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Homeostatic levels of nonstructural carbohydrates after 13 yr of drought and irrigation in *Pinus sylvestris*

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Summary

- Nonstructural carbohydrates (NSCs) are important for the growth and survival of trees. Drought may lead to a decrease in tree growth and to NSC depletion, whereas increased soil moisture in otherwise dry ecosystems may increase growth and NSC concentrations.
- A long-term (13 yr) irrigation experiment was conducted in a *Pinus sylvestris*-dominated forest located at the dry margin of the species in southern Switzerland. We measured the relative leaf area, growth, NSCs, needle δ^{13}C, [N] and [P] in trees on control and irrigated plots.
- Irrigation resulted in higher growth rates and carbon isotope discrimination, but did not alter NSC levels. Growth and NSC decreased with decreasing leaf area in both treatments, but NSC did not correlate with leaf-level gas exchange indices, such as foliar δ^{13}C, [N] or [P]. A legacy effect was shown, as trees with initially low leaf area had limited ability to respond to prolonged irrigation.
- The NSC constancy across treatments provides evidence that carbohydrate storage may stay constant when climate changes are sufficiently slow to allow acclimation. Moreover, we speculate that total leaf area, rather than leaf gas exchange per unit leaf area, drives the variation in whole-tree carbohydrate dynamics in this system.

Introduction

Drought causes reduced forest productivity, and increasing duration and frequency of drought events can turn forest ecosystems from carbon (C) sinks into sources (Ciais *et al.*, 2005). Trees store large amounts of assimilated C in their sapwood and roots, as well as in needles in the case of conifers (Hoch, 2015). These storage pools can be used for growth, maintenance metabolism and defense (Chapin *et al.*, 1990). As drought impairs photosynthetic C assimilation, C storage pools may play a major role in supporting tree functions during such periods. Although our general knowledge on C storage and remobilization dynamics in trees is increasing, there is still an ongoing debate on how, and to what extent, C reserves play a role in sustaining function and growth under stressful conditions, as well as during tree recovery after stress (McDowell, 2011; Sala *et al.*, 2012; Palacio *et al.*, 2014; Gessler & Treydte, 2016).

For trees, C storage mostly occurs in the form of sugars and starch which make up nonstructural carbohydrates (NSCs) (Dietze *et al.*, 2014), whereas other compounds, such as lipids, play a minor role in most species (Hoch *et al.*, 2003). NSCs buffer the imbalance between the supply and demand of C across plant tissues on diurnal and seasonal scales (Li *et al.*, 2002; Hoch *et al.*, 2003; Hartmann & Trumbore, 2016). In periods of drought, NSC pools in different plant organs can serve as buffers

to compensate for the decreased C assimilate supply as a result of decreased photosynthesis (McDowell, 2011; Sala *et al.*, 2012; Hoch, 2015). C storage is considered to be a passive process that only occurs when the C supply is higher than the demand (Sala *et al.*, 2012), but is also thought to be actively regulated in balance with other C sinks, such as growth and defense (Chapin *et al.*, 1990; McDowell, 2011; Dietze *et al.*, 2014). There is presently intensive discussion on whether C storage is 'passive' or 'active' or both (Sala *et al.*, 2012; Wiley & Helliker, 2012; Hartmann & Trumbore, 2016), and whether NSC depletion under extreme conditions, and thus C starvation, is a driving mechanism for reduced tree survival under drought (Sala *et al.*, 2012; Klein, 2015).

Theory and data both suggest that the higher sensitivity of growth (sink activity) than photosynthesis (source activity) to long-term drought (Körner, 2015) leads growth to cease first during drought, followed by photosynthetic decline (McDowell, 2011). These differing temporal patterns in photosynthetic supply and demand allow the accumulation of NSC during the initial phase of a drought period or at very mild drought stress (trees are sink limited), and may eventually result in NSC depletion if drought persists over longer periods when respiratory, metabolic and, perhaps, hydraulic C demands exceed the supply by photosynthesis (trees become source limited) (McDowell *et al.*, 2013; Hoch, 2015). This hypothesis can possibly explain the seemingly

contradictory results found in the literature, ranging from NSC decrease during drought, as repeatedly shown in Scots pine (*Pinus sylvestris*), to no changes at all or even increases in NSC (Adams *et al.*, 2017).

Patterns of NSC concentration under long-term drought vs the release of such drought exposure under field conditions have not been extensively studied. We also lack information on the interrelationship between NSC, growth, tissue nutrient concentrations and crown conditions under such long-term drought vs non-drought exposed conditions (but see von Arx *et al.*, 2017). Decreasing leaf area (in some literature, referred to as 'crown transparency', a surrogate for relative leaf area (Dobbertin, 2005; Gottardini *et al.*, 2016)) is correlated with long-term growth decrease (Timofeeva *et al.*, 2017), and also with stem sapwood NSC after a drought event (Galiano *et al.*, 2011; Camarero *et al.*, 2015). In the first years of release from long-term drought, high crown defoliation impedes the growth recovery of trees (Eilmann *et al.*, 2013); thus, reduced relative leaf area (relative to a tree with maximum leaf area in a forest) may also limit NSC storage dynamics after irrigation is commenced. Moreover, tissue nutrient depletion resulting from impaired nutrient uptake during drought may exacerbate the adverse effects of long-term reduced water availability by impairing leaf-level photosynthesis (Gessler *et al.*, 2016).

At the Pfynwald, the largest Scots pine-dominated forest in Switzerland, located in a dry inner alpine valley, several drought events within recent decades have caused large-scale mortality of Scots pine trees (Allen *et al.*, 2010; Rigling *et al.*, 2013). Together with low growth rates (Dobbertin *et al.*, 2010), this indicates that the stand grows at the dry edge of the distribution of Scots pine. A unique long-term (13 yr) irrigation experiment at this forest site allowed us to study the long-term effects of chronically increased soil water content on seasonal NSC dynamics in Scots pine, compared with many studies which have used short-term treatments or single extreme (natural) drought events (Adams *et al.*, 2017).

The conceptual framework on NSC trajectories proposed by McDowell (2011) was based on evidence from short-term droughts (e.g. seasonal to a few years), but NSC dynamics may differ after 13 yr of treatment that allow the acclimation to new soil water conditions. We based our a priori hypotheses on the assumption that, despite any acclimation, NSC dynamics would respond to treatments and crown conditions. First, we hypothesized that trees would be source limited during long-term drought, resulting in lower NSC concentrations in dry (control) relative to irrigated trees after 13 yr of treatment based on their presumed locations within the hypothesized NSC curve (Fig. 1). Second, we predicted that NSC would be correlated with relative leaf area. Alternatively, NSC may be better correlated with indices of leaf-specific gas exchange (we note that this is possibly a false dichotomy as both total photosynthetic leaf area and gas exchange per unit leaf area may influence NSC). Lastly, we hypothesized that the legacy effects of low leaf area before irrigation could constrain growth and NSC responses to irrigation after 13 yr. As most studies have determined the carbohydrate levels when drought reaches its maximum, but have neglected the

role of winter NSC in growth and survival of drought-stressed trees, we sampled multiple tissues (needles, roots and sapwood) throughout multiple seasons to better test our hypotheses at both the whole-tree and seasonal scales.

