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Research paper

Drought stress, growth and nonstructural carbohydrate dynamics of pine trees in a semi-arid forest

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In trees exposed to prolonged drought, both carbon uptake (C source) and growth (C sink) typically decrease. This correlation raises two important questions: (i) to what degree is tree growth limited by C availability; and (ii) is growth limited by concurrent C storage (e.g., as nonstructural carbohydrates, NSC)? To test the relationships between drought, growth and C reserves, we monitored the changes in NSC levels and constructed stem growth chronologies of mature *Pinus halepensis* Miller trees of three drought stress levels growing in Yatir forest, Israel, at the dry distribution limit of forests. Moderately stressed and stressed trees showed 34 and 14% of the stem growth, 71 and 31% of the sap flux density, and 79 and 66% of the final needle length of healthy trees in 2012. In spite of these large reductions in growth and sap flow, both starch and soluble sugar concentrations in the branches of these trees were similar in all trees throughout the dry season (2–4% dry mass). At the same time, the root starch concentrations of moderately stressed and stressed trees were 47 and 58% of those of healthy trees, but never <2% dry mass. Our results show that all the studied trees maintain a fairly good coordination between C supply and demand, and even during prolonged drought there is more than one way for a tree to maintain a positive C balance.

Keywords: carbon balance, carbon transport, phloem, tree rings.

Introduction

Maintaining a positive carbon (C) balance during drought is often considered a major challenge for trees (Galiano et al. 2011, Mitchell et al. 2013). Yet, it is unresolved whether trees actually suffer from C supply limitations during drought, because their ability to invest C may be constrained more than uptake (Körner 2003). When C limitation is assumed, the classical reasoning starts with stomatal closure and thus, diminished C uptake, while at the same time maintenance respiration continues or may even increase if concurrent warming takes place (Maseyk et al. 2008a, Adams et al. 2009, Klein et al. 2011, Poyatos et al. 2013). Soil water exploration through root proliferation, xylem refilling (generally assumed to involve some C cost, Sala et al. 2012) and osmoregulation then depends on C reserves. While all plants store C, growth constraints

commonly cause the accumulation of C reserves as is well documented from unicellular plants to trees (Estiarte et al. 1999, Würth et al. 2005). Therefore, drought-induced reduction in growth can lead to increases in C reserves under some conditions (Sala and Hoch 2009). It was recently suggested that C reserve formation is not only an overflow response or a precautionary measure during periods of abundant C uptake, but may even become enhanced during periods of C shortage (Sala et al. 2012, Wiley and Helliker 2012). That view is in conflict with the evidence from crops to trees that meristematic processes are far more sensitive to drought stress than photosynthetic C acquisition (Körner 2003, Muller et al. 2011, Palacio et al. 2014). At the onset of water shortage, or during mild or short drought, nonstructural C pools have been found to rise in multiple tree species (Körner 2003, Würth

et al. 2005, Sala et al. 2010, Mitchell et al. 2013), and even the combination of drought and defoliation did not cause substantial long-term C deficits in Mediterranean pines (although a transient decrease was observed; Palacio et al. 2014). During prolonged and very severe drought, trees might enter a phase of reserve reduction, either systemically or in specific organs (McDowell et al. 2008, 2011, Mitchell et al. 2013). Should C starvation come into play, other effects have been presumed to follow, such as decreased phloem transport due to loss of hydraulic conductivity (McDowell et al. 2011) and nutrient deficiency, although the empirical evidence is lacking. Increasing atmospheric CO₂ concentrations at otherwise unchanged supplies of other resources are reducing the likelihood of such C starvation (Oren et al. 2001, Körner et al. 2005).

Recently reported drought impacts on forests include increased tree die-off in multiple locations across the globe (Allen et al. 2010 and references therein), transient declines in forest productivity (Baldochi 1997, Ciais et al. 2005, Granier et al. 2007, Dorman et al. 2013), decrease in tree growth (Leuzinger et al. 2005) and higher sensitivity to attack by biotic agents (Allen et al. 2010). Such impacts may be exacerbated if expectations for increases in intensity or higher frequency of drought events in many regions worldwide are realized (Alpert et al. 2006, Burke et al. 2006, Christensen et al. 2007, Zhang et al. 2007). The physiological causes of drought-induced tree die-off usually involve tissue desiccation, but not necessarily problems with the tree's C balance. In this respect, some questions are still open: to what degree can C reserves such as nonstructural carbohydrate (NSC) support tree activities like respiration under drought? How frequently and under which conditions is the utilization of NSC limited by phloem transport? What are the upper and lower limits of C storage (i.e., in % of wood tissue dry mass (d.m.))? Do trees either enhance stores or utilize them under drought stress? Answers to each of these questions can have large consequences at the ecosystem scale. For example, if C storage is a net outcome of the discrepancy between meristematic and stomatal sensitivity to drought, trees would enter the severe phase with a surplus of C, thus enabling higher resilience to drought (O'Brien et al. 2014). As another example, there may be a lower limit for the use of stored nonstructural C (i.e., a quasi-sequestration of reserves, Millard et al. 2007), meaning that reserves cannot be entirely mobilized under extreme conditions. It is also possible that the inner parts of starch granules are not accessible to degrading enzymes (Srichuwong and Jane 2007) or that sugars cannot be transported in the phloem due to xylem embolism. In fact, it is very likely that C reserve compounds that have additional metabolic or osmotic functions (e.g., low-molecular weight carbohydrates) will never decrease to zero in living cells, even in plants dying of C starvation. Nevertheless, should a dying tree approach that low threshold of zero or residual NSC, then C starvation can be identified as a cause of

mortality. Alternatively, NSC may stay high and hydraulic failure might take place, especially if some level of stomatal conductance is maintained even under prolonged drought.

One approach to test the relationships between drought, drought stress, growth and C reserves is to monitor the changes in NSC levels in trees growing at the dry limit of forest existence and relate these changes to measurements of growth and hydraulic functioning. The Aleppo pine (*Pinus halepensis* Miller) forest in Yatir, Israel, is such an extreme case, with a mean annual precipitation of 285 mm and 6–8 months of continuous seasonal drought (Grünzweig et al. 2003, Rotenberg and Yakir 2010). Trees in this forest are well adjusted to the harsh local conditions of a long-term steady drought, as opposed to sporadic drought events in milder sites. During the long dry season, photosynthesis is not completely inhibited, but is restricted to morning and late afternoon; however, new needles grow exclusively in the dry period (Klein et al. 2005, Maseyk et al. 2008b). Two consecutive years with annual precipitation of 73 and 60% of the annual mean (2008–09) were followed by mortality of 5–10% of all trees in Yatir forest, with surviving trees showing various levels of stress (Klein et al. 2014). This low precipitation period, on top of the steady seasonal drought, permitted an extreme drought stress study that acknowledges the important role of climate extremes (rather than changes in the mean) in tree function (Reyer et al. 2012).

