

Carbohydrate content governs growth-storage allocation during recovery from carbon depletion

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Summary

- How plants prioritize carbon (C) allocation between growth and storage remains an outstanding question, limiting predictions of forest C cycling and ecosystem resilience following periods of C limitation.
- To address this gap, we experimentally depleted carbohydrate reserves via low-CO₂ conditions and quantified C allocation to reserves and growth during recovery at ambient CO₂ in *Fagus sylvatica* juveniles.
- Trees with lower carbohydrate reserves following C depletion prioritized C allocation to storage, whereas trees replete with carbohydrates favored growth, demonstrating that internal carbohydrate status governs allocation priorities during recovery from periods of C limitation.
- Our findings show that storage-growth allocation during recovery from C limitation is driven by internal carbohydrate status, identifying plant carbohydrate concentration as a key variable for parameterizing the substrate-dependent sink strength that current process-based vegetation models largely omit.

Introduction

Plants store nonstructural carbohydrates (NSC) to buffer fluctuations between carbon (C) supply and demand (Chapin *et al.*, 1990). These reserves support all carbon-demanding plant functions, including primary metabolism, osmoregulation, and growth, and constitute a substantial component of total plant carbon pools (Hoch *et al.*, 2003; Martínez-Vilalta *et al.*, 2016). Stress events such as drought, herbivory, and shading can severely deplete NSC reserves and reduce growth. Following drought, gross primary productivity (GPP) recovers substantially faster than radial growth (Cabon *et al.*, 2022), with GPP legacy effects in forests lasting *c.* 1 yr (Yu *et al.*, 2025), while radial growth legacies can persist up to 4 yr (Anderegg *et al.*, 2015). Reduced growth during recovery from such stressors is often attributed to the allocation of C toward replenishing stress-depleted NSC reserves (Sala *et al.*, 2012; Wiley *et al.*, 2013; Cabon *et al.*, 2022), or toward repairing drought-damaged hydraulic tissue (Trugman *et al.*, 2018). To this effect, experimental studies frequently observe an initial accumulation of NSC during recovery from drought (Rehshuh *et al.*, 2022; Alongi *et al.*, 2025). However, this NSC accumulation may be due to the confounding effects of drought-induced hydraulic dysfunction, where residual xylem embolism and phloem dysfunction following stress limit water supply to growing tissues, restricting growth and causing

apparent NSC accumulation. Understanding such poststress allocation dynamics is particularly consequential given that drought-induced reductions in NSC pools can persist for years after stress (Hagedorn *et al.*, 2016; Gessler & Zweifel, 2024). However, the mechanisms determining allocation priorities following C-limiting stress events remain poorly understood and are largely absent from process-based vegetation models, with this deficiency cited as a major limitation in our ability to predict forest carbon dynamics under increasingly frequent climate extremes (Cho *et al.*, 2022).

Resolving debates about C allocation during or following periods of C limitation requires isolating internal NSC and growth from C assimilation. Studies utilizing shading and defoliation to experimentally induce NSC depletion have demonstrated that trees typically prioritize the allocation to NSC over growth during periods of C limitation (Hartmann *et al.*, 2015; Maguire & Kobe, 2015; Weber *et al.*, 2019). However, these methods can also complicate the interpretation of C allocation dynamics through inadvertent metabolic side effects, such as disrupted light-mediated stomatal aperture and transpiration following darkness or defoliation. While more difficult due to greater technical requirements, treatment with low CO₂ similarly induces NSC depletion but maintains or even promotes transpiration while minimizing destructive sampling (Engineer *et al.*, 2016). Prior low-CO₂ studies have further supported

preferential C allocation to NSC storage over growth during periods of C limitation (Hartmann *et al.*, 2015; Huang *et al.*, 2019), although poststress allocation dynamics have not been investigated. To our knowledge, Weber *et al.* (2018) is the only study to examine C allocation following recovery from C limitation (shading treatment). However, without concurrent gas-exchange measurements to characterize whole-plant C balance dynamics, it remains unclear whether allocation patterns between growth and storage reflect (1) continued limitations in C supply during recovery, (2) the extent of NSC depletion incurred during stress, or (3) regulation by internal carbohydrate reserve status. Identifying what drives C allocation will improve our understanding of the physiological underpinnings of multiyear growth lags and reduced forest productivity (Trugman *et al.*, 2018; Yu *et al.*, 2025).

This controlled experimental study was designed to disentangle how C is allocated between growth and storage during recovery from C depletion. To this end, we exposed 2-yr-old potted *Fagus sylvatica* either to an ambient or low CO₂ atmosphere for several days using whole-tree gas flux chambers, and then restored ambient CO₂ during a recovery period while monitoring C fluxes as well as C allocation to storage and growth. Despite uniform low-CO₂ conditions and a consistent decline of NSC concentrations, biological variation in pretreatment carbohydrate reserves and respiration rates yielded substantial individual differences in whole-plant total NSC concentration following low-CO₂ treatment, providing a natural reserve gradient to investigate allocation priorities during recovery. We tested the following hypothesis: H₀: (null hypothesis) that trees allocate consistently between storage and growth regardless of internal carbohydrate reserve status, implying allocation is governed by fixed fractions of photosynthesis; H_A: (alternate hypothesis) that allocation shifts dynamically with reserve status, such that trees with a lower concentration of NSC reserves allocate proportionally more C to storage relative to growth during recovery (Fig. 1).

Materials and Methods

Plant material

We obtained 50 bare-root two-year-old *Fagus sylvatica* L. juveniles (HkG 81024) from a tree nursery in March 2025 (Forstbaumschulen Gracklauer, Gunzenhausen, Germany). Juveniles were placed in individual 5.7-l pots containing a C-free inorganic substrate mixture of fine quartz (0.1–1.2 mm), medium grain sand (1–2.5 mm), perlite, and vermiculite (2 : 2 : 2 : 1), with 12 g extended-release fertilizer and 2 g micronutrient mix (Osmocote® 5 8-9 M, Micromax® Premium; ICL Specialty Fertilizers, Geldermalsen, Netherlands). From March 2025 to June 2025, juveniles were kept outside an experimental glasshouse facility in Garmisch-Partenkirchen, Germany (708 m above sea level, 47°28'32.9"N, 11°3'44.2"E), and were watered to field capacity twice-weekly. At the start of June 2025, juveniles were then moved inside the glasshouse facility for 1 wk before placement in the tree flux chambers to acclimate to glasshouse conditions with watering maintained.