Materials and Methods

A 13-yr irrigation experiment was conducted in the Pfynwald forest (46°18'N, 7°36'E, 615 m above sea level), the largest Scots pine (*Pinus sylvestris* L.)-dominated forest in Switzerland, located in the dry inner-Alpine valley of the river Rhone, close to the dry edge of the natural distribution of Scots pine. The Pfynwald is a naturally regenerated forest, but past forest practices have favored the regeneration of Scots pine over other species, such as *Quercus pubescens*. Climatic conditions are characterized by a mean annual temperature of 10.1°C and an annual precipitation sum of *c.* 600 mm. Scots pine forests in the Valais are regularly subjected to drought- and heat-induced mortality (Bigler *et al.*, 2006; Allen *et al.*, 2010; Rigling *et al.*, 2013). The average tree age is *c.* 100 yr and the forest has a mean canopy height of 10.8 m, a stand density of 730 stems ha^{-1} and a basal area of 27.3 m^2 ha^{-1} (Dobbertin *et al.*, 2010). The soil is shallow parendzina, characterized by very low water retention and high vertical drainage (Brunner *et al.*, 2009).

The experimental site (1.2 ha; 800 trees) is divided into eight plots of 25 × 40 m^2 each, separated by a 5-m buffer zone. The irrigation of *c.* 600 mm yr^{-1} was applied at night on four plots between April and October, from the year 2003 onwards, with 1-m-high sprinklers using water from a nearby channel parallel to
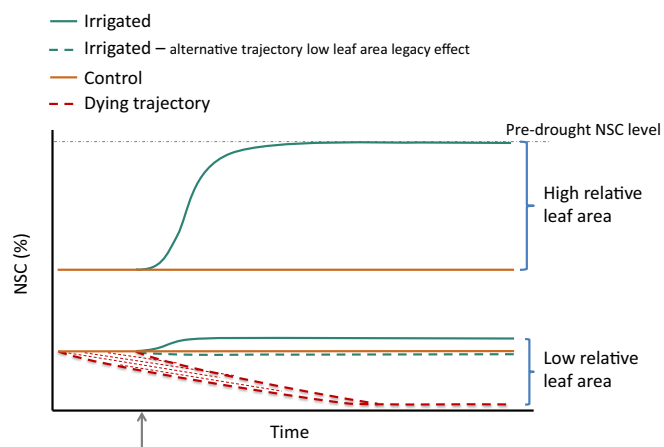
Fig. 1 Possible trajectories of long-term tree nonstructural carbohydrate (NSC) concentrations in irrigated and control plots, as an elaboration on the modeled representation of short-term NSC dynamics during drought for generic trees posed in McDowell (2011). We hypothesize that NSC will be lower in dry (control) relative to irrigated trees, but that NSC is also correlated with relative leaf area. This interaction may result in a wide spectrum of NSC levels in trees with or without drought stress and with different crown conditions. Drought release (grey arrow) may bring a trees' NSC pools back to pre-drought levels, but historical crown legacy may reduce the capability to recover carbon reserves fully (green solid line) or even at all (green dashed line) after drought release. Lastly, trees may reach a point of no return that only leads to mortality, caused by hydraulic failure, carbon starvation or biotic factors.

the experimental plot, fed by the Rhone river. Nutrient input through irrigation has been proven to be minor (Thimonier *et al.*, 2005, 2010). The control plots thus represent the drought-exposed situation, whereas the treatment results in drought release. The volumetric soil water content was monitored hourly in one control and one irrigated plot using time domain reflectometry (Tektronix 1502B cable tester, Beaverton, OR, USA) at soil depths of 10, 40 and 60 cm at four different locations per plot (Supporting Information Fig. S1). Air temperature, relative humidity (Sensirion SHT-21; Sensirion AG, Stäfa, Switzerland) and precipitation (Tipping Bucket Rain Gauge; R.M. Young, Traverse City, MI, USA) were measured and stored with a time resolution of 10 min. Tree height and stem diameter (diameter at breast height, DBH) were measured in 2003, 2009 and 2014 by manual calipering. Crown transparency (the surrogate for relative leaf area) was measured once a year from 2003. Crown transparency assessment was performed as described by Dobbertin *et al.* (2004) by visual rating of the crown transparency (also termed defoliation) using reference photographs ranging from 0% (= a fully foliated tree) to 100% (= a dead tree (for more details, see Dobbertin *et al.* (2004)). This assessment is not a strict crown transparency assessment as tree crown foliage is judged relative to the optimum foliage of a tree of the same species. Relative leaf area (hereafter called 'leaf area') was calculated using 100% minus the crown transparency.

Sampling

Thirty control and 23 irrigated trees from five different relative tree leaf area classes (1 = 100–80% leaf area, 2 = 80–60% leaf area, ..., 5 = 20–0% leaf area), as determined in 2014, were randomly selected (*n* = 6 per class, except for the irrigated plots, where *n* was 2 and 1 for classes 4 and 5, respectively). The trees were randomly distributed over the four plots per treatment. Only trees that were alive and carried at least 10% green needles in February 2015 were included. Sampling took place on 24–25 February, 8–9 June and 26–27 October 2015. Sapwood samples of the trunks of all 60 trees were taken with an increment corer (5 mm in diameter) at 1, 3 and 5 m above the ground, on the south side of the tree. Two cores were taken per height, with a distance of 20 cm above each other. The first 2 cm of sapwood from the cambium was used and the bark and phloem were removed. The two sapwood cores were pooled. At 7 and 9 m above the ground, small sun-exposed branches were cut. A small piece of branch wood was collected, and 1-, 2- and 3-yr-old needles were separated and pooled for the 7- and 9-m collections. A main root located close to the trunk with a diameter of *c.* 1 cm was collected at a distance of *c.* 30 cm from the stem base. Fine roots (< 2 mm) were collected by following the root to a distance of *c.* 60 cm from the stem base. All samples were immediately placed on dry ice to stop enzymatic activity.

Measurements

NSC All stem, root and needle materials were dried at 60°C until a stable weight was achieved and then ground to a fine powder. NSCs were analyzed following the protocol described by Wong (1990) adapted according to Hoch *et al.* (2002). NSCs are defined here as low-molecular-weight sugars (glucose, fructose and sucrose) plus starch. Ten to 12 mg of ground material were boiled in 2 ml of distilled water for 30 min. After centrifugation, an aliquot of 200 µl was treated with Invertase and Isomerase from baker's yeast (Sigma-Aldrich, St Louis, MO, USA) to degrade sucrose and convert fructose into glucose. The total amount of glucose (sugars) was determined photometrically at 340 nm in a 96-well microplate photometer (HR 7000; Hamilton, Reno, NE, USA) after enzymatic conversion to gluconate-6-phosphate (hexokinase reaction, hexokinase from Sigma Diagnostics, St Louis, MO, USA). The total amount of NSC was measured by taking 500 µl of the extract (including sugars and starch) incubated with a fungal amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich) for 15 h at 49°C to digest starch into glucose. Total glucose (corresponding to NSC) was determined photometrically as described above. The concentration of starch was calculated as NSC minus free sugars. Pure starch and glucose, fructose and sucrose solutions were used as standards, and standard plant powder (Orchard leaves; Leco, St Joseph, MI, USA) was included to control the reproducibility of the extraction. NSC concentrations are expressed on a percentage dry matter basis. Because all samples were run in a single laboratory with no change in protocol during the laboratory processing of samples, issues with the comparison of results across methods or laboratories were obviated (Quentin *et al.*, 2015).

