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Research paper

Effects of mistletoe removal on growth, N and C reserves, and carbon and oxygen isotope composition in Scots pine hosts

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Most mistletoes are xylem-tapping hemiparasites, which derive their resources from the host's xylem solution. Thus, they affect the host's water relations and resource balance. To understand the physiological mechanisms underlying the mistletoe–host relationship, we experimentally removed *Viscum album* ssp. *austriacum* (Wiesb.) Vollmann from adult *Pinus sylvestris* L. host trees growing in a Swiss dry valley. We analyzed the effects of mistletoe removal over time on host tree growth and on concentrations of nonstructural carbohydrates (NSC) and nitrogen (N) in needles, fine roots and sapwood. In addition, we assessed the $\delta^{13}C$ and $\delta^{18}O$ in host tree rings. After mistletoe removal, $\delta^{13}C$ did not change in newly produced tree rings compared with tree rings in control trees (still infected with mistletoe), but $\delta^{18}O$ values increased. This pattern might be interpreted as a decrease in assimilation (A) and stomatal conductance (g_s), but in our study, it most likely points to an inadequacy of the dual isotope approach. Instead, we interpret the unchanged $\delta^{13}C$ in tree rings upon mistletoe removal as a balanced increase in A and g_s that resulted in a constant intrinsic water use efficiency (defined as A/g_s). Needle area-based concentrations of N, soluble sugars and NSC, as well as needle length, single needle area, tree ring width and shoot growth, were significantly higher in trees from which mistletoe was removed than in control trees. This finding suggests that mistletoe removal results in increased N availability and carbon gain, which in turn leads to increased growth rates of the hosts. Hence, in areas where mistletoe is common and the population is large, mistletoe management (e.g., removal) may be needed to improve the host vigor, growth rate and productivity, especially for relatively small trees and crop trees in xeric growth conditions.

Keywords: mistletoes, nitrogen, nonstructural carbohydrates, soluble sugars, stable carbon isotope, stable oxygen isotope, starch.

Introduction

About 1% of all angiosperm species are parasitic and ~40% of them parasitize the aboveground parts of their host plants (shoot parasites) (Norton and Carpenter 1998). Mistletoes are the predominant group of angiosperm shoot parasites, and they are found widely in boreal and temperate forests, tropical rain forests and arid woodlands (Norton and Carpenter 1998). Under current predictions of global climate changes, mistletoes are expected to expand their geographical ranges (Rigling et al. 2010). As an example, Dobbertin et al. (2005*a*) showed that pine mistletoe (*Viscum album* ssp. *austriacum*) shifted its altitudinal distribution upward by 200 m in the Swiss Rhone Valley due to climate warming during the past century.

Most mistletoes are xylem-tapping plants that derive water and nutrients from the host's xylem solution (Schulze and Ehleringer 1984, Ehleringer et al. 1985, Schulze et al. 1991,

Popp and Richter 1998, Bannister and Strong 2001, Zuber 2004). Thus, mistletoes may exacerbate the effects of environmental stress such as drought and limited resources on host trees (Rigling et al. 2010, Sangüesa-Barreda et al. 2012), leading to increased host mortality (Dobbertin et al. 2005*b*, Dobbertin and Rigling 2006).

Water and nutrient relationships between mistletoes and their hosts have been studied extensively (Glatzel 1983, Orozco et al. 1990, Pate et al. 1990, Küppers et al. 1992, Flanagan et al. 1993, Hosseini et al. 2007, Zweifel et al. 2012). The parasitic plants use two pathways to receive mineral nutrients from the hosts: (i) a passive pathway caused by the parasite's transpiration, where the transport of nutrients occurs with water flow from the host to the parasite, and (ii) an active pathway in which the haustorium actively draws nutrients from the host to the parasitic plant (Lamont 1983). Mistletoes derive nitrogen (N) from their hosts, resulting in lower N concentrations in mistletoe-infected trees than in uninfected trees within a forest community (Ehleringer et al. 1986*a*, Bell and Adams 2011). Nitrogen products extracted by dwarf mistletoe were found to comprise up to 20% of the total N storage in host *Acacia nilotica* (L.) Delile forests in Sudan (Gibson 1967). The N concentration in mistletoes was found to be three to four times higher than that in their hosts (Küppers et al. 1992, Schulze et al. 1994), suggesting that overuse of N by mistletoes may result in N-depletion in the hosts, especially under stressed growth conditions.

Compared with water and nutrients, the carbohydrate relationship between mistletoes and the host is more complicated (Bell and Adams 2011), because all mistletoes are hemiparasites, bearing evergreen leaves that carry out photosynthesis. It has been proposed that mistletoes affect the host carbon (C) balance in various ways (Bickford et al. 2005, Wang et al. 2008, Logan et al. 2013). Mistletoes are able to extract C compounds from the host's xylem (Reblin et al. 2006) and thus directly decrease C availability in the host. In addition, mistletoes absorb water from hosts, which may induce closure of the stomata (Zweifel et al. 2012) and reduce the photosynthetic activity in the leaves of the hosts (Rigling et al. 2010). Therefore, mistletoes can directly reduce the host's ability to gain C under drought-stressed conditions. For example, Zweifel et al. (2012) found that mistletoe infection created a leak in the water flow system of hosts and thus caused stomatal closure and lower transpiration and assimilation rates. Mistletoe-induced decreases in host leaf N may also reduce host photosynthesis, as leaf N concentrations are significantly positively correlated with photosynthetic rates (Marshall et al. 1994, Meinzer et al. 2004). These effects may result in or intensify C starvation and mortality of the hosts (Dobbertin and Rigling 2006, Rigling et al. 2010).

Previous studies have mainly concentrated on mistletoe effects on growth, nutrient and carbohydrate concentrations, and water use efficiency (WUE) at the branch level. In particular, these investigations have compared either mistletoe-infected and uninfected branches within a host tree (Hosseini et al. 2007, Lo Gullo et al. 2012, Logan et al. 2013), or infected and uninfected branch parts of a given branch (Ehleringer et al. 1986*b*, Tennakoon and Pate 1996, Rigling et al. 2010, Tennakoon et al. 2011). Other studies have compared the physiological properties of mistletoes with those of branches they had infected (Küppers et al. 1993, Bannister and Strong 2001, Escher et al. 2004, 2008). These branch-level experiments may underestimate the effects of resource reallocation and mobilization, as well as the feedback mechanisms within the whole host tree. Only a few studies have compared mistletoe effects on growth and physiological parameters at the whole-tree level by comparing mistletoe-infected trees with trees that were never infected (Ehleringer et al. 1986*b*, Marshall et al. 1994, Sala et al. 2001, Miller et al. 2003, Sangüesa-Barreda et al. 2012). However, these tree-level results may be biased by the mistletoe infection history of the infected trees and the specific biological properties (e.g., resistance) of the trees that were never infected.