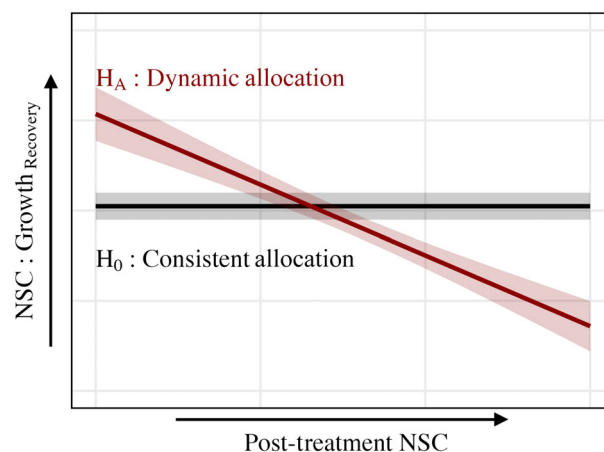


Fig. 1 Conceptual framework for competing hypotheses of growth-storage carbon allocation during recovery from carbon limitation. Two hypothesized relationships between post-treatment nonstructural carbohydrate (NSC) concentration and the ratio of recovered NSC to recovered secondary growth (NSC : Growth_{Recovery}). Under H₀ (consistent allocation, black line), this ratio does not vary with the severity of NSC depletion at the end of treatment. Under H_A (dynamic allocation, dark red line), trees that end treatment more depleted preferentially reallocate recovered carbon toward NSC replenishment over growth, producing a negative relationship. Shaded bands are illustrative 95% confidence intervals. Axes are unitless: this panel depicts the two competing predictions tested empirically in Fig. 5, not measured data.

We used *F. sylvatica* as a model species because its NSC pool is dominated by soluble sugars and starch, whereas lipids and sugar alcohols contribute only a minor fraction of the seasonal carbon reserves (Hoch *et al.*, 2003), allowing for the estimation of total NSC pools using standardized methods (Landhäusser *et al.*, 2018). For details on NSC quantification, see the [Non-structural carbohydrate quantification](#) section.

Tree flux chamber system

We used a custom-built whole-tree gas-flux system with 19 individual chambers, each separated into temperature-controlled transparent aboveground ($n = 19$) and opaque belowground ($n = 19$) compartments (Birami *et al.*, 2018). The belowground compartment consisted of opaque polyvinyl chloride cylinders (30 cm diameter, 20 cm height, 14.1 l volume), while the aboveground compartment consisted of transparent acrylic glass cylinders (29 cm diameter, 35 cm height, 23.1 l volume). Each chamber was sealed around the tree stem to prevent air exchange between above- and belowground compartments. The C-free potting substrate (see the [Plant material](#) section) ensures belowground CO₂ efflux represented root respiration only (Birami *et al.*, 2018; Rehschuh *et al.*, 2022).

Supply air (Air_{supply}) was continuously delivered to the tree flux system by an oil-free screw compressor at controlled H₂O and CO₂ concentrations. During the treatment phase, ambient control chambers received 433.1 ± 0.1 ppm CO₂ while low CO₂ treatment chambers received 58.0 ± 0.1 ppm CO₂, corresponding to the approximate point of zero net daytime assimilation in

aboveground compartments (Fig. 2a). Low- CO_2 concentrations were achieved using a molecular sieve desiccant (SEDAPAC-MZ; FST GmbH, Essen, Germany), which scrubbed CO_2 from $\text{Air}_{\text{supply}}$. During the recovery phase, both treatments received 417.6 ± 0.9 ppm CO_2 . H_2O vapor concentration was maintained at 14.0 ± 0.0 mmol mol $^{-1}$ throughout the experiment. Absolute $[\text{CO}_2]$ and $[\text{H}_2\text{O}]$ of $\text{Air}_{\text{supply}}$ were measured using a gas analyzer (LI-840; Li-Cor, Lincoln, NE, USA) connected to a differential analyzer (LI-7000; Li-Cor) to quantify concentration differences between $\text{Air}_{\text{supply}}$ and the air returning from the chambers ($\text{Air}_{\text{sample}}$). $\text{Air}_{\text{sample}}$ was drawn at 0.02 mol air min $^{-1}$ from each chamber and compartment in sequence via multiplexer valves and routed to the differential gas analyzer. Air samples were measured in 10-s intervals for 1 min per chamber compartment, with the final measurement utilized to allow for signal stabilization due to switching of air streams between compartments. CO_2 and H_2O fluxes in the chamber compartments remained close to steady-state conditions due to uninterrupted ventilation with $\text{Air}_{\text{supply}}$. Each chamber was measured sequentially every ~40 min, with *c.* 36 measurements per chamber compartment per day. Molar flow rates of $\text{Air}_{\text{supply}}$ were adjusted to enhance CO_2 signal detection, particularly during low CO_2 treatment and for belowground respiration measurements. During the treatment phase, aboveground flow rates of $\text{Air}_{\text{supply}}$ averaged 0.24 mol air min $^{-1}$ for ambient chambers and 0.18 mol air min $^{-1}$ for low CO_2 chambers, while belowground flow rates were 0.06 mol air min $^{-1}$ for both treatments. During recovery, all aboveground chambers received 0.36 mol air min $^{-1}$ and belowground chambers 0.06 mol air min $^{-1}$. Gas analyzers were

calibrated at the beginning of and end of the treatment phase using zero CO_2 and H_2O air following manufacturer recommendations.

Two empty chambers (one per treatment) containing pots with carbon-free substrate in belowground compartments were operated as reference blanks. All gas-exchange measurements were corrected by subtracting from the corresponding treatment's blank chamber flux at the closest timepoint. Average offsets during the experiment were 3.4 ± 0.1 ppm CO_2 for ambient aboveground, 16.1 ± 0.4 ppm CO_2 for ambient belowground, 2.0 ± 1.1 ppm CO_2 for low CO_2 aboveground, and 14.2 ± 0.3 ppm CO_2 for low CO_2 belowground compartments. Higher belowground offsets reflected lower flow rates (enhancing signal strength) and minor microbial activity in the wetted potting substrate.

Aboveground air temperature was regulated by fast-response thermocouples (5SC-TT1-36-2 M; Newport Electronics GmbH, Deckenpfronn, Germany) and maintained at $23.5 \pm 0.1^\circ\text{C}$ during the day and $18.4 \pm 0.1^\circ\text{C}$ at night. Photosynthetically active radiation was provided by LED plant growth lamps (LED-KE 400 VSP; DHLicht, Wuelfrath, Germany) at 418 ± 6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for a 10-h photoperiod (07:00–17:00 h), which supplemented natural daylight passing through the glasshouse (average daylength in June 15.9 h; Supporting Information Fig. S1). Root-zone temperature and moisture were monitored with temperature sensors (TS 107; Campbell Scientific Inc., Logan, UT, USA) and moisture sensors (10HS; Meter Group, Pullman, WA, USA), logged every 30 min. Reference and chamber gas-exchange fluxes during the experiment are provided in Table S1.