Needle δ^{13}C and C, nitrogen (N) and phosphorus (P) contents Two milligrams (± 0.1 mg) of ground needle material were weighed into tin cups and converted to CO_2 and N_2 in an elemental analyzer *Euro EA* (Hekatech GmbH, Wegberg, Germany), connected to an isotope ratio mass spectrometer (IRMS; Delta V Advantage, Thermo Scientific, Bremen, Germany), to determine the C and N contents and the isotopic compositions. Laboratory standards and international standards with known δ^{13}C values were used for the calibration of the measurements, resulting in a precision of 0.2‰. The isotopic ratios in all samples were expressed in δ notation (‰) relative to the international standard Vienna Pee Dee Belemnite (VPDB). C and N contents were assessed as a percentage relative to dry weight, and total P as per mille relative to dry weight. Total P was determined on acidified samples and measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Optima 7300 DV analysis; Perkin Elmer Inc., Waltham, MA, USA) after pressure digestion with HNO_3 and HF.

Growth The length increment of the sun-exposed shoots was measured for the last 5 yr on two branches per tree. A subsample (*c.* 30–50 needles) of the last three generations of needles from one sun-exposed branch was scanned with a flatbed scanner and the respective fresh and dry weights were measured. Specific leaf area, total leaf area, length and width were calculated from the scans using the free software PIXSTAT v.1.2.0.0 (Schleppi, 2011).

Gas exchange and leaf water potential In the summer of 2016, 1 yr after the extensive sampling campaign for NSC measurements (due to time restrictions the year before), pre-dawn leaf water potential and gas exchange measurements were carried out. These measurements were taken on 24 of 53 trees sampled in 2015. In both treatments, 12 trees were selected, evenly divided over leaf area classes 1, 3 and 4–5. Leaf water potential was measured before sunrise, between 03:30 and 05:30 h. With a pole pruner, a small twig was cut from a branch at *c.* 9 m aboveground. The bark was removed to make it easier to distinguish between water and resin. Leaf/twig water potential was measured using a Scholander bomb (Model 600 pressure bomb; PMS Instrument Company, Albany, NY, USA) in steps of 0.05 mPa. Gas exchange (stomatal conductance and A_{max}) was measured using an LI6400 Portable Photosynthesis System (LiCor Inc., Lincoln, NE, USA) in June, July and October of 2016. A branch of *c.* 50 cm in length was cut with a pole pruner and immediately placed in water. Approximately 20 needles were enclosed in the 2×3 cm chamber and photosynthesis (A_{max}) was measured under 400 µmol mol^{-1} CO_2, 1200 photosynthetically active radiation, 60–70% relative humidity (RH) and 22°C.

Statistical analysis

To validate that the selected trees were a good representation of the population, DBH and diameter growth rate of the whole population and the subset used for NSC and other analyses were compared in a paired Student's *t*-test, separately for the different combinations of treatment (irrigation (I), control (C)) and leaf area class groups. In addition, initial DBH in 2002 was compared between control and irrigated plots with a Student's *t*-test, and was found to be significantly different ($P < 0.001$, C = 19.95 cm, I = 22.33 cm). To correct for these differences, we calculated the relative DBH increment from the start of the experiment $[(DBH - DBH_0)/DBH_0 \times 100\%]$, where DBH is the diameter at breast height in cm in 2015 and DBH_0 is DBH at the onset of irrigation in 2003.

Concentrations were log-transformed and, where necessary, other transformations were carried out to obtain the normality of residuals (transformations are given in Tables S3, S4 and S5). Sugars, starch, $\delta^{13}C$, N and P content were analyzed using linear mixed effect models employing maximum likelihood (*lmer* function; LME4 package, R v.3.2.2, R Core Team, Vienna, Austria), and *P* values were calculated based on Satterthwaite's approximations. Fixed factors were treatment (Tr), leaf area class (Cl), season (S), tissue (T) and all two-way interactions. DBH was also considered as a factor in the complete model, but, because it gave non-significant results in all analyses (Table S1), we simplified the model. Tree individual was used as repeated measure factor. Separate analyses were carried out for three tissue groups: (roots, two tissues; wood, three tissues; needles, three generations). The normality of residuals was checked and corrections were made by transformation of the data. Least-square means were calculated for specific differences of significant

factors. Correlation analyses were carried out for NSC and growth, with $\delta^{13}C$, N and P.

Leaf area and tree diameter data were analyzed for all trees in the experimental plot for the years 2002–2014, with linear mixed effect models as indicated above. Fixed factors were year (Y) (for DBH: 2002, 2009, 2015; for leaf area: yearly 2003–2016), treatment (Tr), current leaf area class (Cl) (as indicated in 2014) and their interactions. Tree individual was used as a random factor for repeated measures. Shifts of trees to higher or lower leaf area classes were not taken into account.

Gas exchange and leaf water potential data were also analyzed with linear mixed effect models, with treatment, class and date as fixed factors and tree individual as random factor.

Tree biomass was estimated using allometric equations developed for Scots pine (Forrester *et al.*, 2017). For all tissues (foliage, branch, stem and root mass), the same equation type was used: $[\log_e(biomass) = \log_e(b0) + b1 \times \log_e(DBH)]$. The parameter values used can be found in Table S2. As these equations were developed for average trees, and trees with different crown conditions were included in this study, needle biomass was scaled according to the crown condition. For this, the assumption was made that the average leaf area is *c.* 80%, based on the average leaf area (between 2005 and 2013) for Scots pine in the whole of Switzerland as measured in the Sanasilva forest inventory (A. Gessler, unpublished). Total NSC pools of needles, branch, stem and roots were determined using the calculated biomass and measured NSC concentrations of 1-yr-old needles (N1), 9-m branch wood, 1-m stem wood and coarse roots (CR), and the total tree NSC pool was calculated as the sum of the four tissues. For stem wood, we considered only the outer 2 cm of sapwood in the calculation, as it has been shown that NSC concentrations decline radially from the cambium towards the pith (Hoch *et al.*, 2003). These are estimates and we do not know the exact amounts of NSC in the entire stem. Student's *t*-test was used to test the treatment differences for the total NSC pools of the trees.

Linear regression models were carried out to compare growth with starch and sugar concentrations in winter in all tissues. If necessary, growth was log-transformed.

All statistical calculations were performed with R v.3.2.2 (R Core Team, 2015).

Results

Irrigation did not significantly affect starch concentrations in any tissue examined in the summer season (needles, wood, roots; Fig. 2b; Table S3). Sugar levels, however, were lower with irrigation in the needles and roots (Fig. 2a; Table S3). In stem wood, irrigated and control trees showed comparable sugar levels. Needle $\delta^{13}C$ values were significantly ($P < 0.001$) higher in control trees than in irrigated trees in summer in all three needle age classes examined (Fig. 3; Table S4). No significant treatment effect was found for needle N or P concentrations (Fig. 3; Table S4).