To better understand the C- and N-balance and WUE in mistletoe-infected trees, we carried out a long-term (8 years) mistletoe removal experiment in a dry valley in Switzerland. In the research area, increased summer temperatures and rain shortage (Rigling et al. 2002, Dobbertin et al. 2005*b*), coupled with the high transpiration rates of mistletoes (Zweifel et al. 2012), may lead to drought-induced C-depletion (Scharpf et al. 1988) and water deficit in host trees (Dobbertin and Rigling 2006). We compared mistletoe-infected Scots pine trees (MIT) with trees where mistletoes (*V. album* ssp. *austriacum*; Noetzli et al. 2003) were removed (MRT). We studied tree needle morphology, shoot growth and stem radial growth, as well as mobile C and N concentrations (starch (ST), sugars and total N) in needles, fine roots and stem sapwood. Moreover, we assessed the tree ring stable C and oxygen (O) isotope composition in tree rings produced 6 years before and 6 years after mistletoe removal. We expected that mistletoe removal would reduce the risk of C-depletion and water deficit in host trees growing in a dry environment. We tested the specific hypotheses that (I) mobile carbohydrate and N concentrations of host trees would increase upon mistletoe removal and (II) the removal of mistletoe would lead to higher assimilation rates, as well as higher stomatal conductance (g_s), in the host trees, and that these changes would be imprinted in tree ring growth and tree ring $\delta^{13}C$ and $\delta^{18}O$ signals (Scheidegger et al. 2000, Gessler et al. 2014).

Materials and methods

Study site, stand and mistletoe treatment

The study site is situated in Pfynwald (46°19′27″N, 7°34′40″E, 610 m above sea level) in the inner-Alpine valley of Valais,

Switzerland. According to climate data gained from the nearest climate station to the site, mean annual precipitation was 657 mm (Zweifel et al. 2009) with large interannual variation, and mean annual temperature was ~9.7 °C over a 50-year period (Dobbertin et al. 2010).

The Pfynwald forest (>10 km^2) is dominated by Scots pine (*Pinus sylvestris* L.) with occasional *Quercus pubescens* Willd. The forest is 90–100 years old, and pine trees have a diameter at breast height (DBH) of ≥12 cm and a mean height of ~11 m. The understory consists of shrubs and young *Q. pubescens*, grasses, mosses and a few dwarf shrub species. The soil type is a Rendzic Leptosol with limestone as the parent material (Rigling et al. 2010).

We selected 12 *P. sylvestris* trees of similar age (90–100 years old) and size (10–12 m in height and 15–20 cm in DBH), similar growth performance and similar intensity of mistletoe infection in 2004. The growth performance and mistletoe infection intensity were visibly estimated. We randomly selected 6 out of those 12 trees to completely remove all attached mistletoe plants from the branch surfaces in September 2004 (after the 2004 growing season), and we repeated removal in September 2007 and 2010. A mean fresh weight of mistletoes of 2.04 ± 0.57 kg was removed from each tree. These six trees were defined as mistletoe-removed trees (MRT). The other six trees were controls with all mistletoes intact (MIT).

Sampling and growth measurements

On 12 July 2012, one south-oriented and one north-oriented leading branch, each with a branch base diameter of >2 cm, were taken from the upper crown section of each tree, i.e., 12 branches were collected for MRT and MIT, respectively. Only unshaded, healthy and undamaged branches with intact terminal buds were selected.

Annual shoot growth was measured along the collected branches. We measured the annual shoot increment for each tree from 1999 to 2010 (i.e., spanning 6 years before to 6 years after the mistletoe treatment occurred in after the growing season 2004). Scots pine shoots can be dated accurately by counting back the internodes or age classes of the branches from the branch tip (Kozlowski 1971, Li et al. 2006). In the present study, the age of needles and shoot segments in each selected branch was determined using marks from overwintering buds on the branch and by counting annual needle cohorts (Li et al. 2001). For example, the length of shoot segments along the leading branch emerged in 2011, 2010 and 2009 was defined as the shoot increments for 2011, 2010 and 2009, respectively. Needles that emerged in 2011 (i.e., attached to the 2011 shoot segment), 2010 and 2009 were named as 1-, 2- and 3-year-old needles, respectively.

Different-aged needles (1-, 2- and 3-year-old) were sampled separately from the collected branches mentioned above for each sample tree. We only took 1- to 3-year-old needles for the present study since very few needles reached an age of 4 years. The current-year needles (i.e., needles emerged in 2012) were also not sampled because they were not mature at the sampling time (12 July 2012), and thus, their carbohydrate concentrations were considered to be highly dynamic and not stable over time (Li et al. 2002). About 25 fresh needles for each of the three needle age classes for each tree (mixed sample collected from the two branches per tree) were scanned at 600 d.p.i. resolution (Epson V500, NSK Ltd., Tokyo, Japan), and the needle length and the projected area were then calculated automatically with the software WinSeedle (Regents Instruments Inc., Quebec, Canada). The specific leaf area per dry weight (SLA) and the leaf mass per unit leaf area (LMA) were calculated.

Using a 5-mm-diameter increment corer, two tree ring cores (one from the south-stem side and one from the north-stem side) were taken from each MIT and MRT at 1.0–1.3 m stem height above the ground surface. These cores were used for measuring tree ring width and stable isotope composition. Annual tree ring width was measured from 1999 to 2010 (as done for shoot growth, spanning 6 years before to 6 years after mistletoe treatment) on a Lintab measuring table (Rinntech, Heidelberg, Germany), and analyzed using the TSAP software (Rinntech).

The tree rings in MRT formed before mistletoe removal (end of 2004) and in MIT over the whole period were very narrow. In order to obtain the minimum amount of material necessary for $\delta^{13}C$ and $\delta^{18}O$ analyses, and thereby assess differences in the isotope composition between MRT and MIT over time, we combined the tree rings formed during 3-year periods (1999–2001, 2002–04, 2005–07 and 2008–10) from two cores per tree and pooled the material as a mixed sample per tree ($n = 6$).