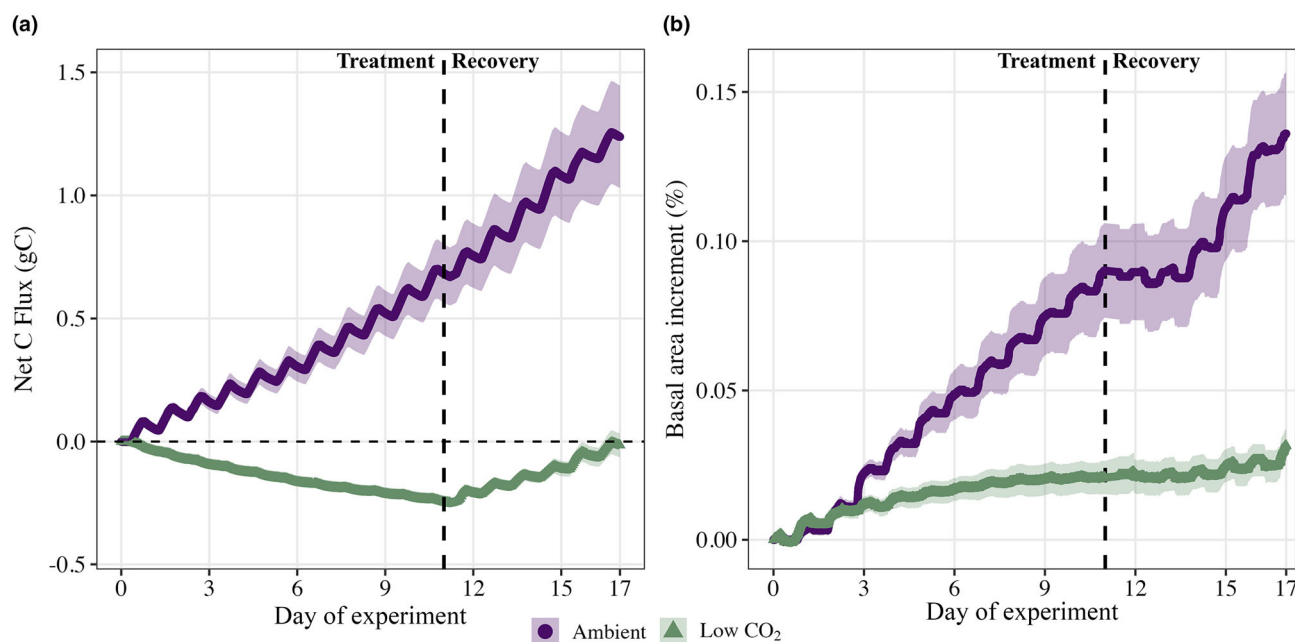


Fig. 2 Net carbon balance and growth in *Fagus sylvatica* diverge between treatments during the depletion and recovery periods. (a) Cumulative net carbon (C) flux (net photosynthesis minus respiration, gC). Positive values indicate net C uptake (photosynthesis exceeding respiration); negative values indicate net C release (respiration exceeding photosynthesis). (b) Basal area increment (%) across the 17-d experiment for ambient (purple circles) and low- CO_2 (green triangles) treatments. The vertical dashed line marks the end of the treatment period (day 11) and the start of recovery; the horizontal dashed line in (a) marks zero net C balance. Points and lines show treatment means $\pm$ SE.

Experimental design and treatment protocol

Before the experiment, a cohort ($n=18$) was randomly selected and destructively harvested to estimate total biomass of trees used in the chamber study. These trees averaged 11.71 ± 1.04 g total dry weight (6.63 ± 0.85 g aboveground, 5.07 ± 0.96 g belowground; root:shoot ratio 0.77 ± 0.29). We assumed an initial whole-plant NSC concentration of 6–10% dry weight (Hoch *et al.*, 2003; Chuste *et al.*, 2020) and a carbon fraction of 0.42 in carbohydrates, yielding an estimated 0.30–0.49 g C stored as NSC per tree. To induce substantial NSC depletion while maintaining tree vitality, we targeted a median net carbon deficit during treatment of $c. 0.25$ g C in low CO₂ trees.

Seventeen seedlings from the remaining cohort were randomly selected and assigned to either ambient CO₂ control ($n=5$) or low-CO₂ treatment ($n=12$) and acclimated in chambers under ambient conditions for 5 d before treatment initiation (initial basal area 38.64 ± 4.9 mm², measured at chamber placement). The unbalanced design was chosen to maximize statistical power for examining within-treatment relationships of allocation patterns during recovery, with ambient controls serving primarily to verify treatment effectiveness. Point dendrometers (DD-S; Ecomatik, Dachau, Germany; 1.5 µm resolution) were installed at $c. 5$ -cm stem height ($n=5$ ambient, $n=10$ low CO₂) to measure relative changes in diameter at 30-min intervals. Growth derived from dendrometer measurements follows the zero-growth concept (Zweifel *et al.*, 2016), where growth is determined to have occurred only when previous maximum diameter measurements are surpassed (to separate from daily stem shrinkage). Absolute diameters were calculated relative to initial caliper measurements and converted to basal area (mm²). All chambers were drip-irrigated twice-daily (06:00 h, 18:00 h; Rain Bird, Azusa, USA) with 100 ml water, maintaining volumetric soil water content of $c. 27.5\%$ throughout the experiment.

The low-CO₂ treatment phase began on 11 June 2025 and continued until 22 June 2025. Low-CO₂ treatment was terminated when the median cumulative net carbon balance across low-CO₂ trees reached 0.245 ± 0.019 g C. At this point, all chambers were returned to ambient CO₂ concentrations (417.6 ± 0.9 ppm) to initiate the recovery phase. The recovery phase continued until the median cumulative net carbon balance of low-CO₂ trees returned to approximately zero, ending with final harvest on 28 June 2025. The treatment median was used as the stopping criterion for both phases to ensure approximately equal numbers of individuals above and below the threshold at each transition, thereby maximizing variation in carbon deficit severity and recovery status for examining allocation priorities.