In this study, we test the relationships between drought stress, stem growth and NSC dynamics in a mature forest setting. We analyzed branch and root NSC during the 2012 dry season in *P. halepensis* trees of three stress levels in the semi-arid Yatir forest. An additional measurement was taken in the dry season of 2013, including trees that had died very recently. Stem growth history of the trees was reconstructed from tree rings. With this experimental design, we sought to answer the following research questions: (i) did growth completely stop at all levels of drought stress? (ii) Were tissue concentrations of NSC lower (due to stronger restrictions of photosynthesis) or higher (due to stronger restrictions of C sinks) in drought-stressed than healthy trees? And (iii) was NSC completely depleted in drought-stressed trees? The results will help explore whether growth is limited during drought by a general C shortage, a C shortage for growth because of competition with storage, or if C shortage is not causally involved in growth cessation under drought.

Materials and methods

Site and climate description

Our study was conducted in Yatir forest, a 45-year-old *P. halepensis* plantation located at the northern edge of the Negev desert, Israel (31°20'N, 35°20'E). The forest covers an area of 2800 ha and lies on a predominantly light brown Rendzina soil (79 ± 45.7 cm deep), overlying chalk and

limestone bedrock. Stand density is ~ 300 trees ha^{-1} , mean tree height is 10.2 ± 2.49 m and mean diameter at breast height (DBH) is 19.8 ± 5.61 cm, leading to an average leaf area index of ~ 1.50 . The climate is hot and dry (40-year average mean annual temperature and precipitation are 18°C and 285 ± 88 mm, respectively). Rainfall is strongly restricted to the period between October and April, and hence the local hydrological year is defined between 1 October of the previous calendar year and 30 September of the current year. In 2008, 2009 and 2011, annual precipitation amounts were smaller than the long-term mean by ~ 1 SD or more (Figure 1).

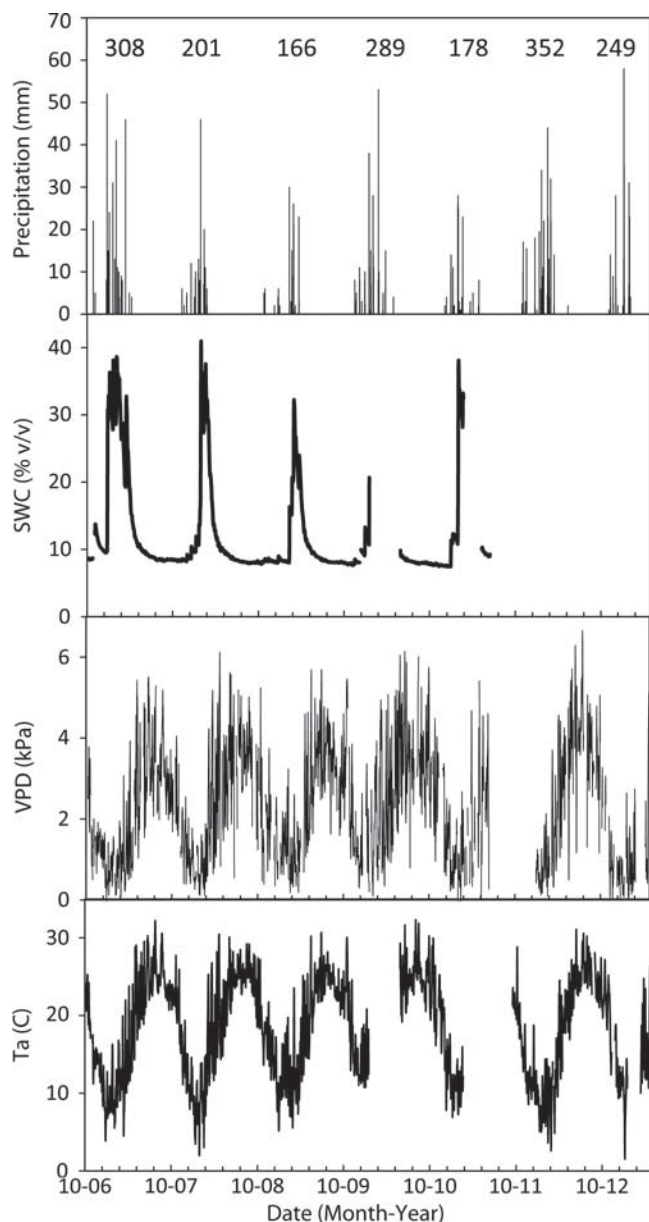


Figure 1. Site environmental variables in 2007–13: daily precipitation (annual amounts noted); daily mean soil water content at 30 cm below surface (SWC); daily maximum vapor pressure deficit (VPD); daily mean air temperature over the forest canopy (T_a). Gaps in the curves are due to technical impediments.

Such drought years are characterized by smaller rain events, as in 2009 and 2011, or a shorter wet season as in 2008. In 2000, an instrumented flux tower was installed in the geographic center of the forest, allowing continuous measurements of major environmental variables (Rotenberg and Yakir 2010). Particularly, soil moisture has been measured continuously using time domain reflectometry sensors (TRIME, IMKO, Inc., Ettlingen, Germany), installed horizontally in three different pits dug in three locations, 5–70 m from the flux tower. All measurements and samples reported here were taken within the flux tower footprint, 20–100 m from the tower base.