For comparing the effects of mistletoe removal (before and after treatment) in MRT, two additional tree ring cores (also one from the south-stem side and one from the north-stem side at 1.0–1.3 m stem height) were taken from each MRT. These tree rings were separated at a 1-year resolution from 1999 to 2010 (6 years before to 6 years after mistletoe removal), and wood samples with the same age were pooled for pairs of adjacent MRT ($n = 3$), to obtain the minimum amount of material necessary for $\delta^{13}C$ and $\delta^{18}O$ analyses.

The samples were milled, followed by the simultaneous delignification and removal of noncellulosic polysaccharides using an acetic acid : nitric acid mixture, as described by Brendel et al. (2000). This method was used to minimize losses from the small samples. After cellulose purification, the samples were dried in the Pyrex test tubes until a constant weight was reached (Brendel et al. 2000).

Samples for chemical analysis (N and carbohydrate concentrations) included 1-year-old needles, fine roots and stem sapwood. One-year-old needles were collected from the two selected branches per tree and pooled together to get a mixed sample for each tree ($n = 6$ trees). To collect fine roots from MRT and MIT, we first found coarse roots originating from each tree,

and then we collected fine roots (<0.5 cm in diameter) attached to those coarse roots ($n = 6$ trees). Two short tree ring cores (one from the south-stem side and one from the north-stem side) were taken from each tree at a stem height of ~50 cm above the ground surface. To ensure the sapwood analyzed was formed after the mistletoe treatment, the outer six tree rings of the two cores were separated from older rings and pooled as a single sample per tree ($n = 6$). These samples were frozen in liquid N, freeze-dried under vacuum conditions, ground and stored at −20 °C until chemical analysis was conducted.

Tree ring $\delta^{13}C$ and $\delta^{18}O$ analyses

The dry and finely ground tree ring samples were weighed (~0.4–0.6 mg) into tin capsules for $\delta^{13}C$ analysis and were weighed (0.5–0.7 mg) into silver capsules for $\delta^{18}O$ analysis. Prepared capsules were analyzed at the Paul Scherrer Institute in Switzerland (Ecosystem Fluxes Group, PSI, Zurich, Switzerland). Samples were combusted in a High Temperature Conversion/Elemental Analyzer (Flash EA-1112, Carlo Erba, Milan, Italy) coupled to a mass spectrometer (DELTA plus XL, Thermo Finnigan, Bremen, Germany).

Carbon isotopic values were expressed in δ notation relative to the Vienna Pee Dee Belemnite standard (Coplen 2011). For oxygen, δ notation relative to the Vienna Standard Mean Ocean Water was used. The precision for measurements as determined by repeated measurements of standards was better than 0.1‰ for both isotopes. The rates of C fixation (A) and g_s are the primary factors determining photosynthetic C isotopic discrimination and therefore C isotopic composition ($\delta^{13}C$). Thus, the intrinsic WUE can be derived from the C isotope composition according to Farquhar et al. (1982) and Seibt et al. (2008).

Total soluble sugar and starch measurements

The powdered material (0.1 g) of needles, fine roots and stem sapwood was put into a 10 ml centrifuge tube, where 5 ml of 80% ethanol was added. The mixture was incubated at 80 °C in a water bath shaker for 30 min and then centrifuged at 4000 r.p.m. for 5 min. The pellets were extracted two more times with 80% ethanol. Supernatants were retained and combined for soluble sugar (SS) determinations using the anthrone method. Ethanol was removed by evaporation and then sugars were dissolved in distilled water. An aliquot of the water–sugar solution was hydrolyzed in 5 ml of 0.4% anthrone solution (4 g anthrone in 1000 ml 95% H_2SO_4) in a boiling water bath for 15 min. After cooling, the sugar concentration was determined spectrophotometrically (ultraviolet–visible spectrophotometer 752S, Cany Precision Instruments Co., Ltd, Shanghai, China) at 620 nm (Li et al. 2008*b*). Glucose was used as a standard. The SS concentration in fine roots, sapwood and needles was calculated on a dry mass basis (SS_{mass}, % d.m.) and in needles concentrations were additionally calculated on a projected needle area basis (SS_{area}, g m^{-2}).

The ethanol-insoluble pellet was used for ST extraction. Ethanol was removed by evaporation. Starch in the residue was released in 2 ml distilled water for 15 min in a boiling water bath. After cooling to room temperature, 2 ml of 9.2 mol l^{-1} $HClO_4$ was added. Starch was hydrolyzed for 15 min. Four milliliters of distilled water were added to the samples. Samples were then centrifuged at 4000 r.p.m. for 10 min. The pellets were extracted one more times with 2 ml of 4.6 mol l^{-1} $HClO_4$. Supernatants were retained, combined and filled to 20 ml with distilled water. The ST concentration was measured spectrophotometrically (ultraviolet–visible spectrophotometer 752S) at 620 nm using anthrone reagent and calculated by multiplying glucose concentrations by the conversion factor of 0.9 (Li et al. 2008*b*). Glucose was used as a standard. The ST concentrations in needles, fine roots and sapwood were calculated on a dry mass basis (ST_{mass}, % d.m.), and concentrations in needles were additionally expressed on a projected needle area basis (ST_{area}, g m^{-2}).

Total N analysis

The total N concentration in needles, roots and sapwood was determined in finely ground oven-dried samples by the micro Kjeldahl procedure, using $CuSO_4$, K_2SO_4 and H_2SO_4 for digestion (Li et al. 2008*a*). The concentration of NH_3 was determined on an auto-analyzer using the indophenol-blue colorimetric method (Li et al. 2008*a*). The N concentrations in fine roots and sapwood were expressed on a dry mass basis (N_{mass}, % d.m.), and concentrations in needles were expressed both on a dry mass basis and on a projected needle area basis (N_{area}, g m^{-2}).