Carbon balance calculations

Molar CO₂ fluxes (F_{CO_2} , µmol CO₂ s⁻¹) for each chamber compartment were calculated from the concentration differences between Air_{sample} and Air_{supply} after correcting for water vapor dilution:

$$F_{\text{CO}_2} = \dot{m} \times \left(\frac{C_{\text{sample}}}{1 - W_{\text{sample}}} - \frac{C_{\text{supply}}}{1 - W_{\text{supply}}} \right) \times 10^6 \quad \text{Eqn 1}$$

where $\dot{m}$ is molar flow rate (mol s⁻¹), C_{sample} and C_{supply} are CO₂ mole fractions (mol mol⁻¹), and W_{sample} and W_{supply} are H₂O mole fractions (mol mol⁻¹) of that air stream. Positive values of F_{CO_2} indicate net CO₂ uptake (net assimilation), while negative values indicate net CO₂ release (net respiration).

Molar CO₂ fluxes were converted to a mass-based carbon flux (C_{min} , g C min⁻¹):

$$C_{\text{min}} = \frac{F_{\text{CO}_2} \times M_C \times 60}{10^6} \quad \text{Eqn 2}$$

where $M_C = 12.01$ g mol⁻¹ is the molar mass of carbon.

Hourly carbon exchange (C_{hourly} , g C h⁻¹) for each compartment was calculated by averaging C_{min} over each hour and multiplying by 60 min. Whole-tree net carbon exchange (C_{net}) was obtained by summing hourly aboveground ($C_{\text{aboveground}}$, g C h⁻¹) and belowground ($C_{\text{belowground}}$, g C h⁻¹) fluxes:

$$C_{\text{net}} = C_{\text{aboveground}} + C_{\text{belowground}} \quad \text{Eqn 3}$$

Cumulative carbon accumulation ($C_{\text{cumulative}}$, g C) over the experimental period was calculated by integrating hourly values from the start of low-CO₂ treatment (11 June 2025) through final harvest (28 June 2025):

$$C_{\text{cumulative}}(t) = \sum_{i=1}^t C_{\text{hourly},i} \quad \text{Eqn 4}$$

where t is time in hours since experiment initiation and $C_{\text{hourly},i}$ is the hourly carbon exchange at hour i .

Tissue sampling and biomass determination

Leaf, branch, and root tissue samples were collected from all trees at three timepoints: pre-treatment (10 June), post-treatment (22 June), and recovery (28 June). At the pre-treatment and post-treatment timepoints, single samples collected for each tissue represented a fraction of total compartmental biomass (branch: $2.27 \pm 0.05\%$; leaf: $10.98 \pm 0.15\%$; root: $1.56 \pm 0.27\%$), minimizing the impact of destructive sampling on subsequent measurements. At the third (recovery) timepoint, trees were harvested in full: all remaining leaf, stem, and root tissue was removed from chambers and separated into compartments. All tissues were oven-dried at 65°C for 72 h and weighed to determine total compartmental biomass.

To account for tissue removed during destructive sampling at pre- and post-treatment timepoints, total biomass was calculated as the sum of final harvest biomass (recovery) plus the cumulative dry weight of all previously collected tissue samples. This approach was justified by the minimal secondary growth during the entire experimental period (treatment + recovery), with the basal area of ambient trees growing only $0.14 \pm 0.02\%$ and low-CO₂ trees growing $0.03 \pm 0.01\%$ (derived from dendrometer measurements). We note that we were unable to quantify

root or primary apical growth, although due to the compact experimental window occurring after leaf out (when apical elongation predominantly occurs) (Packham *et al.*, 2012), we expect these contributions to be minimized. Using constant biomass across timepoints therefore introduced negligible error in NSC content calculations.

Water potential and photosynthetic pigment measurements

Predawn branch water potential (Ψ) was measured on all individuals post-treatment (22 June) and postrecovery (28 June) using a pressure chamber (Model 600D; PMS Instruments, Corvallis, OR, USA) to confirm that treatments did not induce changes in nighttime xylem tension. Measurements were conducted on excised branches containing at least one leaf. Spectral pigment indices (Table S2) were measured on the excised leaf using a PolyPen RP 410 (Photon System Instruments, Drásov, Czech Republic) during predawn to identify potential treatment effects on photosynthetic capacity. Excised branches and leaves were then utilized for NSC determination.

Nonstructural carbohydrate quantification

Collected tissues were microwaved in three 60-s increments immediately after harvest to halt metabolic activity, then dried at 60°C for 72 h and ground to fine powder. Starch and soluble sugar (sucrose + fructose + glucose) concentrations were quantified using a standardized enzymatic method (Landhäusser *et al.*, 2018). Free sugar concentrations were determined after conversion to glucose-6-phosphate via invertase and isomerase. Starch concentration was determined by hydrolyzing with α -amylase and amyloglucosidase to convert starch to glucose-6-phosphate. Dehydrogenase was used to oxidize glucose-6-phosphate to gluconate-6-phosphate, and absorbance was read at 340 nm on a 96-well microplate photometer (Epoch 2; Agilent, Santa Clara, CA, USA). Absorbance at 340 nm reflects NADPH generated by the dehydrogenase reaction, stoichiometric with the amount of glucose-6-phosphate oxidized. Total NSC was calculated as the sum of free sugars and starch, reported as concentration (mg g^{-1} DW). All samples were analyzed in duplicate. Samples with > 10% discrepancy between duplicate measurements were reanalyzed. To minimize analytical variation among sampling dates, all samples from a given individual (pretreatment, post-treatment, and recovery) were analyzed on the same microplate.

Using measured NSC concentrations and tissue dry weights, we calculated biomass-weighted average NSC concentrations for leaves, aboveground woody tissues (branch samples), and belowground woody tissues (root samples). Whole-plant NSC concentrations were derived from weighted averages across these three compartments.

Carbon allocation determination

To quantify allocation priorities during recovery, we calculated changes in NSC content (mg g^{-1} DW) and basal area increment

(mm^2) between the end of treatment (22 June) and end of recovery (28 June) for low- CO_2 trees. We standardized both metrics in two ways: (1) relative to total biomass, and (2) relative to cumulative net carbon gain during recovery. For the latter, NSC concentration changes were converted from mg g^{-1} DW to absolute g NSC using total biomass, allowing direct comparison with net carbon fluxes measured by gas exchange. Additionally, we calculated the ratio of NSC gain to basal area increment as a direct metric of allocation trade-offs. We examined relationships between these allocation metrics and three measures of carbon status: (1) whole-plant NSC concentration post-treatment, (2) change in NSC mass during treatment, and (3) cumulative net carbon balance during treatment, to determine whether internal carbohydrate status, the extent of reserve depletion, or the absolute carbon deficit influenced allocation priorities during recovery.