Leaf area was positively related to sugar and starch levels in wood and roots in summer (Fig. 2a,b; Table S3). This trend was largely driven by lower values in two classes with the lowest leaf
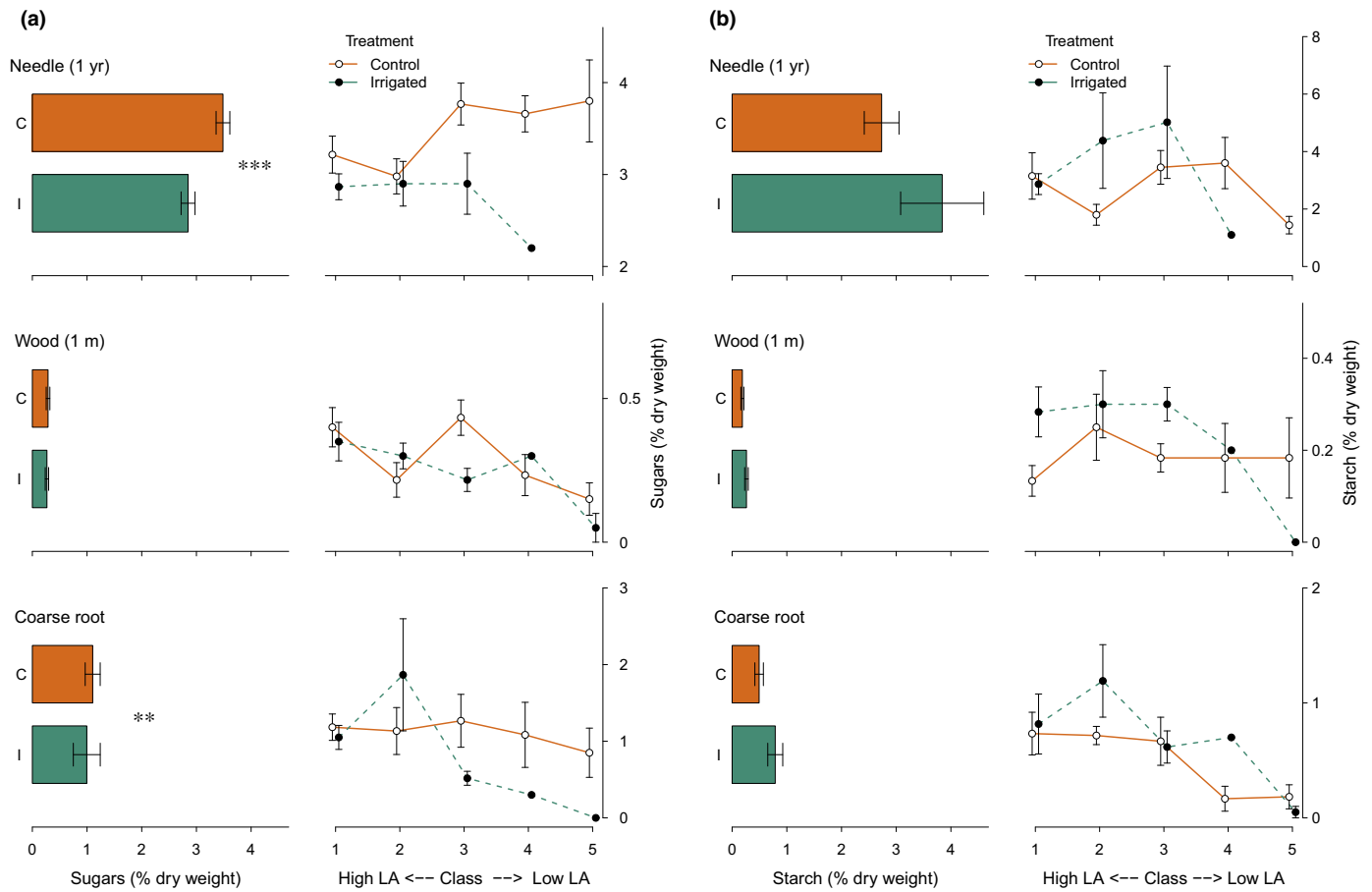
(a)

(b)

Fig. 2 (a) Sugar and (b) starch concentrations in 1-yr-old needles, stem wood at 1 m height and coarse roots of *Pinus sylvestris* in summer. In the left panels, the comparison between control (C) and irrigated (I) plots is shown, on average, for all leaf area classes. Asterisks show significant differences between control and irrigated trees (**, $P < 0.01$; ***, $P < 0.001$). Right panels show concentrations in the three tissues in summer, plotted against relative leaf area (LA) classes for irrigated (green, dotted line) and control (orange, solid line) plots. Bars show $\pm$ SE of the mean. $n = 6$ except for irrigated classes 4 ($n = 1$) and 5 ($n = 2$). In summer, the irrigated trees in class 5 had no needles, resulting in a missing data point.
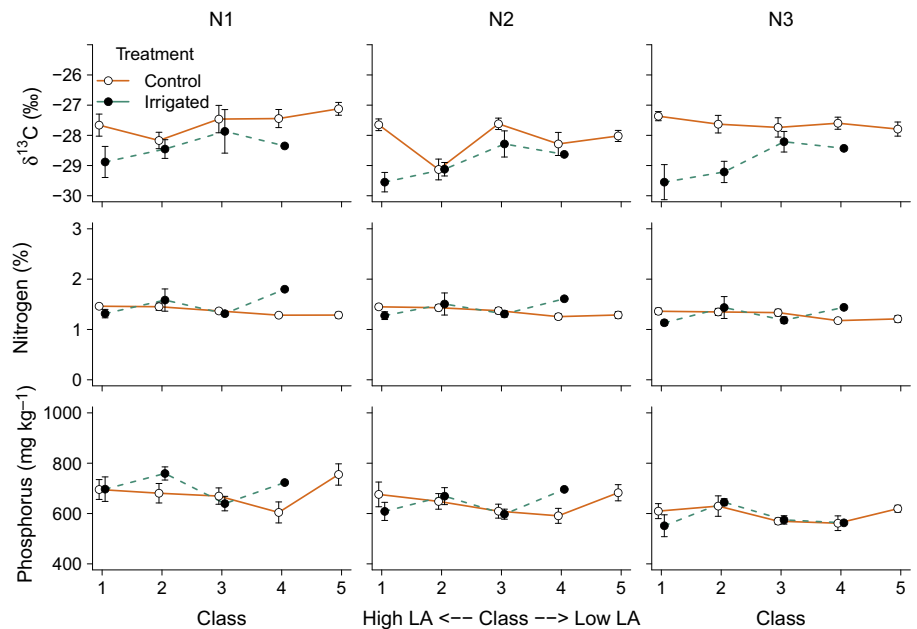

Fig. 3 Summer levels of δ^{13}C, nitrogen and phosphorus in three generations of needles of *Pinus sylvestris*, plotted against leaf area (LA) classes, for control and irrigated plots. Bars show $\pm$ SE of the mean. $n = 6$ except for irrigated classes 4 ($n = 1$) and 5 ($n = 2$). In summer, the irrigated trees in class 5 had no needles, resulting in a missing data point.

area (classes 4 and 5). In the needles of control trees, however, decreasing leaf area was associated with increasing sugar levels (Fig. 2a; Table S3). No significant effects of leaf area were found for needle N or P concentrations (Fig. 3; Table S4), but $\delta^{13}C$ was higher in trees with low leaf area than in those with high leaf area ($P = 0.007$), and $\delta^{13}C$ values increased more rapidly with decreasing leaf area in irrigated trees relative to control trees ($P = 0.017$, Fig. 3; Table S4). NSC was mostly negatively correlated with needle nutrients and positively correlated with $\delta^{13}C$ (Fig. 4). Growth was positively correlated with needle nutrients (N and P) and negatively correlated with $\delta^{13}C$ values (Fig. 4). For all treatment and leaf area effects, similar patterns were found during the autumn and winter seasons, with some exceptions (Figs S2, S3).

Irrigation increased leaf area, especially during the first 4 yr after the start of the treatment (Figs 5a, S4; Table S5), but only trees that are currently in classes 1, 2 and 3 showed a clear positive treatment response in the first 4 yr after irrigation, and this stabilized after 2007 (Fig. 5a; Table S5). Trees from classes 4 and 5 in 2014 had an average initial leaf area of *c.* 40% and, independent of treatment, their leaf area decreased (Fig. 5a; Table S5). The relative DBH increment increased significantly with irrigation in leaf area classes 1 and 2, but, in classes 3–5, the effect of irrigation was not significant (Table S5b), showing that the differences between control and irrigated trees (and hence the positive effect of irrigation) decreases with decreasing leaf area (Fig. 5b; Table S5). The

relative DBH increment was lower in trees with low leaf area in both treatments (Fig. 5b; Table S5b). Shoot growth also decreased with decreasing leaf area in both treatments, but was not influenced by irrigation (Fig. 5c; Table S5).