Data analysis

The relative growth rate (shoot and tree ring) was defined as growth changes, expressed in percent, using the yearly value from 2005 to 2010 relative to the pretreatment mean value averaged over 6 years (1999–2004) for MRT and MIT, respectively. Nonstructural carbohydrate (NSC) is defined as the sum of ST and SS for each sample within each category (Chapin et al. 1990, Li et al. 2001). The SS/ST ratio is defined as the concentration ratio of SS to ST, and NSC/N ratio is the concentration ratio of NSC to N within each sample. All data ($\delta^{13}C$, $\delta^{18}O$, NSC, ST and total SS) were checked for normality by Kolmogorov–Smirnov tests. Repeated measurements analyses of variance (RM-ANOVAs) were used and determined that both mistletoe treatment (MRT vs MIT, all $P < 0.05$) and time after treatment (all $P < 0.01$) significantly affected tree growth (6 years after treatment for shoot and tree ring growth and 3 years after treatment for needle parameters) and values of $\delta^{13}C$ and $\delta^{18}O$ (results of RM-ANOVAs not shown due to consistently significant effects). Therefore, one-way ANOVAs were used to test differences in variables between MRT and MIT, and independent-samples t-tests (two-tailed) were performed to examine the difference in variables between pretreatment and

posttreatment values within MRT or MIT. To eliminate the effects of annual environmental variations on shoot increment, tree ring width, $\delta^{13}C$ and $\delta^{18}O$ across the study period of 12 years, values of these parameters in MRT were compared with those in MIT. All statistical analyses were performed using SPSS 17 for windows (SPSS Inc., Chicago, IL, USA).

Results

Host tree growth

Mistletoe removal tended to increase the needle length, mean single needle area and LMA of host trees (Table 1). Mean needle length increased by 63.1% ($P < 0.05$), 27.6% ($P < 0.10$) and 29.6% ($P < 0.05$) for 1-, 2- and 3-year-old needles, respectively, in MRT compared with those in MIT (Table 1). Mean single needle area increased by 76.4% ($P < 0.05$), 30.2% ($P < 0.10$) and 38.2% ($P < 0.05$) for 1-, 2- and 3-year-old needles, respectively, in MRT compared with those in MIT (Table 1). Similarly, the mean needle LMA in MRT was 12.7% ($P < 0.05$), 5.6% ($P > 0.10$) and 5.9% ($P > 0.10$) higher than those in MIT for 1-, 2- and 3-year-old needles, respectively (Table 1).

Tree ring width in both MRT and MIT decreased until 2003, when it increased (Figure 1a). For the 6 years (1999–2004) before the mistletoe treatment took place, the mean annual tree ring width was 78.33×10^{-2} mm for MIT and 70.33×10^{-2} mm for MRT (10.2% smaller for MRT; $F = 0.714$, $P > 0.05$; Figure 1a). An opposite trend occurred after treatment, when MIT (65.94×10^{-2} mm) had a 9.5% smaller mean tree ring width than MRT (72.86×10^{-2} mm) for the 6 years from 2005 to 2010 ($F = 1.175$, $P > 0.05$; Figure 1a). The yearly standardized relative tree ring growth rate was always greater in MRT than in MIT after treatment (Figure 1b).

The mean annual shoot increment length in MRT did not significantly differ from that of MIT before treatment (MRT > MIT, $P > 0.05$; Figure 2a). After mistletoe removal, the mean annual shoot increment length was significantly larger (+29.1%; $P < 0.05$) in MRT (3.9 cm) than that in MIT (3.0 cm) (Figure 2a). The yearly standardized relative growth rate of shoot growth was always higher in MRT than in MIT after mistletoe removal (Figure 2b).

Nitrogen and mobile carbohydrate concentrations

MRT had significantly higher needle area-based concentrations of N, SS and NSC than MIT. Only ST did not display a significant difference between MRT and MIT (Figure 3). Needle N_{area}, SS_{area} and NSC_{area} in MRT were 28.6% ($P < 0.05$), 32.1% ($P < 0.05$) and 17.3% ($P < 0.05$) higher than those in MIT, respectively (Figure 3).

MRT needles had significantly lower ST_{mass} (−26.5%, $P < 0.05$; Figure 4c) and NSC_{mass} (−11.4%, $P < 0.05$; Figure 4d) but higher SS_{mass} (+5.5%, $P < 0.05$; Figure 4b) than MIT needles. Nitrogen (Figure 4a) and ST (Figure 4c) concentrations in

Table 1. Mean values (±1 SD, $n = 6$ trees) of LMA, needle area and needle length from 1- to 3-year-old needles in MRT and MIT. One-way ANOVAs were performed to examine statistical differences in the mean values of each needle parameter between MRT and MIT within each age class. Significant differences at $P < 0.05$ are highlighted in bold, and changes in percent are given in parentheses and were calculated using (MRT-mean − MIT-mean) × 100/MIT-mean.

Needles age	Mean LMA (g m^{-2})				Mean single needle area (mm^2)				Mean length (mm)			
	MRT	MIT	F	P	MRT	MIT	F	P	MRT	MIT	F	P
1 year	290.49 ± 11.08	257.69 ± 9.20	2.313	**0.043**	26.74 ± 2.32	15.16 ± 3.79	6.781	**0.026**	31.10 ± 2.09	19.07 ± 3.83	7.596	**0.02**
2 years	314.57 ± 9.16	297.73 ± 8.13	1.375	0.199	32.04 ± 2.42	24.61 ± 3.19	3.438	0.093	35.49 ± 2.50	28.05 ± 2.97	3.676	0.084
3 years	326.78 ± 6.07	308.49 ± 11.22	1.433	0.182	35.07 ± 1.87	25.37 ± 3.31	6.52	**0.029**	37.42 ± 2.40	28.87 ± 2.77	5.445	**0.042**
Mean	310.61 (+7.9%)	287.97			31.28 (+44.1%)	21.71			34.67 (+36.9%)	25.33		