Sample sizes varied by metric due to data availability: NSC-based analyses included all low- CO_2 trees ($n = 12$), growth-based analyses included trees with dendrometers ($n = 10$), and NSC : growth ratio analyses included only trees exhibiting measurable secondary growth during recovery ($n = 8$). The two trees excluded from ratio analyses showed no detectable growth during recovery and had very low post-treatment NSC concentrations (< 1% DW), consistent with severe carbon limitation preventing growth resumption.

Statistical methods

All analyses were conducted in R v.4.4.1 (R Core Team, 2024).

Treatment effects on NSC concentrations (soluble sugars, starch, and total NSC) were assessed separately for each tissue type (leaf, branch, root, and whole plant) using linear mixed-effects models (LMER, Bates *et al.*, 2014; LMERTEST, Kuznetsova *et al.*, 2017) with sampling time (pre-treatment, post-treatment, and recovery), treatment, and their interaction as fixed effects and tree identity as a random intercept to account for repeated sampling of the same individuals over time. NSC data were log-transformed before analysis to improve conformity to normality and homogeneity of variance. Time $\times$ treatment interactions were evaluated using Type III F -tests with Satterthwaite-approximated denominator degrees of freedom, and treatment contrasts at each sampling time were estimated from the fitted models using estimated marginal means (EMMEANS, Lenth *et al.*, 2024). Water potential measurements were compared between treatments using two-sample t -tests. Statistical significance was assessed at $\alpha = 0.05$ for all tests.

Spectral pigment indices were analyzed using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations to test for treatment effects, implemented using the VEGAN package (Oksanen *et al.*, 2025). Homogeneity of multivariate dispersion among treatments was assessed using PERMDISP.

Growth responses during the experiment were quantified as both absolute and percent basal area increment per day for each tree. To account for repeated measurements, growth rates were analyzed using linear mixed-effects models (LMER, Bates

et al., 2014) with tree identity as a random effect. Treatment-specific growth rates were estimated using estimated marginal trends (EMTRENDS, Lenth *et al.*, 2024), and contrasts between treatments were computed separately for the treatment and recovery periods. Statistical inference was based on asymptotic confidence intervals and significance at $\alpha = 0.05$. To evaluate whether differences in initial tree size confounded treatment effects, all models were additionally fit with each tree's initial stem diameter included as an additional covariate (Table S3).

Results

The low- CO_2 treatment (58 ppm for 11 d) imposed a cumulative whole-tree C deficit across individuals (-0.25 ± 0.02 g C), quantified from continuous gas-exchange measurements (Fig. 2a; individual plant C balance per chamber compartment available in Fig. S2). By the end of treatment, low- CO_2 trees showed a 68.6% reduction in whole-plant total NSC concentration relative to ambient controls (linear mixed-effects model, $P < 0.001$;

Fig. 3a; Table S4), with reductions in both starch (-82.4% , $P = 0.003$) and soluble sugars (-59.6% , $P < 0.001$; Fig. 3b,c; Table S4). These reductions were largest in leaf (total NSC: -87.0% , $P < 0.001$) and branch tissue (-78.6% , $P = 0.001$), whereas the root NSC reduction was smaller and not statistically significant (-37.0% , $P = 0.267$; Fig. 3f; Table S4). Despite an immediate negative C balance, low- CO_2 trees initially grew during treatment, though both aboveground growth (Ambient – Low: 0.0066 , % BA increase d^{-1} , $P < 0.001$) and root respiration declined progressively over the treatment period (Figs 2b, S2b; Table S5). Daytime transpiration did not differ significantly between treatments during low CO_2 (-20.7% , mixed-effects model, $t = -1.65$, $\text{df} = 16$, $P = 0.118$; Fig. S1a).

When CO_2 returned to ambient concentrations, trees largely regained photosynthetic function without any detectable water stress (predawn water potential, Ambient – Low: -0.11 MPa, $P = 0.368$ post-treatment; -0.09 MPa, $P = 0.379$ postrecovery; Table S6), a difference in daytime transpiration (-14.9% , mixed-effects model, $t = -0.65$, $\text{df} = 16$, $P = 0.528$), or evidence of leaf