Characterization of drought stress level

Following the two consecutive drought years of 2008 and 2009 (Figure 1), stress became visible across most of Yatir forest and 5–10% of all *P. halepensis* trees died in the course of 2010 and 2011 (Klein et al. 2014). At the study plot, as in other parts of the forest, there were large variations in the trees' foliage density and greenness. Some trees seemed unaffected by the drying conditions, while neighboring trees had shorter and chlorotic needles (although not defoliated). These naturally occurring variations can be related to microenvironment heterogeneity (e.g., soil depth and soil water availability), increased intraspecific competition and genotypic differences, or other, as yet unknown, causes. In April 2012, 15 trees of various foliage density and greenness were selected (Table 1). To quantify the foliage density and greenness as indicators of the drought stress level of these trees, we developed a scale of foliage vitality based on a combination of two needle properties, similar in approach to Saffell et al. (2014). At the

Table 1. Individual characteristics of the 15 sampled *P. halepensis* trees (mean (SE)), in descending order by the foliage vitality index. DBH, diameter at breast height; Chl, chlorophyll; conc., concentration. Drought stress levels: H, healthy; M, moderately stressed; and S, stressed.

| Tree ID | DBH (cm) | Needle length (cm) | Needle Chl conc. (mg g d.m.^{-1}) | Foliage vitality ($\mu\text{g Chl. needle}^{-1}$) | Stress level class |
|---------|----------|--------------------|--|---|--------------------|
| 71 | 21.82 | 5.90 (0.20) | 1.40 (0.14) | 20.64 | H |
| 72 | 17.68 | 7.05 (0.17) | 1.10 (0.04) | 21.48 | H |
| 70 | 22.61 | 6.50 (0.11) | 1.13 (0.07) | 24.00 | H |
| 2 | 21.66 | 6.30 (0.08) | 1.05 (0.01) | 26.87 | H |
| 1 | 21.34 | 6.75 (0.25) | 0.94 (0.05) | 25.19 | H |
| 100 | 25.16 | 5.50 (0.20) | 1.08 (0.08) | 14.62 | M |
| 24 | 10.83 | 5.60 (0.15) | 0.95 (0.05) | 14.50 | M |
| 102 | 14.97 | 4.70 (0.13) | 0.99 (0.05) | 17.42 | M |
| 10 | 16.88 | 4.85 (0.26) | 0.92 (0.05) | 19.45 | M |
| 17 | 14.17 | 4.50 (0.00) | 0.99 (0.13) | 15.19 | M |
| 51 | 13.54 | 3.95 (0.28) | 1.06 (0.04) | 7.60 | S |
| 54 | 11.78 | 3.80 (0.19) | 1.04 (0.02) | 13.70 | S |
| 101 | 14.33 | 4.50 (0.11) | 0.58 (0.06) | 12.85 | S |
| 103 | 8.28 | 4.10 (0.07) | 0.62 (0.04) | 8.55 | S |
| 8 | 14.65 | 3.85 (0.21) | 0.61 (0.09) | 8.34 | S |

needle level, needle length was measured and needle chlorophyll concentration was determined after extraction using the 'wet ball-milling' method (O'Neill and Cresser 1980, Klein et al. 2013). The latter was chosen because trees exposed to severe drought commonly appear more yellowish (chlorotic).

One-year-old needles were collected in three replications of 10 each, from each of the 15 study trees. Following length measurement, needles (50 mg leaf tissue per sample) were cut into 5 mm segments and immersed in 1 ml of 80% aqueous acetone solution together with two bearing balls (diameter = 3 mm). Specimens were ground using a ball mill (Retsch, Hann, Germany) at a frequency of 25 s⁻¹ until full extraction was achieved (~5 min), followed by centrifugation at 14,000 rpm for 10 min. The supernatant of each specimen was transferred into a 96-well plate, where its absorbance was measured at 663.6 and 646.6 nm using a tunable micro-plate reader (Molecular Devices, Sunnyvale, CA, USA). Concentrations of chlorophylls *a* and *b* were determined following subtraction of the absorbance of a blank sample (containing solution but not leaf sample) according to Porra et al. (1989). Results were expressed on a dry mass basis following the drying of remaining needles in the sample at 60 °C for 48 h.

Following the chlorophyll measurements, foliage vitality was determined for each of the 15 trees from a single needle chlorophyll content (in µg chlorophyll needle⁻¹), i.e., the product of the mean needle length, mean chlorophyll concentration and an average d.m. per needle length of 3.26 ± 0.08 mg d.m. cm⁻¹. Trees were classified according to the foliage vitality index into one of three stress level classes: healthy (H; vitality > 20), moderately stressed (M; 20 > vitality > 14) and stressed (S; vitality < 14), five trees at each stress level (Table 1). The mean DBH (±SE) were 21.0 ± 0.9, 16.4 ± 2.4 and 12.5 ± 1.1 cm for H, M and S trees, respectively; hence, because all the trees are of the same age, they had a history of differential growth, related to their position along the drought stress gradient (see below).

Sap flow measurement

At the whole tree level, sap flow was monitored continuously in one tree of each stress level class. Sap flow data came from an existing measurement plot that was established in September 2009 and reported in Klein et al. (2014), where the methodology is fully described. Although sap flow was monitored in 16 trees, drought stress level was measured in only three of these trees (one per stress level), to minimize potential effects of the destructive sampling (trunk cores, root samples, twigs and needles) on the stand-level water-use measurement. Briefly, thermal dissipation sensors (Granier and Loustau 1994) were applied, and calibrated with commercial heat balance sensors (EMS, Brno, Czech Republic; Cermak et al. 2004). The radial gradient of sap velocity in these trees was quantified in an earlier study (Cohen et al. 2008), indicating a quasi-linear decrease from maximum sap velocity at 5 mm below

the cambium down to zero at 40 mm. To account for size differences between individual trees, sap flow rates (SF, cm³ h⁻¹) were transformed into sap flux densities (SFD, cm³ h⁻¹ cm⁻²), dividing SF values by the individual sapwood area.

Tree-ring analysis

Stem cores were sampled on 5 December 2013 from all 15 trees, five of each stress level, including one S tree that died during 2013, and one additional dead tree. From each tree, two trunk cores were collected at 1.3 m and at 0°N and 180°S using a 200-mm increment borer (core diameter 5.15 mm; Haglof, Sweden). Tree cores were dried at 80 °C for 72 h and scraped using a scalpel for better reading of the tree-ring structure. Ring widths of the past 30 years were measured at a precision of 1/100 mm using LINTAB tree-ring station connected to TSAP-Win software (Rinntech, Heidelberg, Germany) equipped with a binocular microscope (Leica, Heerbrugg, Switzerland). The 30 ring widths measured from each core were cross-matched graphically, comparing intra- and inter-tree cores. The mean ring width for each tree and year was used in the calculation of the basal area increment (BAI). Stem growth chronologies were constructed by averaging the BAI sequences from each stress level.