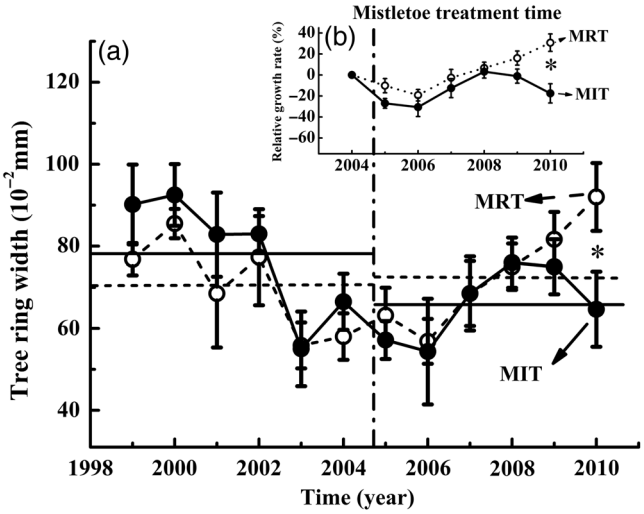

Figure 1. Tree ring width (a) and annual relative growth rate (b) for both mistletoe-removed trees (MRT, $n = 6$ trees) and mistletoe-infected trees (MIT, $n = 6$ trees) during the period from 1999 to 2010 (mean values ± 1 SD). (a) Shown are tree ring width in MRT (open circles) and MIT (filled circles), and mean tree ring width in MRT (broken horizontal lines) and MIT (continuous horizontal lines) for the 6 years before and 6 years after mistletoe removal (conducted at the end of the growing season 2004). The relative tree ring growth rate (b) was defined as tree ring width changes, expressed in percent, using the yearly tree ring width from 2005 to 2010 relative to the pretreatment mean tree ring width averaged over 6 years (1999–2004) for MRT and MIT, respectively. Asterisks indicate a significant difference ($P < 0.05$) in mean values between MRT and MIT.

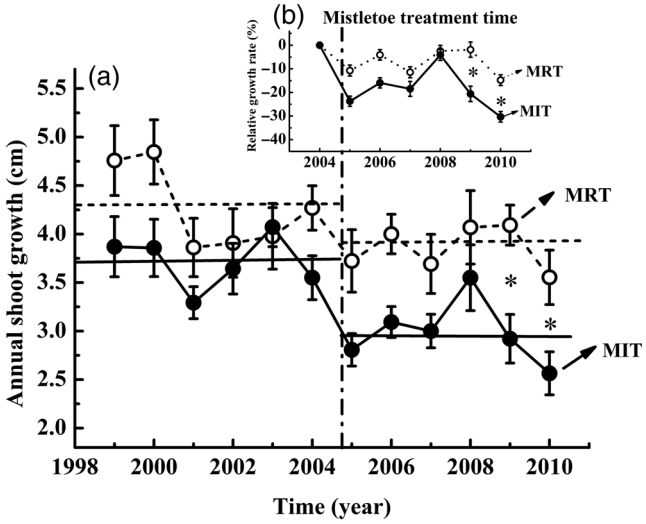

Figure 2. The annual shoot growth increment (a) and annual relative growth rate (b) for both mistletoe-removed trees (MRT, $n = 6$ trees) and mistletoe-infected trees (MIT, $n = 6$ trees) during the period from 1999 to 2010 (mean values ± 1 SD). (a) Shown are shoot growth increment in MRT (open circles) and MIT (filled circles), and mean shoot growth increment in MRT (broken horizontal lines) and MIT (continuous horizontal lines) for the 6 years before and 6 years after mistletoe removal (conducted at the end of the growing season 2004). The relative growth rate (b) was defined as growth changes, expressed in percent, using the yearly shoot growth increment from 2005 to 2010 relative to the pretreatment mean growth increment averaged over 6 years (1999–2004) for MRT and MIT, respectively. Asterisks indicate a significant difference ($P < 0.05$) in mean values between MRT and MIT.

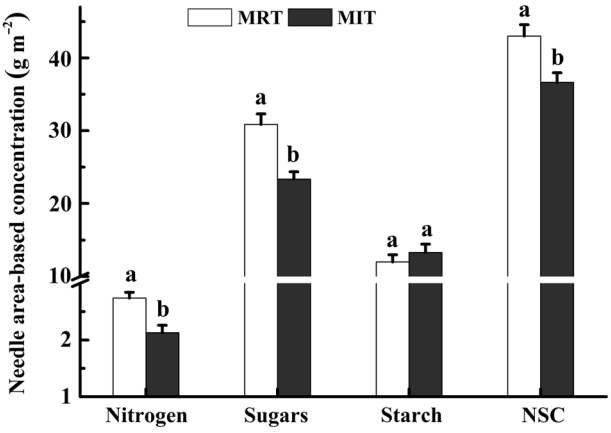

Figure 3. Mean concentration (±1 SD, $n = 6$) of total N, SS, ST and NSC in 1-year-old needles expressed on a projected area basis (g m^{-2}) in mistletoe-removed trees (MRT, white columns) and mistletoe-infected trees (MIT, black columns). Different letters indicate statistically significant ($P < 0.05$) differences in mean values between MRT and MIT for a given variable.

sapwood did not differ between MRT and MIT (both $P > 0.05$), but MRT sapwood had significantly higher SS (+31.3%, $P < 0.05$; Figure 4b) and NSC (+23.3%, $P < 0.05$; Figure 4d) concentrations than MIT sapwood. MRT roots had significantly lower N (–19.4%, $P < 0.05$; Figure 4a) and SS (–4.7%, $P < 0.05$; Figure 4b) concentrations but significantly higher ST (+27.6%, $P < 0.05$; Figure 4c) and NSC (+8.7%, $P < 0.05$; Figure 4d) concentrations than MIT roots.

MRT had a significantly higher SS/ST ratio than MIT in needles and wood, but a significantly lower ratio in roots (see Figure S1a available as Supplementary Data at *Tree Physiology* Online). The needle NSC/N ratio did not differ between MRT and MIT, but MRT had a significantly higher NSC/N ratio in sapwood and roots than MIT (see Figure S1b available as Supplementary Data at *Tree Physiology* Online).