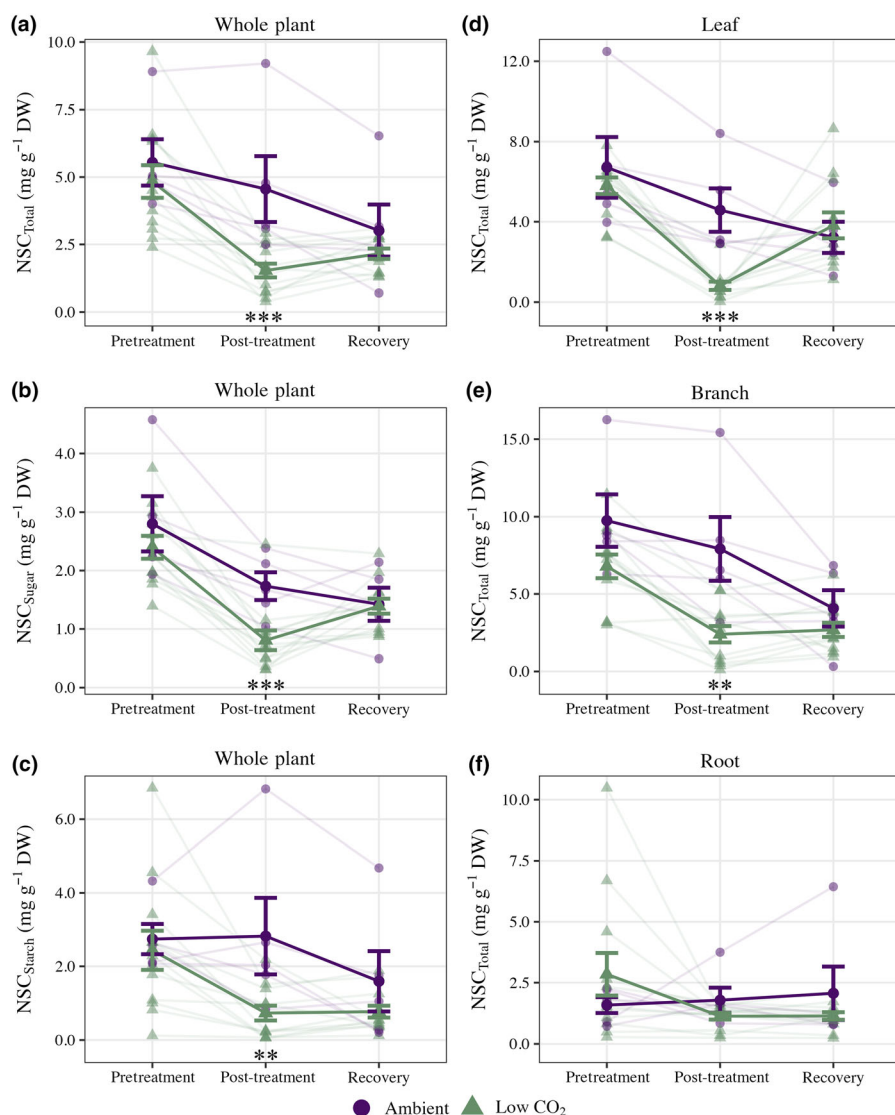
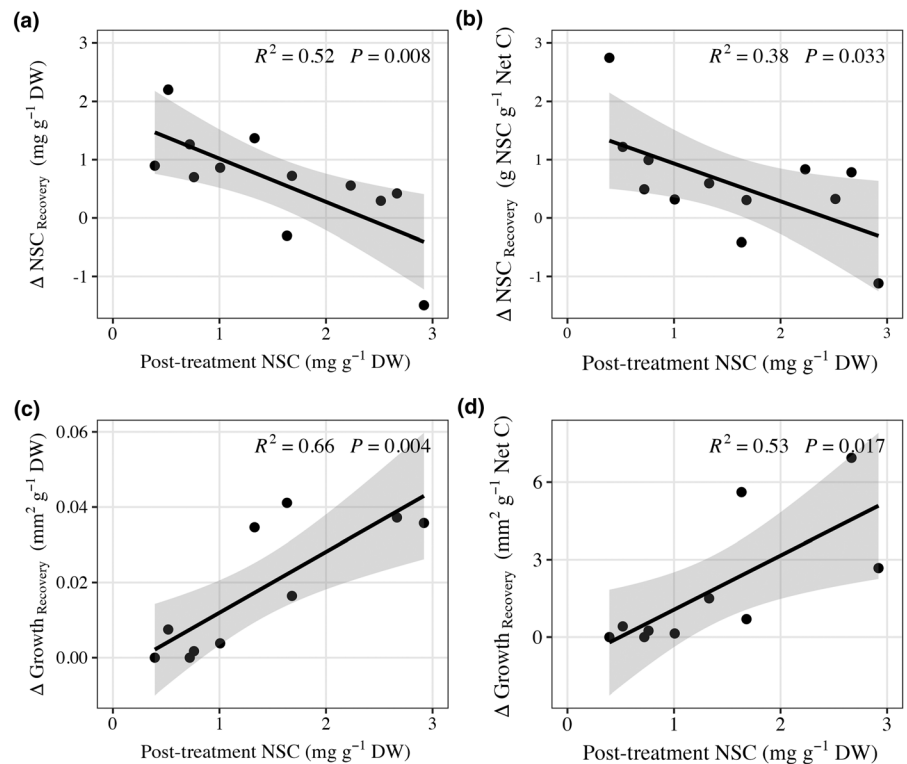


Fig. 3 Nonstructural carbohydrate (NSC) concentrations in *Fagus sylvatica* across treatment and recovery, by component and by tissue. Mean $\pm$ SE NSC concentration (mg g^{-1} dry weight, DW) for ambient (purple circles) and low- CO_2 (green triangles) treatments at Pretreatment, Post-Treatment, and Recovery sampling points. Left column: whole-plant NSC partitioned into (a) total NSC ($\text{NSC}_{\text{Total}}$), (b) soluble sugars ($\text{NSC}_{\text{Sugar}}$), and (c) starch ($\text{NSC}_{\text{Starch}}$). Right column: total NSC concentration (sugars + starch) in the (d) leaf, (e) branch, and (f) root. Thin faded lines show individual trees; thick lines with error bars show treatment means $\pm$ SE. Asterisks denote the significance of the treatment effect (Low CO_2 vs Ambient) from linear mixed-effects models (Supporting Information Table S4): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Sample time $\times$ treatment interaction statistics and pairwise treatment contrasts for each tissue/compartment combination are reported in Tables S4 and S8.

Fig. 4 Post-treatment nonstructural carbohydrate (NSC) concentration predicts the magnitude of recovery-phase NSC and growth gain in *Fagus sylvatica*. Relationships between whole-plant NSC concentration at the end of the treatment period (Post-Treatment NSC, mg g^{-1} DW) and recovery in the low- CO_2 treatment, expressed per unit dry weight (left column) or per unit net carbon assimilated during recovery (right column): (a) change in whole-plant NSC concentration during recovery ($\Delta\text{NSC}_{\text{Recovery}}$, mg g^{-1} DW); (b) $\Delta\text{NSC}_{\text{Recovery}}$ expressed per gram net C assimilated (g NSC g^{-1} Net C); (c) change in growth during recovery ($\Delta\text{Growth}_{\text{Recovery}}$, $\text{mm}^2 \text{g}^{-1}$ DW); (d) $\Delta\text{Growth}_{\text{Recovery}}$ expressed per gram net C assimilated ($\text{mm}^2 \text{g}^{-1}$ Net C). Solid lines show ordinary least-squares linear regressions; shaded bands show 95% confidence intervals; R^2 and P -values are from the fitted model shown in each panel.



pigment damage (spectral PERMANOVA, treatment effect: $R^2 = 0.059$, $P = 0.41$ post-treatment; $R^2 = 0.046$, $P = 0.51$ post-recovery; Fig. S3; Tables S2, S7). Growth remained restricted throughout the six-day recovery in low- CO_2 trees (Ambient – Low: 0.0031 , % BA increase d^{-1} , $P < 0.001$; Fig. 2b; Table S5). Stem secondary growth appeared diminished for 2 d following tissue sampling at both pre- and post-treatment timepoints in both treatments, likely reflecting a temporary wound response from sampling (Fig. S4). While NSC pools in low- CO_2 trees recovered to ambient levels in all tissues (all recovery-period treatment contrasts $P \geq 0.20$; Table S4; Fig. 3), ambient trees displayed continuous NSC utilization throughout the experiment. Time $\times$ treatment interaction tests further indicated that the treatment effect on NSC concentration varied significantly over time for whole-plant total NSC, starch, and soluble sugars, and for leaf total NSC and starch, and branch total NSC (all $P \leq 0.038$); however, no such interaction was detected for any root NSC compartment (all $P \geq 0.066$; Table S8).