Wood material and NSC analysis

Small branches (five 2–3 mm diameter of xylem) were sampled from each of the 15 study trees every 2–3 weeks between 3 May and 30 October 2012. Branchlets had green needles at their terminal parts, and were cut from larger branches in lower tree crowns, 1–3 m above ground. All 11 sampling dates were during the dry season, i.e., after the last rain event that ended on 16 March 2012, and before the first rain event of the following wet season, on 11 November 2012. On three sampling dates (17 May, 26 July and 11 September 2012), three roots (2–5 mm diameter) were also sampled from two to three trees of each stress level class. Roots were dug at 5–10 cm soil depth, 30–50 cm from the stem base. Additional sampling of branches and roots took place on 20 May 2013, this time including two trees that had recently died: one tree that was classified as stressed in summer 2012 and had died since, and one tree that was not sampled before. The time of death was estimated as recent, i.e., within the last 2 months, since (dry) needles had not yet been shed, and the bark was still intact. Within 2 h from sampling, the xylem of all root and branch specimens was dried at 60 °C for 72 h. Between February and June 2013, all samples were ground using a ball mill (Retsch) at a frequency of 25 s⁻¹ until tissues had turned into fine powder (~5 min). Nonstructural carbohydrate analyses followed the method by Wong (1990), modified as described in Hoch et al. (2002). Dried wood powder (8–12 mg) was extracted with 2 ml deionized water at 100 °C for 30 min. An aliquot of each sample extract was taken for the determination of low-molecular weight

carbohydrates using invertase (from Baker's yeast, Sigma-Aldrich, Buchs, Switzerland) to break sucrose into glucose and fructose. Glucose and fructose were converted into gluconate-6-phosphate using glucose hexokinase (Sigma Diagnostics, St Louis, MO, USA) and phosphogluconate isomerase (from Baker's yeast, Sigma-Aldrich). The total amount of formed gluconate-6-phosphate was determined as the increase in NADH + H⁺ using a photometer (HR 700; Hamilton, Reno, NE, USA). For NSC determination, the remaining extract was incubated at 40 °C for 15 h with dialyzed clarase, an amylase from *Aspergillus oryzae* (Enzyme Solutions Pty Ltd, Crydon South, Victoria, Australia) to break starch into glucose. Nonstructural carbohydrate was determined as the total amount of glucose as described above. Starch content was calculated as total NSC minus free sugars. All concentrations were calculated on a % d.m. basis.

Statistical analysis

The effect of drought stress level on measured parameters was tested by suitable statistical tests using JMP (Cary, NC, USA), with $\alpha = 0.05$ across all tests. Differences in needle length and chlorophyll concentration were tested by independent *t*-tests; differences in BAI of specific years were tested by Tukey–Kramer HSD; and differences in BAI across years, starch, soluble sugars and NSC concentrations were tested by multiple analysis of variance (MANOVA), with years or sampling dates as repeated measures. Multiple analysis of variance results also include *P* values for the effect of time and the interaction between stress level and time. Comparison of the mean response in studied parameters across stress level classes was done by *t*-tests.

Results

Drought stress levels

Following the two consecutive drought years of 2008 and 2009 in Yatir forest, soil water availability decreased (Figure 1). For example, annual maximum SWC decreased from 37.9% v/v in 2007 to 32.2% v/v in 2009 (October 2008–September 2009), and the number of days with SWC > 17% v/v (the threshold for water extraction at the main rhizosphere soil layer) decreased from 99 in 2007 to 41 in 2009. Differences among trees of the three drought stress levels (healthy, H; moderately stressed, M; and stressed, S) were quantified at the needle level (Table 1). On average, 1-year-old needles of M and S trees were 21 and 34% shorter and had 4 and 16% less chlorophyll than needles of H trees, respectively. The effect of stress level on needle length was significant, but not the effect on needle chlorophyll concentration (Table 2). Trees of the different stress classes also differed in water-use patterns. Figure 2 shows sap flow rates of one tree per stress level class normalized by the sapwood area and stem diameter, respectively, of each individual

Table 2. The statistical significance (*P* value) of the effect of droughts stress level, time and their interaction on studied parameters. Significant effects are in boldface. Chl, chlorophyll; conc., concentration; NSC, non-structural carbohydrates.

| Parameter | Stress level | Time | Stress level × time |
|-----------------------|--------------|------------------|---------------------|
| <i>t</i> -test | | | |
| Needle length | 0.010 | | |
| Needle Chl. conc. | 0.670 | | |
| MANOVA | | | |
| Basal area increment | 0.319 | <0.001 | 0.219 |
| Branch NSC | 0.290 | 0.009 | 0.210 |
| Branch starch | 0.713 | 0.005 | 0.032 |
| Branch soluble sugars | 0.311 | 0.083 | 0.713 |
| Root NSC | 0.028 | 0.404 | 0.462 |
| Root starch | 0.121 | 0.138 | 0.246 |
| Root soluble sugars | 0.009 | 0.252 | 0.493 |

tree. During late April, the xylem of a healthy tree transported water at a rate of 320 cm³ day⁻¹ cm⁻², compared with 228 and 100 cm³ day⁻¹ cm⁻² in M and S trees, respectively. Sap flow of the S tree also had a 1–2 h shorter diurnal cycle, due to the slower increase in the morning and the early decrease in the evening, but this tree still reached a third of the sap flow density of an H tree.

Stem growth patterns

Tree-ring analysis of stem wood cores from all sample trees showed their stem growth patterns during the past 30 years (Figure 3). The annual BAI was usually lower in S trees than others, but the differences between stress levels were overall not significant (Table 2), also for individual years, until 1999. During the consecutive drought years of 1999–2000, BAI of the M and S trees decreased to 52 and 5% that of the H trees (H and S significantly different; $P < 0.025$). Trees of both stress classes recovered during the following 7 years, but not to the growth level of H trees: differences were not significant at the $\alpha = 0.05$ level; however, the *P*-values were smaller than in earlier years. A clear divergence between H and S trees appeared again in 2008 ($P = 0.015$). In 2012, BAIs of M and S trees were 34 and 14% that of the H trees. The difference between the M and the S trees was not significant at any time, and during two consecutive years (2010–11), S trees grew even better than M trees. The annual BAIs of the H trees correlated well with the annual precipitation (*P*), with a linear regression $BAI = 1.2 \times P + 123$ ($r^2 = 0.48$, $P < 0.0001$; Figure 4). M and S trees yielded regressions with slopes relatively similar to those of the H trees, however, with negative intercepts ($BAI = 1.4 \times P - 61$, $r^2 = 0.56$, $P < 0.0001$; and $BAI = 0.9 \times P + 46$, $r^2 = 0.47$, $P < 0.0001$, respectively). Lack of interaction between precipitation and stress level effects on BAI ($P = 0.301$) indicated that the three slopes were not significantly different.