$\delta^{13}C$ and $\delta^{18}O$ values

In MRT tree rings, the mean $\delta^{13}C$ value for wood formed during the 6 years (2005–10) following treatment was significantly lower than the mean value for wood formed in the 6-year period (1999–2004) before treatment ($P < 0.05$; see Figure S2a available as Supplementary Data at *Tree Physiology* Online). In contrast, the mean $\delta^{18}O$ value was significantly higher after mistletoe removal ($P < 0.05$; see Figure S2b available as Supplementary Data at *Tree Physiology* Online). However, the mean $\delta^{13}C$ value in the combined 3-year tree ring material (1999–2001, 2002–04, 2005–07 and 2008–10) decreased for both MRT and MIT from the pretreatment period to the posttreatment period (Figure 5a). No significant difference in $\delta^{13}C$ between MIT and MRT was observed for either the pre- or posttreatment period (Figure 5a). Therefore, the trend of decreasing $\delta^{13}C$ values in both MRT and MIT suggests that this pattern (Figure 5a, Figure S2a available as Supplementary Data at *Tree Physiology*

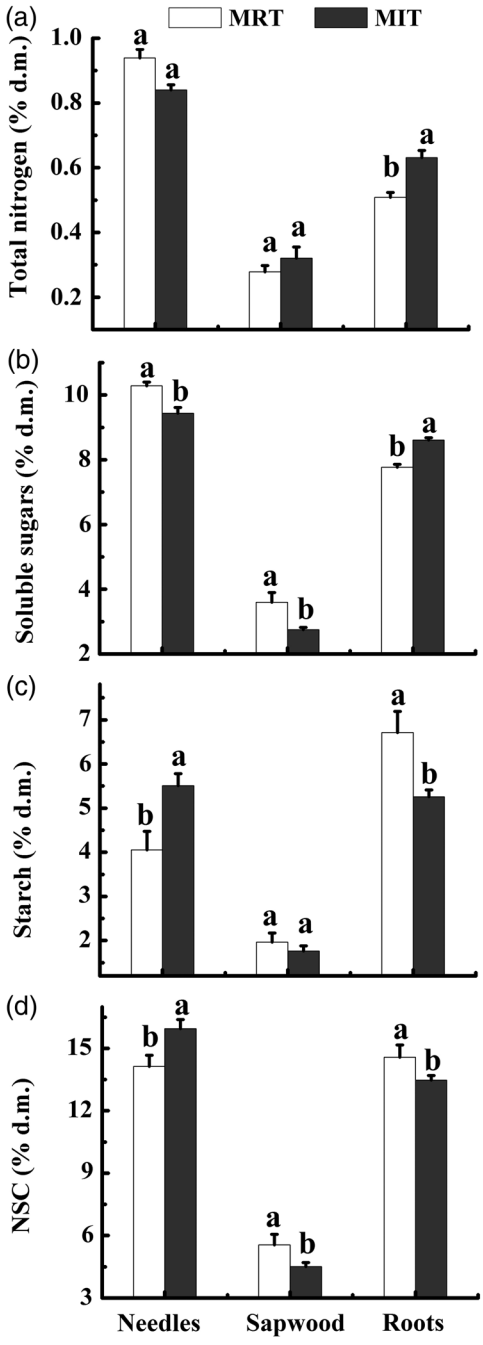

Figure 4. Mean concentration (±1 SD, $n = 6$) of total N (a), SS (b), ST (c) and NSC (d) in 1-year-old needles, stem sapwood and fine roots in mistletoe-removed trees (MRT, white columns) and mistletoe-infected trees (MIT, black columns). Different letters indicate significant differences in mean values between MRT and MIT for a given variable ($P < 0.05$).

Online) was caused by environmental variations over time and was not a result of the mistletoe treatment, since MIT was not subjected to mistletoe removal.

There was no statistically significant difference between MRT and MIT in the mean $\delta^{18}O$ values in the combined 3-year tree ring material over the pretreatment phase (MRT 25.06‰ vs MIT 25.20‰; $P > 0.05$) (Figure 5b). The mean $\delta^{18}O$ values for the posttreatment phase (2005–10) decreased in MIT but increased in MRT (Figure 5b), indicating that mistletoe removal resulted in an increase in $\delta^{18}O$ values in tree rings. The mean $\delta^{18}O$ values of the entire posttreatment phase were significantly ($P < 0.05$) higher in MRT (25.27‰) than in MIT (24.79‰) (Figure 5b). This result indicates that the increase in $\delta^{18}O$ observed in MRT after mistletoe removal (see Figure S2b available as Supplementary Data at *Tree Physiology* Online) was a result of the treatment.

Discussion

Effects of mistletoe removal on host growth

Mistletoe removal resulted in greater needle size, tree ring width and shoot growth in MRT (Table 1, Figures 1 and 2). Consistent negative effects of mistletoes on host growth and survival have been reported in many previous studies (Reid et al. 1994, Stanton 2006, Shaw et al. 2008, Rigling et al. 2010, Logan et al. 2013, Marias et al. 2014). At the same site as our study, Rigling et al. (2010) found that the mean needle length decreased by 20% in mistletoe-infected branches compared with noninfected branches, due to decreased water availability. Moreover, mistletoe infection increased needle loss, leading to a decline in the crown area and the photosynthetically active leaf area (Dobbertin et al. 2010, Zweifel et al. 2012). Mistletoe infection caused a leaf mass loss of >70% in Scots pine at the same site (Rigling et al. 2010) and in other species such as *Eucalyptus* trees in Australia (Reid et al. 1994). Mistletoe infection-induced leaf loss (Rigling et al. 2010) and increased crown transparency (Dobbertin and Rigling 2006) are thus considered to be important factors causing the decline in shoot and diameter growth rates of host trees, as observed in our study (Table 1).

Previous studies described that a reduction in leaf N concentration resulted in smaller leaf size (Logan et al. 1999, Robinson and Gessner 2000). Smaller leaf size and lower leaf N concentration cause a decline in photosynthetic production (Meinzer et al. 2004), which could in turn lead to lower shoot growth rates in mistletoe-infected trees (Ehleringer et al. 1986*b*, Cechin and Press 1993). Increased N availability tends to result in biomass accumulation in branches and wood (Xia and Wan 2008). A recent study showed that the length of current-year shoots decreased by ~60% in both young and mature Qinghai spruce (*Picea crassifolia* Kom.) trees infected by dwarf mistletoe (*Arceuthobium sichuanense*) compared with those of the noninfected trees (Xia et al. 2012). Stanton (2006) showed that severe dwarf mistletoe infection reduced the radial growth rate in hosts by 28–43% compared with moderately or lightly infected trees (ponderosa pine). Therefore, mistletoe removal can enhance the growth rate of host trees. In our study, both the relative tree ring growth rate (see also Shaw et al. 2008) and the relative annual shoot growth rate were higher in MRT during the posttreatment phase than in MIT during the same time period (Figures 1 and 2).