During recovery, allocation between storage and secondary growth strongly depended on the amount of remaining NSC following treatment (Figs 4, 5). NSC-depleted trees showed greater NSC recovery rates and limited or absent radial growth, whereas trees with higher NSC reserves post-treatment showed the opposite pattern of minimal or absent NSC recovery but high growth rates (NSC recovery vs post-treatment NSC: $R^2 = 0.52$, $P = 0.008$; growth recovery vs post-treatment NSC: $R^2 = 0.66$, $P = 0.004$; Fig. 4a,c; Table S9). As a result, we observed a negative relationship between the ratio of ΔNSC to ΔGrowth during recovery and reserve status after depletion treatment ($R^2 = 0.67$, $P = 0.013$; Fig. 5; Table S9). We further evaluated this by

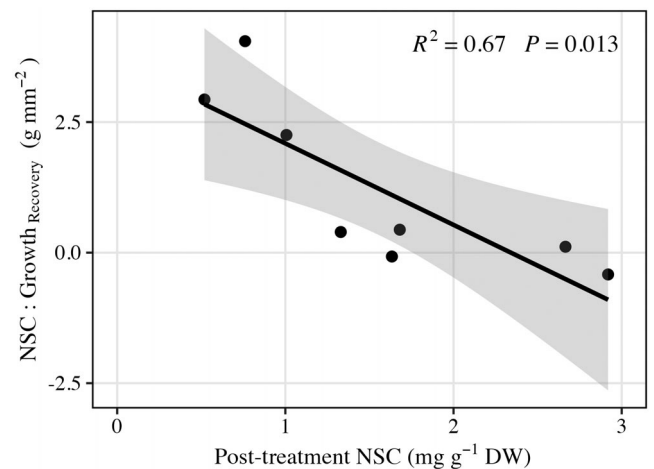


Fig. 5 Nonstructural carbohydrate (NSC): growth recovery ratio declines with post-treatment NSC concentration in *Fagus sylvatica*, confirming the dynamic-allocation hypothesis. NSC : Growth_{Recovery} ratio ($\text{g NSC recovered per mm}^2$ growth) vs Post-Treatment NSC concentration (mg g^{-1} DW) for trees in the low- CO_2 treatment. Each point is one tree. The solid line shows an ordinary least-squares linear regression; the shaded band shows the 95% confidence interval. This empirical relationship corresponds to the H_A (dynamic allocation) prediction diagrammed conceptually in Fig. 1.

standardizing NSC and growth to the net C gas flux during recovery (Fig. 4b,d) and similarly found greater allocation to storage with decreasing post-treatment NSC concentrations (NSC gain per net C: $R^2 = 0.37$, $P = 0.037$; growth gain per net C: $R^2 = 0.53$, $P = 0.018$; Table S9). Notably, neither the absolute

change in NSC mass nor the cumulative respiratory C loss during treatment explained recovery allocation patterns (all $R^2 \leq 0.19$, all $P \geq 0.21$; Table 1).

Discussion

Our results demonstrate that storage-vs-growth allocation during recovery from C limitation falls along a continuum governed by internal NSC status, rather than a fixed fraction of photosynthesis. NSC-depleted trees preferentially replenished reserves during recovery, whereas ambient trees, which retained abundant NSC throughout the experiment, continued to prioritize secondary growth despite a gradual seasonal decline in NSC, consistent with sustained C demand during the peak growing season (Fig. 5). By simultaneously measuring whole-plant gas exchange with NSC status and high-resolution radial growth, these results represent the first identification of internal carbohydrate status as the proximate driver of storage-growth allocation during recovery from C limitation.

Storage prioritization during or following C limitation likely reflects multiple adaptive pressures that together favor reserve preservation over secondary growth allocation. Conservative allocation strategies that prioritize storage over growth may represent plastic or evolutionary responses to minimize the risk of carbon limitation, with populations experiencing severe or frequent depletion events selecting for greater reserve formation at the expense of secondary growth (Wiley & Helliker, 2012). Maintaining adequate reserves provides insurance against recurring stress (Blumstein *et al.*, 2023), as trees with depleted NSC exhibit reduced recovery capacity and higher subsequent mortality risk following drought (Galiano *et al.*, 2011), while storage prioritization may maximize fitness by maintaining a safety margin to respond to subsequent stress events (Stefaniak *et al.*, 2024). Additionally, NSC serves essential metabolic and osmotic functions, such as cell turgor maintenance, that require soluble sugar concentrations to be maintained above minimum thresholds (Martinez-Vilalta *et al.*, 2016; Blumstein *et al.*, 2022). Storage prioritization in severely depleted individuals may therefore reflect restoration of this metabolic safety margin. Storage prioritization as a function of NSC reserve status is consistent with known molecular carbohydrate-sensing pathways, which regulate

C sink activity in proportion to cellular energy status (Feng *et al.*, 2025). Furthermore, this work extends the transcriptional evidence from Huang *et al.* (2021) by suggesting that the synthesis of proteins involved in storage upregulation are likely regulated by tissue NSC concentration rather than the absolute decrease in internal NSC pools or external C assimilation (net C flux) that occur during stress.

Our findings are based on recently transplanted juvenile *F. sylvatica* under controlled conditions. As the isolated mechanisms operate at the level of C balance and sink activity, we expect them to extend to other woody plants, though this remains to be confirmed empirically, and the specific patterns observed here likely vary with ontogeny-dependent and species-specific NSC pool sizes and turnover times. For example, *F. sylvatica* is considered a late-successional species, which typically allocate greater C toward storage than growth in comparison with faster growing pioneer species (Signori-Müller *et al.*, 2022). Therefore, we expect the strength of C allocation toward storage, as well as potential NSC thresholds at which storage is prioritized, to reflect these species-specific strategies. In addition, while the low-CO₂ approach was instrumental in isolating recovery allocation priorities, the imposed CO₂ concentration (58 ppm) is below any level experienced since the evolution of land plants. However, intercellular CO₂ concentrations do reach limiting levels during drought-induced stomatal closure (Brodribb, 1996), as continued Rubisco carboxylation draws down internal CO₂ faster than restricted stomatal conductance can replenish it. Our treatment was intended to simulate this internal C-limited state directly, without the confounding hydraulic and stomatal effects of actual drought. In natural drought recovery, C allocation is further complicated by competing demands for tissue repair, which draw substantially on stored C (Hikino *et al.*, 2022) and may alter the balance between storage refilling and secondary growth in ways our design could not capture. Whether NSC-dependent allocation priorities are maintained, amplified, or overridden when additional demands on substrate exist remains unresolved.

