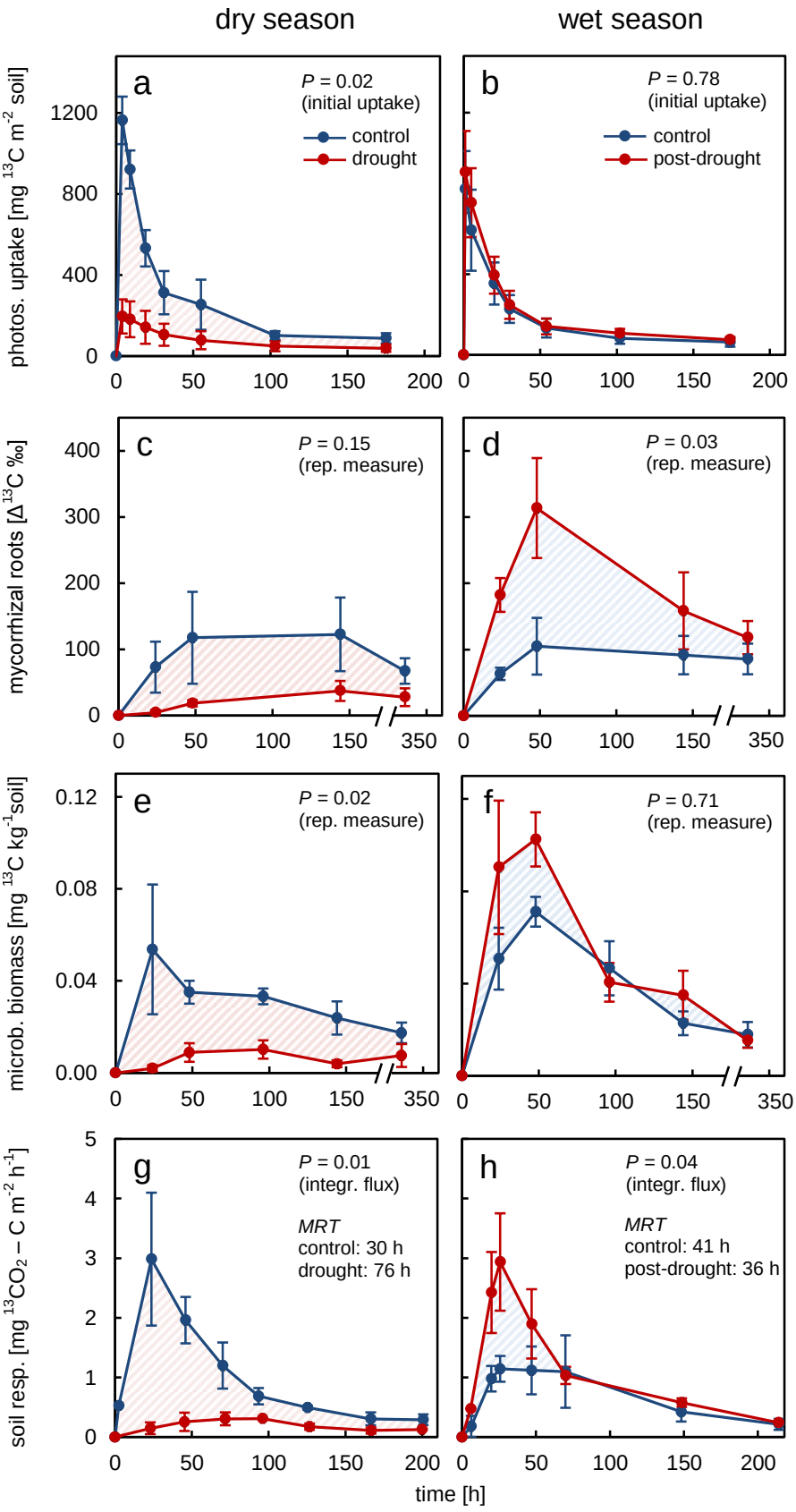
dry season
wet season
a
P = 0.02
(initial uptake)
control
drought
b
P = 0.78
(initial uptake)
control
post-drought
photos. uptake [mg 13C m-2 soil]
c
P = 0.15
(rep. measure)
d
P = 0.03
(rep. measure)
mycorrhizal roots [Δ13C ‰]
e
P = 0.02
(rep. measure)
f
P = 0.71
(rep. measure)
microb. biomass [mg 13C kg-1soil]
g
P = 0.01
(integr. flux)
MRT
control: 30 h
drought: 76 h
h
P = 0.04
(integr. flux)
MRT
control: 41 h
post-drought: 36 h
soil resp. [mg 13CO2 – C m-2 h-1]
time [h]