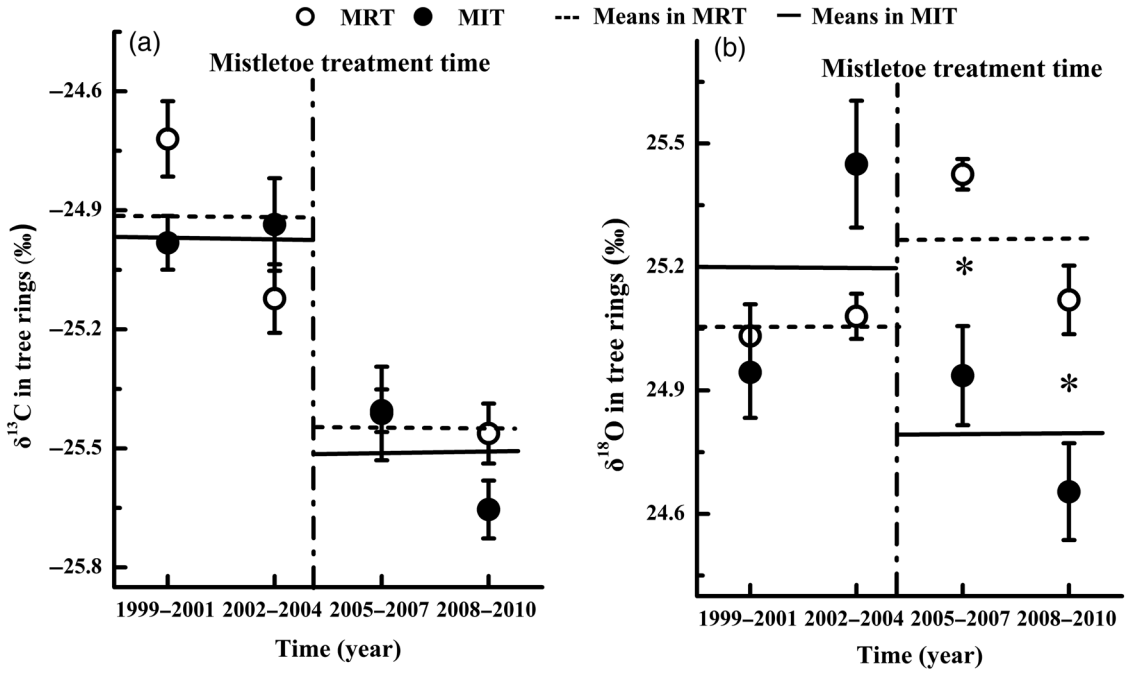

Figure 5. Mean values (±1 SD, $n = 6$) of $\delta^{13}C$ (a) and $\delta^{18}O$ (b) in tree rings pooled for 3 years in both mistletoe-removed trees (MRT) and mistletoe-infected trees (MIT) during the period of 1999–2010. Shown are isotopic values in MRT (open circles) and in MIT (filled circles), and pretreatment and posttreatment mean values of $\delta^{13}C$ and $\delta^{18}O$ in MRT (broken horizontal lines) and in MIT (continuous horizontal lines). Asterisks indicate a significant difference ($P < 0.05$) in mean values between MRT and MIT.

Effects of mistletoe removal on N and C balance in host trees

We expected to find an overall increase in total N concentrations in MRT after treatment (Hypothesis I). However, mistletoe removal resulted in an increase in N in source tissues (i.e., needles; Figures 3 and 4a) but a decrease in N in sink tissues (i.e., sapwood and roots; Figure 4a) compared with MIT. This increase in MRT needle N may be mainly a result of decreased N absorption by mistletoes from the host canopy because mistletoes were removed. The decrease in N in MRT sapwood and roots might be related to N redistribution, accumulation and utilization in the larger photosynthetically active needle mass (or area) in MRT relative to MIT (Table 1). Rigling et al. (2010) measured needle biomass in the same forest stand and found that the needle dry mass of a mistletoe-infected branch was only one-quarter (6.2 g) the amount of a noninfected branch (26.6 g) (see also Tennakoon and Pate 1996, Meinzer et al. 2004).

Previous studies have revealed that host N is consumed by mistletoes and that mistletoe leaves accumulated N significantly, which resulted in lower leaf N concentrations in mistletoe-infected hosts (Schulze and Ehleringer 1984, Ehleringer et al. 1986*b*). For example, the mean leaf N ratio of parasite to host was ~1.6 for *Phoradendron leucarpum* (Raf.) Reveal & M.C. Johnst. on a variety of its hosts (Panvini and Eickmeier 1993), and for *Loranthus europaeus* Jacq. on *Quercus* sp. (Lamont 1983). Ehleringer et al. (1986*b*) found that leaf N was 10.4% higher in uninfected *Juniperus osteosperma* (Torr.) than that in *Juniperus* infected by *Phoradendron juniperinum* Engelm. ex A. Gray. Tennakoon and Pate (1996) noted that mistletoe tissues generally have much higher N concentrations than any parts of the host. In our study, mistletoe removal and the related disappearance of the parasite N-sink increased leaf N concentrations in host trees (Figures 3 and 4a). This effect is beneficial for the hosts because it probably increases C assimilation and thus facilitates growth (Figures 1 and 2), especially under the dry conditions in the present study.

The processes of litterfall and nutrient cycling at the stand level may also be influenced by mistletoe infection. Mistletoe infection-induced host leaf mass loss (Reid et al. 1994, Rigling et al. 2010) led to an increase in accumulation of litter surrounding mistletoe-infected trees. Moreover, mistletoe litterfall contributes to the overall increase in litterfall. March and Watson (2007) found that litter from mistletoes significantly increased overall litterfall by up to 189% in adult *Eucalyptus* forests infected by *Amyema miquelii* (Lehm. Ex Miq.) Tiegh. in Australia. Increased litterfall may imply an increase in nutrient availability in the rhizosphere around mistletoe-infected trees, since nutrients released from litter decomposition are critical for the resupply of soil nutrients (Sullivan et al. 2007). This effect may also have contributed to the increased N concentrations in sapwood and roots of MIT (Figure 4a), since increased litterfall due to mistletoe infection has also been reported at our study site (Rigling et al. 2010). On the other hand, mistletoe-infected trees may

allocate more N from the canopy to the stem and belowground part to promote root growth (McCarthy and Enquist 2007) in order to reach and take up more water for both the host tree and the attached mistletoes. Such an effect may be especially important under dry conditions because mistletoes transpire up to nine times as much water as their hosts (Küppers et al. 1992, Schulze et al. 1994, Zweifel et al. 2012).