Irrigation decreased leaf water potential in all trees, but no treatment effect was seen in A_{max} and g_s. No leaf area class effect was found for leaf water potential, A_{max} and g_s (Fig. S5; Table S6).

Shoot growth was positively correlated with total NSC (sugars + starch) and starch levels in winter in fine roots ($P = 0.040$, $R^2 = 0.1$ for NSC, $P = 0.001$, $R^2 = 0.23$ for starch, Fig. S6) and wood taken from 5-m height ($P = 0.004$, $R^2 = 0.18$ for NSC; $P = 0.048$, $R^2 = 0.11$ for starch; Fig. S6). In fine roots, the correlations were stronger in control trees ($P < 0.001$, $R^2 = 0.48$), whereas, in sapwood, from 5-m stem height, the irrigated trees showed a better fit ($P = 0.018$, $R^2 = 0.3$). In irrigated trees, starch levels in needles ($P = 0.033$, $R^2 = 0.26$) were positively correlated with shoot growth (Fig. S6).

Total NSC pools of the trees in irrigated plots were significantly higher than those of trees in control plots in all seasons (Fig. S7; Table S7).

Discussion

We tested the hypothesis that 13 yr of irrigation, and thus drought release, increases the trees' C storage pool in drought-exposed *P. sylvestris*. In contrast with our hypothesis, NSC
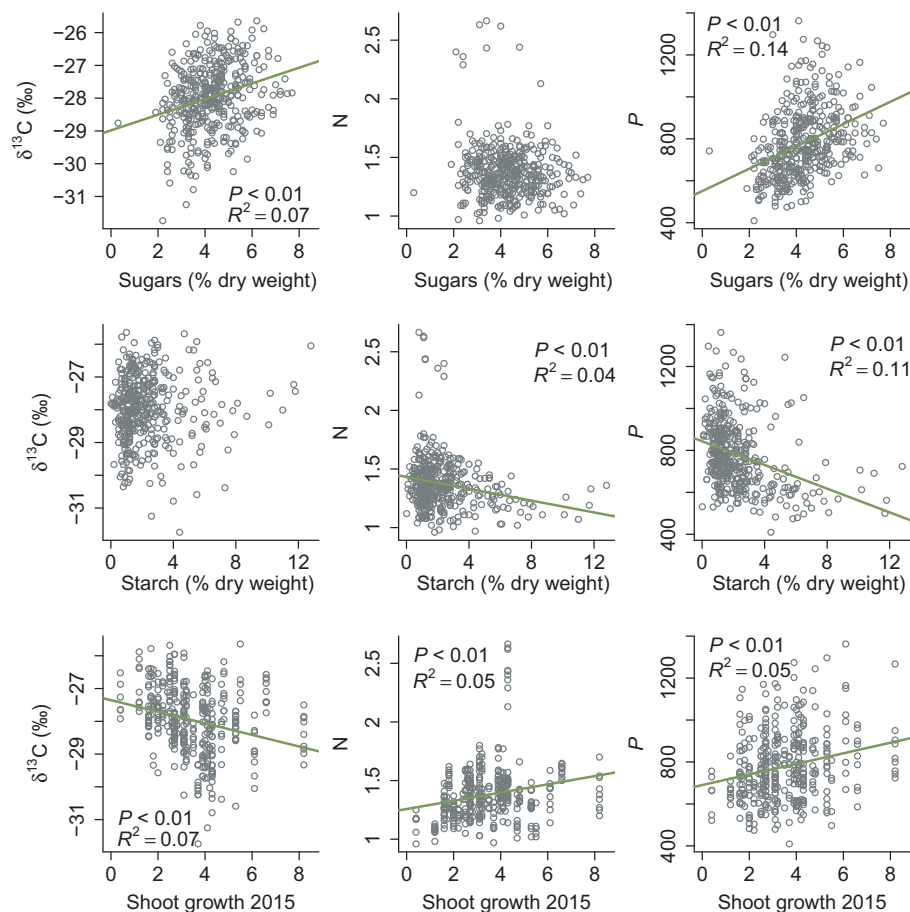

Fig. 4 Correlation between sugars, starch and shoot growth in 2015, on the one hand (x-axes), and $\delta^{13}C$, nitrogen and phosphorus on the other (y-axes). Regression lines are shown when significant.
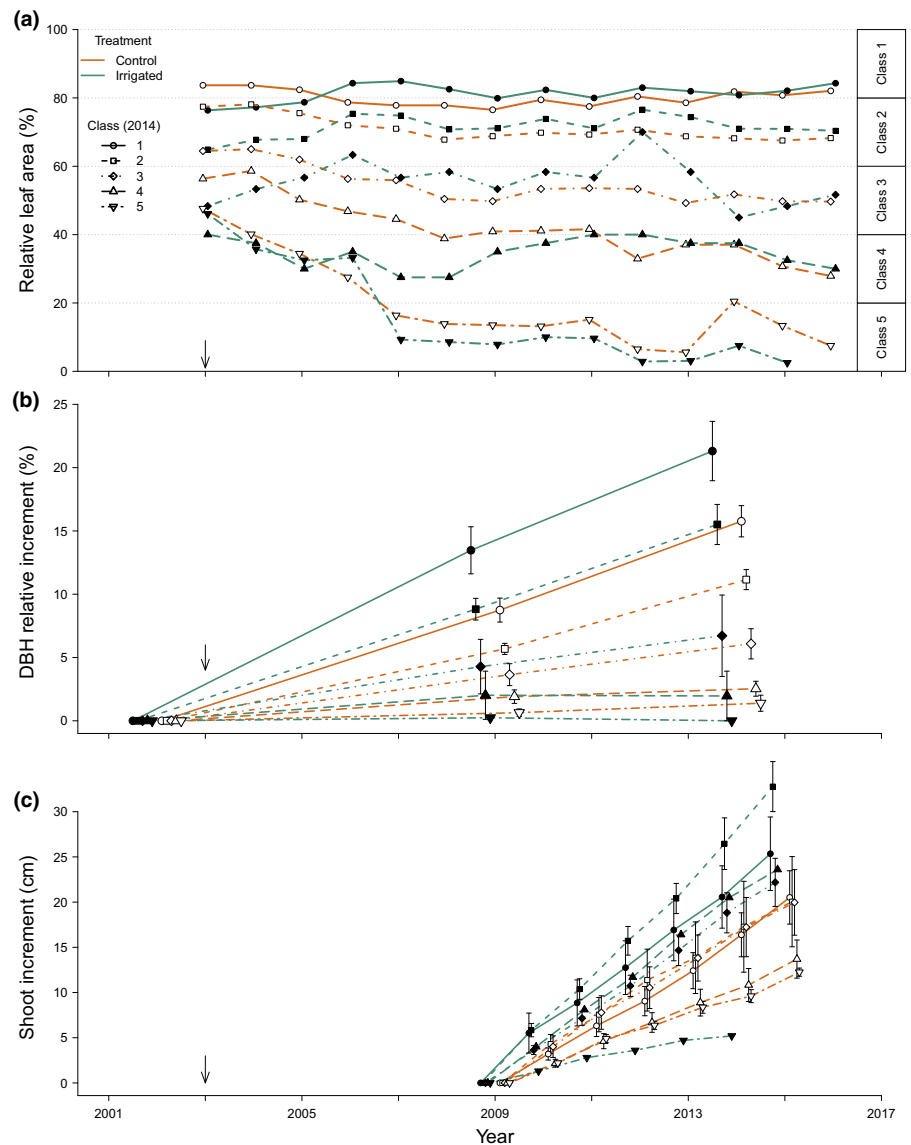
Fig. 5 (a) Development of relative leaf area of *Pinus sylvestris* trees from 2003 to 2015 in control and irrigated plots, divided in five different leaf area classes; (b) cumulative relative increment of diameter at breast height (DBH) of trees in each treatment: leaf area class group; (c) cumulative shoot growth of trees in each treatment: leaf area class group. Bars show $\pm$ SE of the mean. Arrows show the onset of irrigation in 2003.