Figure 3

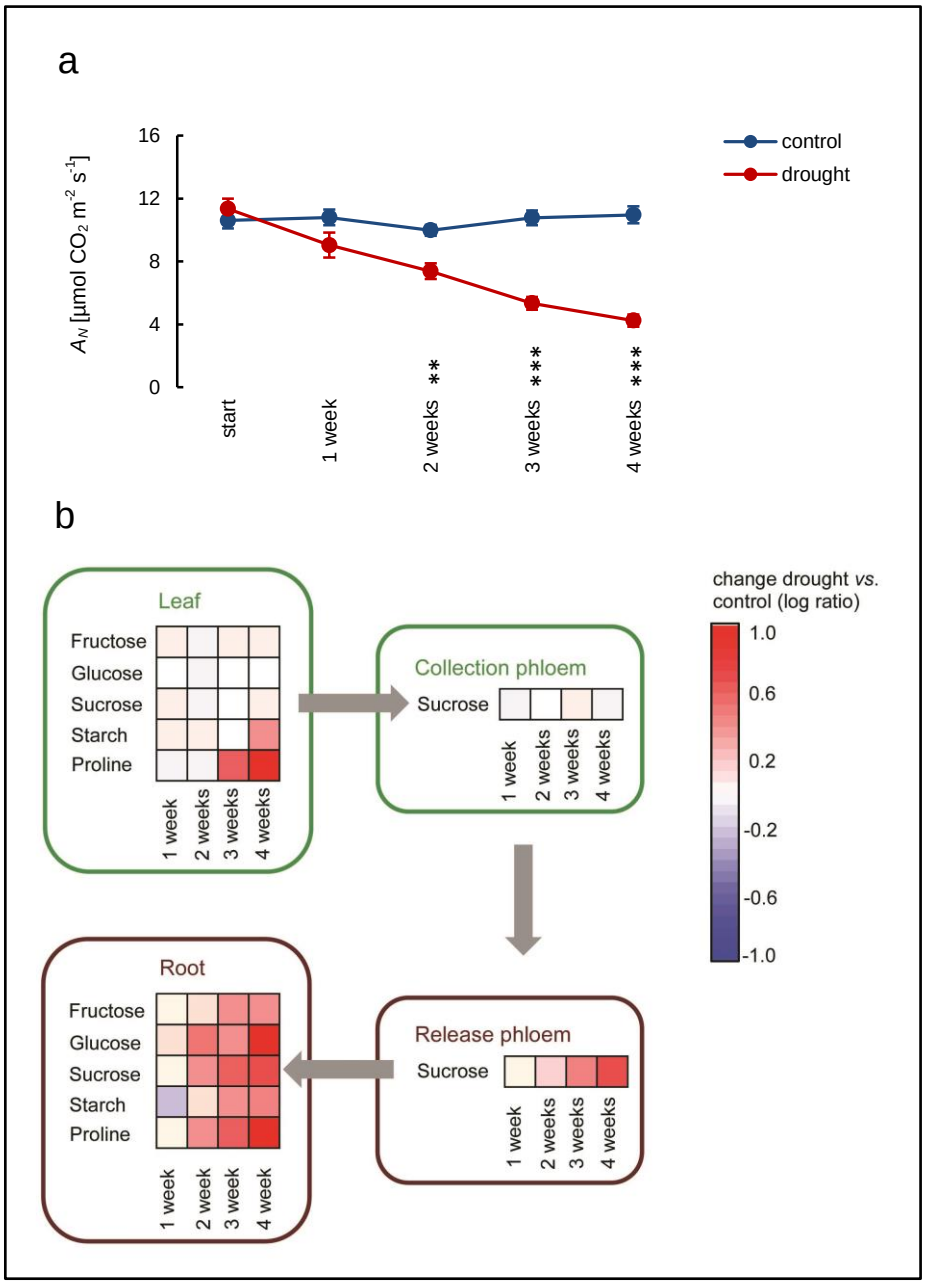
a
16
12
8
4
0
A_N [µmol CO_2 m^{-2} s^{-1}]
control
drought
start
1 week
2 weeks **
3 weeks ***
4 weeks ***
b
Leaf
Fructose
Glucose
Sucrose
Starch
Proline
1 week
2 weeks
3 weeks
4 weeks
Collection phloem
Sucrose
Release phloem
Root
change drought vs. control (log ratio)
1.0
0.6
0.2
-0.2
-0.6
-1.0

Figure 4