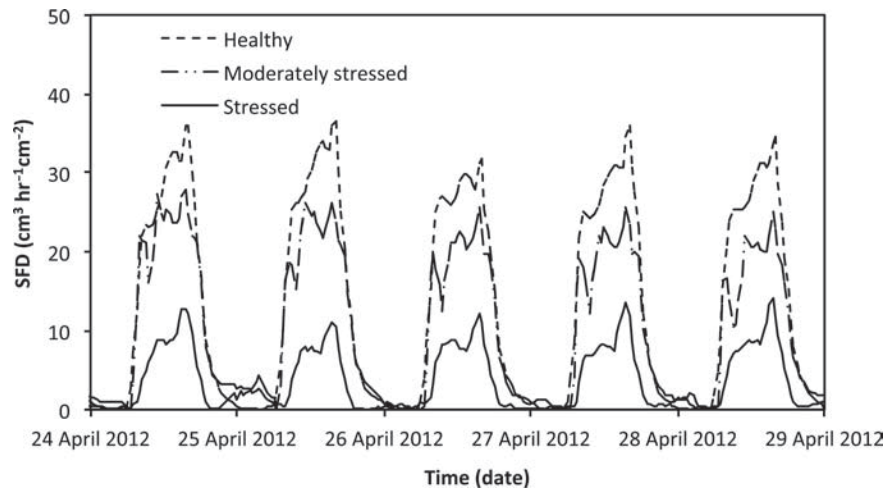


Figure 2. Sap flux density (SFD) of healthy, moderately stressed and stressed *P. halepensis* trees in Yatir forest during 5 days in late April 2012.

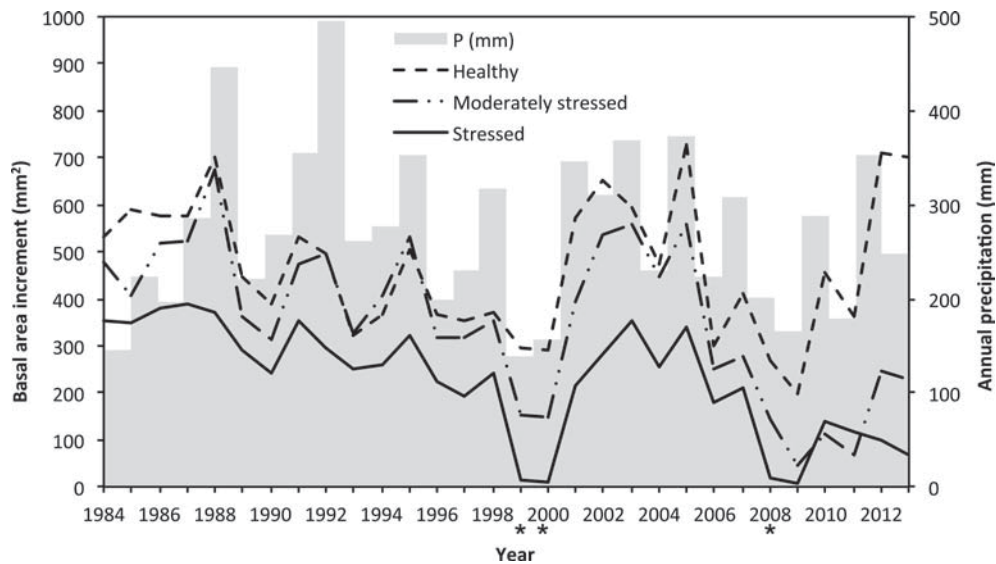


Figure 3. The 1984–2013 stem growth patterns of *P. halepensis* trees characterized in 2012 as healthy, moderately stressed and stressed, with annual precipitation (P) in the background. Values are means ($n = 5$ trees, two cores per tree). Asterisks indicate years when the difference between stress levels was significant by Tukey–Kramer HSD test.

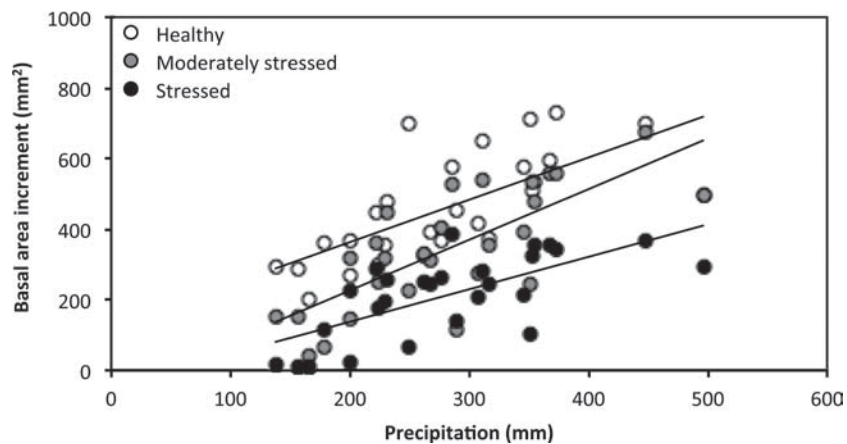


Figure 4. Correlations between basal area increments of *P. halepensis* and annual precipitation in Yatir forest in 1987–2013 for healthy (top line), moderately stressed (middle line) and stressed (bottom line) trees. Each data point is the mean of all trees in a stress level class at a specific year. See the text for regression equations and r^2 .