concentrations did not differ between control and irrigated trees in most cases. Instead, after 13 yr of irrigation, homeostatic NSC levels were found. Needles and root sugars showed even higher concentrations in control trees relative to irrigated trees, which is probably caused by osmotic adjustments (Brodribb & Cochard, 2009; Li *et al.*, 2013; Brunner *et al.*, 2015). We speculate that irrigated trees invested new C preferentially into growth, but not into higher C reserve tissue concentrations; that is, with increased growth the absolute NSC pool increased proportionally, which is in line with von Arx *et al.* (2017), who studied, in the same experiment, the NSC pools in relation to tree ring growth and storage tissues in the sapwood. As a result of the increased biomass production, the total NSC pools of irrigated trees were consequently larger than those of control trees. By contrast, seedlings of the same species prioritized the allocation of new assimilates to storage pools at the expense of growth shortly after drought had stopped, even though the drought did not cause any NSC

depletion (Galiano Pérez *et al.*, 2017). This process might have taken place in our adult trees as well, in the first months or maybe years after the onset of irrigation, but acclimation to long-term irrigation eliminated the short-term need for higher storage input. This is in line with other experiments with Scots pine seedlings that were exposed to two subsequent summer droughts. They also showed homeostatic NSC levels at the end of the second summer (Bachofen *et al.*, 2017). Both our growth and leaf area data support the hypothesis that adult Scots pine acclimates to the new growing conditions reaching a new equilibrium: relative growth rates decreased in 2009–2015 relative to the previous period 2003–2009 in irrigated plots, and the increase in leaf area stabilized after *c.* 4 yr. The increase in soil water content as a result of irrigation (Dobbertin *et al.*, 2010) indicates a decrease in the soil to leaf water potential difference, which allowed trees to increase their total leaf area to maintain high canopy-scale water conductance, as predicted by Darcy's law (McDowell & Allen,

2015). Trees grew towards a new hydraulic limit, with higher growth rates and a higher leaf area. As a result, soil water availability data for the study site showed that irrigated and control plots slowly converged in terms of soil relative water content over the 13-yr duration of the experiment (M. Dawes, pers. comm.). We acknowledge that high leaf area may also offer an increase in drought risk depending on the trees' growth stage, because evapotranspiration loss increases with increasing leaf area.

Although there was no direct effect of drought release on NSC concentrations, leaf area was clearly related to NSC. We found that, irrespective of the treatment, trees with higher leaf area grew faster, had higher NSC concentrations in their roots and, in most seasons, also in the leaves and wood. Decreased leaf area may be a risk factor reducing tree growth under drought and is also an indicator for mortality (Dobbertin & Brang, 2001; Eilmann et al., 2013). The negative influence of defoliation on growth has been reported from several other natural and experimental studies (Galiano et al., 2011; Piper et al., 2015; Puri et al., 2015) and is supported by the growth efficiency theory of Waring (1983). Here, we show that decreasing leaf area not only negatively affects growth, but also C storage. Other studies have also found decreasing NSC levels during defoliation in evergreen and deciduous species (Ericsson et al., 1980; Kolb et al., 1992; Li et al., 2002), but short-term experimental studies normally find a fast recovery after defoliation (Palacio et al., 2012; Puri et al., 2015). However, the trees in such experiments are not pre-exposed to any other stress factor, such as drought. and are not naturally defoliated, as in our study.

Leaf-level indices of gas exchange, such as N and P, and leaf $\delta^{13}C$ were only weakly correlated with NSCs and growth (Fig. 4), suggesting that leaf-level gas exchange was only a weak driver of carbohydrates and growth. This is supported by Feichtinger et al. (2017), who found higher growth rates in response to irrigation, but a high plasticity in gas exchange responses to short- and long-term changes in water availability in Scots pine and European larch. Our results indicate that tree-level leaf area, rather than gas exchange per unit leaf area, best explains the NSC patterns observed. Nutrient availability in the soil was almost unchanged by irrigation (Thimonier et al., 2005, 2010). However, drought can impair soil nutrient uptake and transport to the crown (Rennenberg et al., 2006), and irrigation may thus have led to an improved uptake and transport of nutrients (Feichtinger et al., 2014; Gessler et al., 2016). However, irrigation and leaf area had no impact on the N and P concentrations in needles. We could assume that irrigation may have led to improved soil nutrient uptake, but this increase was balanced by the demand for higher growth compared with the drier control trees, leading to constant tissue concentrations.

A decrease in leaf $\delta^{13}C$ was observed as a result of irrigation. In addition, in irrigated trees, $\delta^{13}C$ increased with decreasing leaf area. The first observation indicated that control trees were indeed exposed to higher drought stress than irrigated trees, confirming the positive effect of additional water on gas exchange (Eilmann et al., 2010). The second observation indicated that even irrigated trees showed signs of drought stress when strongly defoliated. Increasing $\delta^{13}C$ values could be caused by various factors, including increased leaf area and increasing N concentrations. Higher leaf area would lead to lower stomatal conductance per leaf area if other factors were constant, leading to an increase in $\delta^{13}C$ (Mencuccini & Comstock, 1999; Levanič et al., 2011). Most foliar N is stored in Rubisco and therefore directly related to a tree's photosynthetic capacity, reducing the internal CO_2 concentration of the leaf and increasing $\delta^{13}C$. However, as shown above, neither leaf area, stomatal conductance nor N could have caused these increasing $\delta^{13}C$ levels, strengthening the conclusion that these irrigated trees with lower leaf area still suffered from drought stress.

We found large within-population differences in growth and C dynamics that were related to the beginning of the irrigation treatment. Irrigation stimulated growth rates, increased crown cover and reduced the number of trees with strong defoliation symptoms (Fig. S4). Within different leaf area classes, we showed that irrigation mostly positively affected trees with a leaf area higher than 40% (at the onset of the treatment), whereas trees with lower leaf area did not benefit from irrigation, either in terms of growth or crown improvements. The initial leaf area at the beginning of the experiment seems to be a good predictor of growth and C dynamics after 13 yr. In a recent study, Timofeeva et al. (2017) studied living and recently died trees at the Pfynwald site, and found that trees that had died around the year 2013 with low leaf area had exhibited reduced growth since the 1980s. Thus, the process of tree weakening in our study site is a long-lasting and slow process, rather than being triggered by a fast and singular event. Similar to our study, Galiano et al. (2011) investigated mature pines under drought stress which showed different stages of needle loss. In their study, several possible pathways were discussed that could relate NSC to crown foliage loss, and the most likely pathway was that previous drought reduced the amount of green leaves, which, in turn, resulted in lower NSC pools. This pathway leads to a negative feedback (e.g. Manion, 1991), causing trees with a reduced needle area to experience decreasing growth, and therefore limiting the recovery of trees, ultimately resulting in a 'point of no return' after which trees are unable to recover. This idea is corroborated by the finding of a positive correlation between winter NSC storage in fine roots and stem wood (at 5-m height), and shoot growth in the following season (Fig. S6). Overwinter storage is thus crucial for the next season's growth, and reduced NSC accumulation in one year negatively affects growth in the following (Li et al., 2008, 2018). Indeed, we found that the trees that were most defoliated in 2015 (all trees with a relative leaf area of < 20% and some with a relative leaf area of 20–30%) died by 2017. Given the relationship between relative leaf area and NSC, our results also indicate that reduced NSC concentrations are related to, but do not necessarily cause, tree death in Scots pine. This is in agreement with the findings of Adams et al. (2017), who showed that C starvation is a common (but not universal) process associated with tree mortality.