NSC depletion, including the substantial decline in soluble sugars we observed (the osmotically active NSC fraction measured), could plausibly impair turgor maintenance independently of soil or plant water deficit, as NSC-depleted seedlings reach

Table 1 Linear regression statistics for recovery-phase nonstructural carbohydrate and growth allocation as functions of the absolute magnitude of carbon depletion in *Fagus sylvatica* during the low-CO₂ treatment period.

Relationship	<i>n</i>	<i>R</i> ²	<i>F</i>	<i>df</i>	<i>P</i>
ΔNSC _{Recovery} (mg g ⁻¹ DW) vs ΔNSC _{Treatment} (g NSC)	10	0.00	0.00	1, 8	0.959
ΔGrowth _{Recovery} (mm ² g ⁻¹ DW) vs ΔNSC _{Treatment} (g NSC)	10	0.016	0.13	1, 8	0.724
NSC : Growth _{Recovery} ratio (g mm ⁻²) vs ΔNSC _{Treatment} (g NSC)	8	0.135	0.94	1, 6	0.370
ΔNSC _{Recovery} (mg g ⁻¹ DW) vs Net C Flux _{Treatment} (gC)	10	0.104	0.93	1, 8	0.364
ΔGrowth _{Recovery} (mm ² g ⁻¹ DW) vs Net C Flux _{Treatment} (gC)	10	0.116	1.05	1, 8	0.335
NSC : Growth _{Recovery} ratio (g mm ⁻²) vs Net C Flux _{Treatment} (gC)	8	0.001	0.01	1, 6	0.94

DW, dry weight; NSC, nonstructural carbohydrates. ΔNSC_{Treatment} (g NSC) is the absolute decline in whole-plant NSC mass from Pretreatment to Post-Treatment; Net C Flux_{Treatment} (gC) is the magnitude of net carbon lost during the treatment period. ΔNSC_{Recovery} and ΔGrowth_{Recovery} are as defined in Fig. 4; the NSC : Growth ratio is as defined in Fig. 5. None of these relationships were statistically significant (all $P > 0.05$), in contrast to the relationships with Post-Treatment NSC concentration itself (Figs 4, 5).

turgor loss at less negative water potentials than nondepleted seedlings (Sapes *et al.*, 2021). We cannot fully dismiss that reduced turgor contributed to the generally lower daytime transpiration we observed (although the difference in transpiration was not statistically significant), since our water potential measurements were limited to predawn timepoints and do not capture daytime xylem tension. Nonetheless, NSC-altered turgor pressure is unlikely to explain the restricted secondary growth observed here: radial stem growth in trees occurs predominantly overnight (Zweifel *et al.*, 2021), when predawn water potential measurements identified no difference in xylem tension. Similarly, while our data were consistent with a continuous, linear relationship between post-treatment NSC status and recovery allocation, due to our limited sample size we cannot rule out that this relationship is instead nonlinear. Consequently, these results should be used with caution when parameterizing process-based vegetation models. Larger scale studies designed explicitly to test for complex relationships, such as the existence of lower NSC thresholds beyond which allocation priority shifts toward storage, are a valuable direction for future work.

We provide empirical evidence for substrate-constrained allocation dynamics, where storage-vs-growth priorities during recovery are governed by internal NSC status. This mechanism likely contributes to the multiyear forest growth lags commonly observed following stress events, despite rapid GPP recovery (Anderegg *et al.*, 2015; Yu *et al.*, 2025), and represents a key gap in simulating stress legacies in vegetation models (Trugman *et al.*, 2018; Quetin *et al.*, 2024). Most process-based forest models either omit NSC storage pools entirely or treat NSC as a passive overflow of GPP, with growth allocation determined primarily by photosynthesis rather than internal carbohydrate status (Merganičová *et al.*, 2019; Cho *et al.*, 2022), although a small number of models have begun to incorporate NSC as an active, growth-influencing pool (Richardson *et al.*, 2013; Fang *et al.*, 2022). This source-limited paradigm fails to capture storage-dependent allocation dynamics and has been identified as a primary reason models fail to simulate poststress recovery (Cho *et al.*, 2022). The continuous relationship between storage-overgrowth allocation and poststress NSC concentration documented here identifies tissue NSC status as a candidate variable for parameterizing substrate-dependent sink strength in next-generation vegetation models. Testing whether this relationship generalizes across species, ontogenetic stages, and stress types will be an important next step toward improving model prediction of forest C recovery under increasingly frequent climate extremes.

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Competing interests

None declared.

Author contributions

FA and NKR conceived and designed the experiment. FA processed and analyzed all experimental data. FA drafted the manuscript, with assistance from DEMU, MB and NKR. All authors have read and approved of the manuscript.

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Data availability

The gas flux, growth, and NSC data sets are available on PANGAEA (Felden *et al.*, 2023; <https://doi.pangaea.de/10.1594/PANGAEA.993034>, <https://doi.pangaea.de/10.1594/PANGAEA.993035>).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Environmental conditions (H₂O flux, VPD, air temperature, PAR) across the experimental timeline for ambient and low-CO₂ treatments.

Fig. S2 Individual-tree net carbon flux by chamber compartment (aboveground, belowground, net) during treatment and recovery.

Fig. S3 Principal component analysis of leaf spectral reflectance indices showing no treatment-related separation at post-treatment or recovery.

Fig. S4 Cumulative basal area increment across experimental phases, showing transient growth pauses following tissue sampling.

Table S1 Chamber gas-exchange conditions (incoming CO₂/H₂O, fluxes) for reference and sample chambers by treatment, phase, and compartment.

Table S2 Definitions and equations for the leaf spectral reflectance indices used in the PCA.

Table S3 Sensitivity of all treatment effect and regression models to inclusion of initial stem diameter as a covariate.

Table S4 Treatment contrasts for NSC concentration (starch, sugar, total) by tissue and sampling time.

Table S5 Treatment contrasts for basal area growth rate during the depletion and recovery periods.

Table S6 Treatment contrasts for predawn branch water potential at post-treatment and post-recovery.

Table S7 PERMANOVA and dispersion tests for treatment effects on leaf spectral index composition.

Table S8 Sample time × treatment interaction tests for NSC concentration by tissue and compartment.

Table S9 Regression statistics for the recovery allocation relationships shown in Figs 4 and 5.

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