Branch NSC

As the 2012 dry season progressed, branch NSC of all trees decreased, on average from 8.0% d.m. in early dry season to 5.0 and 4.5% d.m. in peak and late dry seasons, respectively (Figure 5). Starch decreased first, followed by soluble sugars. At the end of the dry season, branches of all trees had 2.0% d.m. starch and 2.1–3.6% d.m. soluble sugars (i.e., 4.0–5.9% NSC). These relatively low NSC levels were observed in H trees also in late May 2013 (5% d.m.; data not shown), while M and S trees had even lower NSC concentrations of 3.7 and 3.4% d.m., respectively. Overall, differences in NSC, starch and soluble sugar concentrations among stress classes were not significant, but the seasonal changes in NSC and starch were significant (Table 2). There was also a significant interaction between stress level and time for branch starch.

Root NSC

Root NSC of all trees decreased during the first half of the dry season but not in the second (Figure 5). In H trees, the root NSC decreased from 10.1% d.m. in early dry season to 7.9% d.m. in late dry season. The stress level had a significant effect on root NSC and soluble sugars, but there was no effect on starch (Table 2), and pooling the data per stress class from all sampling dates yielded the NSC concentrations 8.3 ± 1.0 , 3.4 ± 1.2 and $6.7 \pm 0.8\%$ d.m. for H, M and S trees respectively. Unexpectedly, roots of S trees had significantly higher NSC by 1.6–5.0% d.m. than those of M trees. As the dry season progressed, the starch fraction (as % of total NSC) consistently decreased in all trees, e.g., in H trees from 69% in early dry season to 48% in peak, and 29% in late dry season. In S trees, roots started the dry season with 9.5% d.m. NSC and decreased to 3.7% d.m. in peak dry season, also largely at the expense of the starch fraction.

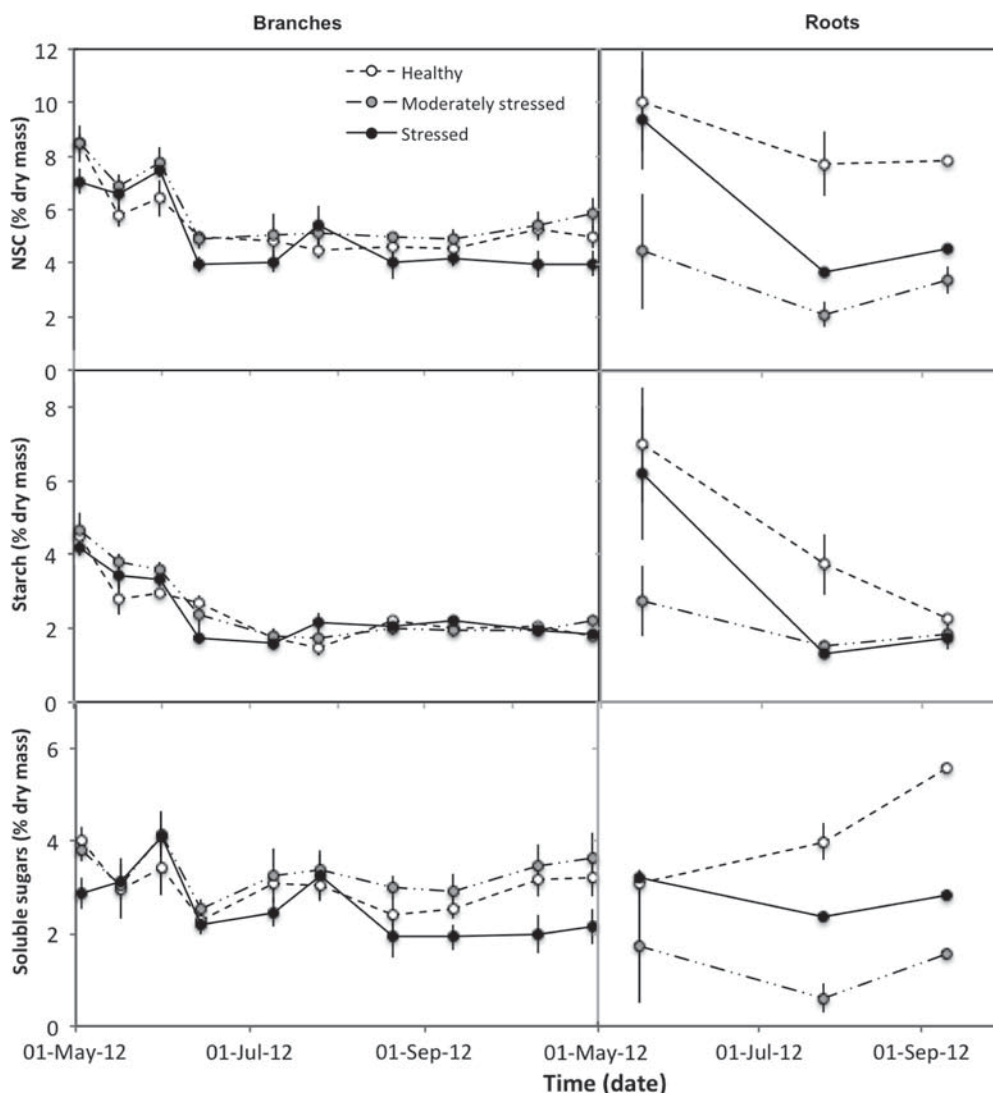


Figure 5. NSC, starch and sugars in branch (left panels) and root (right panels) sapwood of healthy, moderately stressed and stressed *P. halepensis* trees in Yatir forest during the 2012 dry season. Values are means \pm SE ($n = 5$ branches; 2–3 roots).

Interestingly, sugars increased from May to September in H, but not in M and S trees. At the end of the dry season, roots of all trees had 2.0% d.m. starch and various concentrations of soluble sugars, from 1.6% d.m. in M trees to 5.6% d.m. in H trees. These trends prevailed in roots of H, M and S trees in late May 2013, albeit at lower NSC concentrations (6.7, 3.3 and 6.5% d.m., respectively; data not shown).