Our results showed that, after 13 yr of irrigation, the treatment led to higher growth rates and improved crown conditions until new hydraulic limits were reached, but to homeostatic NSC concentrations at the individual tree and stand level. Reduced NSC was related to decreased leaf area, but not to gas exchange per

unit leaf area, suggesting that tree leaf area drives the variation in whole-tree carbohydrate dynamics in this study. Historical crown legacies may drive the within-population variability in growth and survival; the process of tree decline is slow and long term, and the present crown cover data predict tree growth and functioning in the future. The NSC constancy across treatments provides evidence that growth : storage ratios may be kept constant, actively, passively or both (Li *et al.*, 2018), when climate changes are sufficiently slow to allow acclimation.

Author contributions

M-H.L., A.G., A.R., M.S. and L.S. designed the study. L.S. and M-H.L. conducted field sampling. L.S. conducted laboratory analyses with the assistance of G.H., and wrote the first draft of the manuscript with the assistance of A.G., N.G.M. and M-H.L. All authors contributed to the interpretation of the results and to the final version of the manuscript.

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References

Adams HD, Zeppel MJB, Anderegg WRL, Hartmann H, Landhäusser SM, Tissue DT, Huxman TE, Hudson PJ, Franz TE, Allen CD *et al.* 2017. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. *Nature Ecology & Evolution* 9: 1285–1291.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH *et al.* 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259: 660–684.

von Arx G, Arzac A, Fonti P, Frank D, Zweifel R, Rigling A, Galiano L, Gessler A, Olano JM. 2017. Responses of sapwood ray parenchyma and non-structural carbohydrates (NSC) of *Pinus sylvestris* to drought and long-term irrigation. *Functional Ecology* 31: 1371–1382.

Bachofen C, Moser B, Hoch G, Ghazoul J, Wohlgemuth T. 2017. No carbon 'bet hedging' in pine seedlings under prolonged summer drought and elevated CO2. *Journal of Ecology* 106: 31–46.

Bigler C, Bräker OU, Bugmann H, Dobbertin M, Rigling A. 2006. Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems* 9: 330–343.

Brodribb TJ, Cochard H. 2009. Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiology* 149: 575–584.

Brunner I, Graf Pannatier E, Frey B, Rigling A, Landolt W, Zimmermann S, Dobbertin M. 2009. Morphological and physiological responses of Scots pine fine roots to water supply in a dry climatic region in Switzerland. *Tree Physiology* 29: 541–550.

Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C. 2015. How tree roots respond to drought. *Frontiers in Plant Science* 6: 547.

Camarero JJ, Gazol A, Sang G, Vicente-Serrano SM. 2015. To die or not to die: early warnings of tree dieback in response to a severe drought. *Journal of Ecology* 103: 44–57.

Chapin FS, Schulze E, Mooney HA. 1990. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* 21: 423–447.

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A *et al.* 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437: 529–533.

Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R. 2014. Nonstructural carbon in woody plants. *Annual Review of Plant Biology* 65: 667–687.

Dobbertin M. 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. *European Journal of Forest Research* 124: 319–333.

Dobbertin M, Brang P. 2001. Crown defoliation improves tree mortality models. *Forest Ecology and Management* 141: 271–284.

Dobbertin M, Eilmann B, Bleuler P, Giuggiola A, Graf Pannatier E, Landolt W, Schleppi P, Rigling A. 2010. Effect of irrigation on needle morphology, shoot and stem growth in a drought-exposed *Pinus sylvestris* forest. *Tree Physiology* 30: 346–360.

Dobbertin M, Hug C, Mizoue N. 2004. Using slides to test for changes in crown defoliation assessment methods. Part I: Visual assessment of slides. *Environmental Monitoring and Assessment* 98: 295–306.

Eilmann B, Buchmann N, Siegwolf R, Saurer M, Rigling PC. 2010. Fast response of Scots pine to improved water availability reflected in tree-ring width and δ13C. *Plant, Cell & Environment* 33: 1351–1360.

Eilmann B, Dobbertin M, Rigling A. 2013. Growth response of Scots pine with different crown transparency status to drought release. *Annals of Forest Science* 70: 685–693.

Ericsson A, Larsson S, Tenow O. 1980. Effects of early and late season defoliation on growth and carbohydrate dynamics in Scots pine. *Journal of Applied Ecology* 17: 747–769.

Feichtinger LM, Eilmann B, Buchmann N, Rigling A. 2014. Growth adjustments of conifers to drought and to century-long irrigation. *Forest Ecology and Management* 334: 96–105.

Feichtinger LM, Siegwolf RTW, Gessler A, Buchmann N, Lévesque M, Rigling A. 2017. Plasticity in gas-exchange physiology of mature Scots pine and European larch drive short- and long-term adjustments to changes in water availability. *Plant, Cell & Environment* 40: 1972–1983.

Forrester DI, Tachauer IHH, Annighoefer P, Barbeito I, Pretzsch H, Ruiz-Peinado R, Stark H, Vacchiano G, Zlatanov T, Chakraborty T *et al.* 2017. Generalized biomass and leaf area allometric equations for European tree species incorporating stand structure, tree age and climate. *Forest Ecology and Management* 396: 160–175.

Galiano L, Martínez-Vilalta J, Lloret F. 2011. Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytologist* 190: 750–759.

Galiano Pérez L, Timofeeva G, Saurer M, Siegwolf R, Martínez-Vilalta J, Hommel R, Gessler A. 2017. The fate of recently fixed carbon after drought release: towards unravelling C storage regulation in *Tilia platyphyllos* and *Pinus sylvestris*. *Plant, Cell & Environment* 40: 1711–1724.

Gessler A, Schaub M, McDowell NG. 2016. The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* 214: 513–520.

Gessler A, Treydte K. 2016. The fate and age of carbon – insights into the storage and remobilization dynamics in trees. *New Phytologist* 209: 1338–1340.

Gottardini E, Cristofolini F, Cristofori A, Camin F, Calderisi M, Ferretti M. 2016. Consistent response of crown transparency, shoot growth and leaf traits on Norway spruce (*Picea abies* (L.) H. Karst.) trees along an elevation gradient in northern Italy. *Ecological Indicators* 60: 1041–1044.

Hartmann H, Trumbore S. 2016. Understanding the roles of nonstructural carbohydrates in forest trees – from what we can measure to what we want to know. *New Phytologist* 211: 386–403.

Hoch G. 2015. Carbon reserves as indicators for carbon limitation in trees. In: Lüttge U, Beyschlag W, eds. *Progress in botany*. Cham, Switzerland: Springer International Publishing, 321–346.

Hoch G, Popp M, Körner C. 2002. Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos* 98: 361–374.

Hoch G, Richter A, Körner C. 2003. Non-structural carbon compounds in temperate forest trees. *Plant, Cell & Environment* 26: 1067–1081.

Klein T. 2015. Drought-induced tree mortality: from discrete observations to comprehensive research. *Tree Physiology* 35: 225–228.