Discussion

Drought effects on stem growth

Stem growth patterns followed the rate of precipitation as was previously shown for this water-limited forest ecosystem (Klein et al. 2005, 2014) and in other forests (Zeppel et al. 2008, Maseyk et al. 2011, Sarris et al. 2013). Differentiation between the three stress level classes traced back to an earlier drought episode in 1999–2000 (Figure 3). Stem growth stopped completely during 1999–2000 and 2008–09 in S trees but not in H and M trees, providing a negative answer to our first research question whether growth stops at all levels of drought stress. The successive recovery of M and S trees during 2001–07 would most probably hide any phenotypic difference: for example, in 2006 BAI was similar across all trees. This means that the differential responses of trees in 2012 could have been predicted from such dendrological observations, as early as in 1999, and perhaps earlier for S trees. Stem growth of the M and S trees was also lower at low precipitation (P) than that of H trees, with smaller intercepts of regression equations (Figure 4). The positive intercept and relatively high BAI in H trees even in dry years (~300 mm² at P < 250 mm) might indicate that H trees have access to deep water stores (e.g., soil water pockets, cracks, deeper layers and karstic water). The two trees that died in 2012 showed a different behavior from all other groups, with the lowest BAIs in some years (1993–94, 1999, 2008–09) on one hand, and the highest growth among all trees in other years (1988, 1991, 2001–03, 2005) on the other hand (data not shown). The correlation with P was weaker in the two dead trees than in the three stress level classes, the regression had a steeper slope, and the intercept was lower: $BAI = 1.8 \times P - 156$ ($r^2 = 0.39$). It is possible that such large inter-annual growth fluctuations indicate higher vulnerability to drought and involve differences in rooting patterns, but this needs to be tested in a larger number of dying (or dead) trees. The increased growth of H trees following the recovery from the 2008–09 drought is possibly related to the lower competition from their M and S neighbors, i.e., benefiting from higher access to light, water and nutrients at the expense of the weaker trees growing in the same plot.

NSC dynamics during the dry season

Nonstructural carbohydrates decreased as the dry season progressed, but mostly during the first half of the dry season

(after stem growth cessation), and did not change thereafter. The higher NSC levels in the early dry season of 2012 are expected, considering the high photosynthesis rates throughout the wet season, until late April, which appeared to surpass C demand for structural growth (Maseyk et al. 2008b). In Yatir forest, atmospheric evaporative demand becomes severe (VPD > 4 kPa) between early April and late May, and soil water availability in the accessible upper layers drops sharply (SWC < 17% v/v, the water availability threshold in this ecosystem; Klein et al. 2014) between mid-April and mid-May (Figure 1). On average, both these enhancements of moisture stress start around late April. Notably, at that time, C reserves did not increase, as would have been expected from a priority for storage, but they rather decreased (Figure 5). While this decrease cannot be explained by C demand for stem growth, there is still the option of C demand for needle growth, which takes place throughout the dry season. Hoch et al. (2003) showed a similar dynamic in branch sapwood starch in *Pinus sylvestris* L. trees growing in a temperate forest. In that study, starch increased from 3% d.m. in March to 7% d.m. in April and then decreased back to 3% d.m. in the course of the summer growing season, without any drought. Yet needle growth is commonly self-supporting (Sprugel et al. 1991) and mostly relies on current C photosynthate, presumably also in Yatir (Klein et al. 2005).

Aleppo pine root, needle and bark NSC dynamics were studied in Yatir forest in the course of the hydrological year 2001 (Atzmon et al. 2002). In that study, both root and bark NSC increased during the wet season, peaked at the end of the wet season and decreased during the dry season. For comparison with our results, the 2001 root NSC decreased from 17 to 10 and 9% d.m. in early, middle and late dry seasons, respectively. On these dates, starch decreased from 12 to 5 and 3% d.m., while soluble sugars increased from 5 to 5.5% d.m. The starch concentrations reported in Atzmon et al. (2002) are substantially higher than those measured here. Root NSC is highly dependent on root type and age (McClagherty et al. 1982, Würth et al. 2005) and hence this difference could be related to differences in sampling, which was not fully reported in the earlier study. Alternatively, the difference could be related to inter-annual differences in rainfall distribution (as shown here between 2012 and 2013), or could be due to different NSC analysis methodology. However, the relative changes in NSC content and partitioning reported in Atzmon et al. (2002) are similar to our observations during the dry season: (i) NSC decreased as the dry season progressed, mostly during the first half; (ii) soluble sugars increased; and (iii) starch decreased gradually. The NSC levels reported here (1.5–7.0% d.m. starch and 0.6–5.6% d.m. soluble sugars; Figure 5) are similar to those reported for Aleppo pine in other studies (Villar-Salvador et al. 1999, Royo et al. 2001, Fernandes et al. 2003).

NSC, stem growth and drought stress level

In response to the second research question, about the NSC levels in drought-stressed vs healthy trees, the results showed that drought-stressed trees had lower NSC in roots but not in shoots. There was no effect of stress level on branch NSC, and the effect on root NSC was nonlinear (see below). The significant, large differences between trees in stem growth (Figure 3) were not reflected in branch NSC levels. Looking at the NSC levels of individual trees in September 2012, there were no clear relationships with the 2012 stem growth: while NSC levels increased with growth in M and S trees, they decreased in H trees (data not shown). Similarly, the correlation between branch NSC levels in May 2012 and stem growth among all trees yielded a mild negative trend (i.e., slightly lower NSC in fast-growing trees), however with low r^2 of 0.17. Evidently, while stem growth, sap flow density and foliage vitality (μg chlorophyll needle^{-1}) decreased considerably in M trees, and more so in S trees, changes in NSC levels were smaller (Figure 6). Similar NSC levels across trees with various levels of drought stress have already been shown in Mediterranean oaks (Grünzweig et al. 2008). Higher stress sensitivity in stem growth than in NSC at the foliage level has also been reported for Douglas fir in Oregon, USA (Saffell et al. 2014).

The unexpectedly low NSC concentration in the roots of M trees could be explained by one of two alternatives: (1) spatial imbalance between C source (leaves) and C sink (roots) due to constraints in phloem C transport, or (2) temporal imbalance between high C demand for root growth, which might have been especially high in M trees, and C supply, which was decreasing due to the developing stress. Notably, while the first scenario entails some failure in C transport, the second

option precludes it. A spatial imbalance was described in Norway spruce saplings, where induced drought led to a reduction in NSC levels in roots but not in the canopy (Hartmann et al. 2013) and to some extent in potted seedlings of aspen (Galvez et al. 2011, 2013). In Scots pine growing in dry montane terraces, high belowground demand for carbohydrates was related to cessation of stem growth during the growing season (Oberhuber and Gruber 2010). Here, the decoupling between branch and root NSC levels in M trees may suggest a certain level of spatial C imbalance and potentially a phloem transport problem. Yet the root NSC level in M trees increased from 2.1% d.m. to 3.5% d.m. during the second half of the dry season (Figure 5), ruling out that C transport was inhibited.