Kolb TE, McCormick LH, Simons EE, Jeffery DJ. 1992. Impact of pear thrips damage on root carbohydrate, sap, and crown characteristics of sugar maples in a Pennsylvania sugarbush. *Forest Science* 38: 381–392.

Körner C. 2015. Paradigm shift in plant growth control. *Current Opinion in Plant Biology* 25: 107–114.

Levanič T, Čater M, McDowell NG. 2011. Associations between growth, wood anatomy, carbon isotope discrimination and mortality in a *Quercus robur* forest. *Tree Physiology* 31: 298–308.

Li M-H, Cherubini P, Dobbertin M, Arend M, Xiao W-F, Rigling A. 2013. Responses of leaf nitrogen and mobile carbohydrates in different *Quercus* species/provenances to moderate climate changes. *Plant Biology* 15: 177–184.

Li M-H, Hoch G, Körner C. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees* 16: 331–337.

Li M-H, Xiao W-F, Wang S-G, Cheng G-W, Cherubini P, Cai X-H, Liu X-L, Wang X-D, Zhu W-Z. 2008. Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain limitation but not for growth limitation. *Tree Physiology* 28: 1287–1296.

Li M-H, Yong J, Wang A, Li X, Wanze Z, Yan C-F, Du Z, Shi Z, Lei J, Schönbeck L et al. 2018. Active summer carbon storage for winter persistence in trees at the cold alpine treeline. *Tree Physiology*. doi: 10.1093/treephys/tpy020

Manion P. 1991. *Tree disease concepts*. Upper Saddle River, NJ, USA: Prentice Hall.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* 155: 1051–1059.

McDowell NG, Allen CD. 2015. Darcy's law predicts widespread forest mortality under climate warming. *Nature Climate Change* 5: 669–672.

McDowell NG, Fisher RA, Xu C, Domec JCC, Hölttä T, Mackay DS, Sperry JS, Boutz A, Dickman L, Gehres N et al. 2013. Evaluating theories of drought-induced vegetation mortality using a multimodel – experiment framework. *New Phytologist* 200: 304–321.

Mencuccini M, Comstock J. 1999. Variability in hydraulic architecture and gas exchange of common bean (*Phaseolus vulgaris*) cultivars under well-watered conditions: interactions with leaf size. *Australian Journal of Plant Physiology* 26: 115.

Palacio S, Hernández R, Maestro-Martínez M, Camarero JJ. 2012. Fast replenishment of initial carbon stores after defoliation by the pine processionary moth and its relationship to the re-growth ability of trees. *Trees – Structure and Function* 26: 1627–1640.

Palacio S, Hoch G, Sala A, Körner C, Millard P. 2014. Does carbon storage limit tree growth? *New Phytologist* 201: 1096–1100.

Piper FI, Gundale MJ, Fajardo A. 2015. Extreme defoliation reduces tree growth but not C and N storage in a winter-deciduous species. *Annals of Botany* 115: 1093–1103.

Puri E, Hoch G, Körner C. 2015. Defoliation reduces growth but not carbon reserves in Mediterranean *Pinus pinaster* trees. *Trees* 29: 1187–1196.

Quentin AG, Pinkard EA, Ryan MG, Tissue DT, Baggett LS, Adams HD, Maillard P, Marchand J, Landhäusser SM, Lacointe A et al. 2015. Non-structural carbohydrates in woody plants compared among laboratories. *Tree Physiology* 35: 1146–1165.

R Core Team. 2015. *R (3.2.2): a language and environment for statistical computing*. R Foundation for Statistical Computing. [WWW document] URL https://www.r-project.org [accessed 18 April 2018].

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A. 2006. Physiological responses of forest trees to heat and drought. *Plant Biology* 8: 556–571.

Rigling A, Bigler C, Eilmann B, Feldmeyer-Christe E, Gimmi U, Ginzler C, Graf U, Mayer P, Vacchiano G, Weber P et al. 2013. Driving factors of a vegetation shift from Scots pine to pubescent oak in dry Alpine forests. *Global Change Biology* 19: 229–240.

Sala A, Woodruff DR, Meinzer FC. 2012. Carbon dynamics in trees: feast or famine? *Tree Physiology* 32: 764–775.

Schleppi P, 2011. Pixstat 1.2.0.0 [WWW document] URL http://www.wsl.ch/dienstleistungenprodukte/software/pixstat [accessed 9 May 2018].

Thimonier A, Graf Pannatier E, Schmitt M, Waldner P, Walthert L, Schleppi P, Dobbertin M, Kräuchi N. 2010. Does exceeding the critical loads for nitrogen alter nitrate leaching, the nutrient status of trees and their crown condition at Swiss Long-term Forest Ecosystem Research (LWF) sites? *European Journal of Forest Research* 129: 443–461.

Thimonier A, Schmitt M, Waldner P, Rihm B. 2005. Atmospheric deposition on Swiss Long-Term Forest Ecosystem Research (LWF) plots. *Environmental Monitoring and Assessment* 104: 81–118.

Timofeeva G, Treydte K, Bugmann H, Rigling A, Schaub M, Siegwolf R, Saurer M. 2017. Long-term effects of drought on tree-ring growth and carbon isotope variability in Scots pine in a dry environment. *Tree Physiology* 37: 1028–1041.

Waring RH. 1983. Estimating forest growth and efficiency in relation to canopy leaf area. *Advances in Ecological Research* 13: 327–354.

Wiley E, Helliker B. 2012. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytologist* 195: 285–289.

Wong S. 1990. Elevated atmospheric partial pressure of CO_2 and plant growth: II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. *Photosynthesis Research* 23: 171–180.

Supporting Information

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

Fig. S1 Volumetric water content (VWC) of the soil in the Pfynwald experiment.

Fig. S2 Winter levels of sugars and starch in needles, stem and roots in control and irrigated plots.

Fig. S3 Autumn levels of sugars and starch in needles, stem and roots in control and irrigated plots.

Fig. S4 Histogram of distribution of trees with different relative leaf areas in control and irrigated plots.

Fig. S5 Leaf water potential, stomatal conductance and photosynthesis rates in control and irrigated plots.

Fig. S6 Shoot growth correlated to winter total nonstructural carbohydrate (NSC) and starch levels in fine roots, 5-m stem wood and 3-yr-old needles.

Fig. S7 Total nonstructural carbohydrate (NSC) pools in control and irrigated trees in kilograms.

Table S1 Results of the correlation analysis between diameter at breast height (DBH) in 2002 and 2014, and nonstructural carbohydrate (NSC), sugars and starch

Table S2 Parameters derived from the calculation of Forrester *et al.* (2017) of foliage, branch, stem and root biomass of *Pinus sylvestris* trees

Table S3 Results of the linear mixed effect models explaining starch, sugars and total nonstructural carbohydrate (NSC) in needles (three generations), wood (three stem height samples) and roots (coarse and fine)

Table S4 Results of the linear mixed effect models explaining summer δ^{13}C, nitrogen and phosphorus levels in needles

Table S5 Results of the linear mixed effect models explaining relative leaf area, shoot growth, absolute diameter at breast height (DBH) and growth rate of *Pinus sylvestris* trees

Table S6 Results of the ANOVA and linear mixed effect models explaining predawn leaf water potential (LWP), photosynthesis (A_{max}) and stomatal conductance (g_s) in *Pinus sylvestris* trees

Table S7 Results of Student's *t*-test comparing total nonstructural carbohydrate (NSC) pools (in kg) in irrigated and control trees in winter, summer and autumn

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