The possibility of temporal imbalance between C demand and supply is in line with results from a drought experiment on seedlings of the same species (Royo et al. 2001). In that study, there was no drought effect on shoot NSC, with both starch and sugars around 3% d.m., while root sugars decreased from 2.5% d.m. to 0.4% d.m. during severe drought. At the same time, root starch decreased from 4.5% d.m. to 1.3% d.m. during mild drought, but increased up to 5.4% d.m. during severe drought, in agreement with our observation of higher NSC in S than in M trees. High investment in root activity, combined with decreased photosynthetic capacity (due to reduction in foliage vitality, Table 1), could also have reduced root NSC in M trees compared with H and S trees. In contrast, S trees probably had no root activities in terms of growth, and hence, had high NSC concentrations (9.4%) in the early dry season. But as the dry season progressed, NSC levels declined either due to respiration, some growth or export to mycorrhiza. In a drought experiment on Aleppo pine saplings, moderately stressed trees had

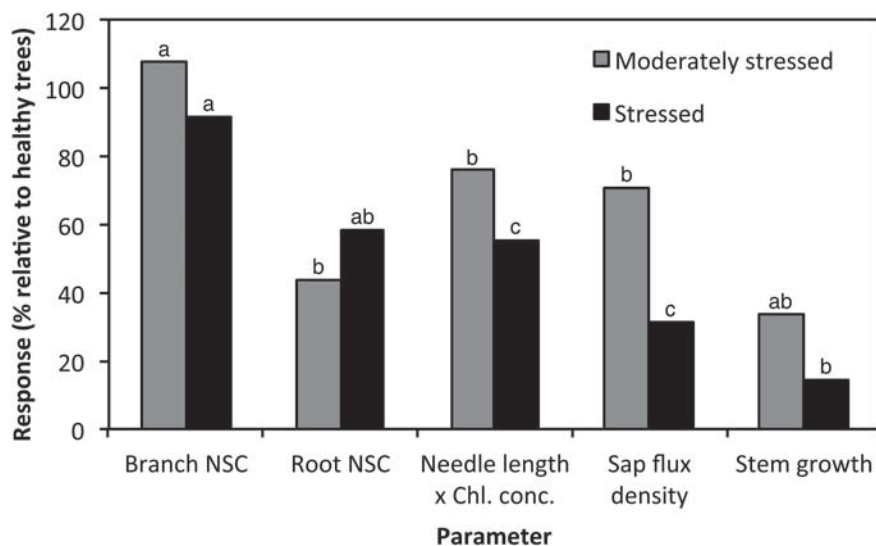


Figure 6. Drought stress level effects on observed parameters, expressed as percentage of the mean value from healthy *P. halepensis* trees in Yatir forest (=100%). Relative responses are for September–October 2012, except for sap flux density, which was measured in April 2012. Relative responses of needle length and chlorophyll concentration were multiplied to serve as proxy for the foliage vitality. Different letters indicate significant differences among stress levels in the absolute values of each parameter by Student's *t* test (healthy = 'a').

the highest projected root area increment, 15% higher than well-watered trees and 55% higher than in severely stressed trees (Klein et al. 2011).

Changes in the relative fractions of starch and soluble sugars in the NSC pool indicate the temporal changes in priorities of the form of NSC in different organs. In branches, starch decreased by 50% during the first half of the dry season without a parallel increase in soluble sugars, suggesting that starch was used for sink activities, such as metabolism, xylem maturation and contemporary needle growth. In roots, however, an increase in soluble sugars occurred in parallel with the decrease in starch. Part of the starch seems to have been hydrolyzed, perhaps for reasons related to osmoregulation or export to other parts of the tree. Yet, this rise in root sugar concentration was not observed in the more stressed M and S trees.

Multiple ways to maintain C balance under drought

Testing the third research question about the potential exhaustion of C reserves, NSC concentration remained >2.1% d.m. at all times in all living trees, regardless of stress levels, and became as high as 9.4% d.m. in the roots of S trees. Moreover, the additional sampling in 2013 indicated that the recovery of C reserves at the end of the 2012 dry season (Figure 5) was not transient, but rather part of a seasonal cycle. The homogeneous minimum 2% d.m. starch concentration maintained in all branches and roots at the end of the dry season is in agreement with NSC analyses in multiple species from various biomes, including four Mediterranean sclerophylls (Körner 2003). In those trees branch NSC decreased from 12% d.m. to 6% d.m. by the end of the growing season and then remained constant. Lower branch and root starch concentration (0.7%) was found only in dead trees (data not shown). If no post-mortem changes occurred, this suggests that: (i) starch was still accessible for sink activities (respiration, growth) in dying trees; and (ii) starch served as a means of last resource, as dying trees might have been able to consume most of the remaining starch. Future research should capture an observation of the NSC level in adult trees at the point of death.

The role of the whole-tree C partitioning in drought-induced tree mortality is the subject of an ongoing discussion (Sala et al. 2010, McDowell et al. 2011, Hartmann et al. 2013). In the trees studied here at the drought limit, it seems that there was little risk of complete exhaustion of NSC, and whole-tree C starvation seems unlikely. The continued sap flow even in the most stressed trees must be accompanied by C gain at the crown level. At the same time, we observed reductions in sink activities in M and S trees: stem growth decreased, and needle growth, presumably relying on current photoassimilates, also decreased. Local C deficiency developed in the roots of M trees in mid dry season, but the maintenance of 2.1% residual C reserve does not indicate depletion. An active transport of C

was probably maintained throughout the dry season and in all trees studied here, as indicated by the active water transport in the phloem-coupled xylem (Figure 2) and the rising NSC concentrations in roots during August (Figure 5).

Overall, our results show that all the studied trees maintain a fairly good coordination between C supply and demand, and the drought stress level affects growth more than it affects C storage. Together, these observations suggest that even under prolonged drought there is more than one way for a tree to maintain a positive C balance: while healthy trees maintain some level of C uptake and continue to grow, stressed trees decrease gas exchange and reduce growth activities dramatically. In both cases, the majority of starch is being consumed during early dry season, but reserves start to build up again at the end of the dry season and up until the next year's dry season.

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Conflict of interest

None declared.

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