Nitrogen is a major component of photosynthetic enzymes that regulate photosynthetic activity (Ripullone et al. 2003), and insufficient N directly decreases photoassimilation (Gruber and Galloway 2008). Loss of needle mass (Xia et al. 2012) in combination with decreases in leaf N concentrations (Figures 3 and 4a) may thus lead to lower concentrations of mobile carbohydrates in MIT relative to MRT, as stated in our first hypothesis. In line with our hypothesis, MRT showed significantly higher mass-based NSC concentrations in sapwood and roots (Figure 4d) and a higher area-based NSC concentration in needles (Figure 3) compared with MIT. However, the mass-based NSC concentration in needles was lower in MRT than in MIT (Figure 4d). This decline in needle mass-based NSC concentration (Figure 4d) may be mainly caused by a dilution effect of increased LMA in MRT compared with MIT (Table 1). In contrast, this greater LMA in MRT led to a smaller specific leaf area (SLA = 1/LMA), and thus, MRT had higher needle area-based NSC concentration than MIT (Figure 3).

Higher NSC concentrations in MRT may be caused by higher photosynthesis, lower NSC utilization or both (Li et al. 2002). Lower NSC utilization in MRT was obviously not the case in our study because these trees needed to use more NSC to support greater growth rates compared with MIT (Figures 1 and 2, Table 1). Increases in photosynthesis in MRT may have mainly resulted from a larger number of photosynthetically active needles, as shown in Rigling et al. (2010), larger needle area per needle (Table 1) and increased leaf N concentrations (Figures 3 and 4a). Meinzer et al. (2004) found that hemlock (*Tsuga heterophylla* (Raf.) Sarg.) branches infected by dwarf mistletoe (*Arceuthobium tsugense* (Rosendahi) G.N. Jones ssp. *tsugense*) had only half as much leaf area as noninfected branches, and needles of the infected branches had only half the N concentration and maximum assimilation rate (A_{max}) as needles of the noninfected branches.

Zweifel et al. (2012) reported leaf stomatal closure of Scots pine infected by *V. album* as a response that reduced water loss in hot and dry growth conditions. This stomatal closure is an important mechanism to prevent cavitation, and is thus essential for host survival, but it also reduces assimilation and C gain (Polle et al. 2001). Meinzer et al. (2004) estimated that the C accumulation rate of *T. heterophylla* heavily infected with *A. tsugense* was up to 60% lower than in uninfected trees. Hence, the C fixation decreases with increasing mistletoe infection level.

A higher ST concentration in MIT needles (Figure 4c) may imply that glucose made by photosynthesis was not invested into growth processes (Figures 1 and 2) but was converted into ST as a storage molecule (Li et al. 2013). An increase in ST concentration may, in turn, downregulate photosynthesis and glucose production (Brugnoli and Farquhar 2000), leading to lower NSC concentrations in MIT compared with MRT (Figure 4d). Watling and Press (2001) proposed that parasites provide a sink for carbohydrates produced by hosts and that some host plants can compensate by increasing photosynthesis via increases in leaf area, delayed leaf senescence and increased Rubisco concentration. In the host trees examined here, however, lower N concentrations observed in MIT suggest that such a compensation did not occur under the dry conditions in the study region.

Not only decreased photosynthesis (Polle et al. 2001, Meinzer et al. 2004, Zweifel et al. 2012) but also extraction of mobile carbohydrates by mistletoes can affect the NSC levels in infected trees. Xylem-tapping mistletoes extract a large amount of carbohydrates from the host (Heizmann et al. 2001, Escher et al. 2008). It has been reported that ~35–78% of the C in mistletoes were directly extracted from the host (Johnson and Choinski 1993, Marshall et al. 1994, Popp and Richter 1998, Escher et al. 2004, Wang et al. 2008). Moreover, previous studies have suggested that branches infected by dwarf mistletoes have higher dark respiration rates than uninfected branches of *Picea mariana* (Mill.) BSP (Clark and Bonga 1970), which might also contribute to higher C consumption in infected trees. Extraction of carbohydrates by mistletoes combined with higher dark respiration would accelerate the depletion of new assimilates and C storage in the host, particularly in drought-stressed trees with decreased photosynthesis (Mcdowell et al. 2008). This mechanism may explain our observations that stem sapwood and roots had lower ST and NSC levels in MIT than in MRT (Figure 4c and d).

Effects of mistletoe removal on $\delta^{13}C$ and $\delta^{18}O$ in host trees

In principle, the dual isotope approach combining $\delta^{13}C$ and $\delta^{18}O$ measurements allows investigators to distinguish the effects of changed g_s and altered maximum assimilation rates (A_{max}) on $\delta^{13}C$ (Scheidegger et al. 2000, Roden and Siegwolf 2012). We hypothesized that the removal of mistletoe would lead to higher A_{max}, which is supported by the observed higher needle N concentrations (Figures 3 and 4a) and the higher needle g_s (Zweifel et al. 2012). The latter point is based on the assumption that the termination of uncontrolled water consumption by the mistletoe allows host trees to control their stomata less strictly and thus also increase C gain. Tree ring $\delta^{13}C$ did not differ significantly between MIT and MRT before and after treatment, indicating that the mistletoes did not significantly affect intrinsic WUE (defined as A/g_s and directly related to $\delta^{13}C$; Farquhar et al. 1982). However, this result could either be due to no change in both A and g_s or to concerted proportional changes in both parameters causing the quotient A/g_s to

remain constant. The increase in $\delta^{18}O$ in MRT compared with MIT in the posttreatment phase (Figure 5b) (accompanied by no change in $\delta^{13}C$) is interpreted by the conceptual Scheidegger model (Scheidegger et al. 2000) as a decrease in both A_{max} and g_s due to the removal treatment. This finding fully contradicts our initial hypothesis that the removal of mistletoe will lead to higher assimilation rates and higher g_s in the host trees, and it also contradicts field measurements of g_s made in Scots pine needles at an adjacent site a few hundred meters away (Zweifel et al. 2012). The pine trees in the study of Zweifel et al. (2012) were the same age as and had similar dimensions to the trees in our study. Moreover, stand structure, soil type and general climatic conditions were fully comparable. Furthermore, the measurements of Zweifel et al. (2012) were performed during the 2004 growing season, which was covered by our tree ring assessments. As a consequence, it is highly reasonable to assume that the gas exchange patterns observed in this previous study are representative for the trees in our present study. In addition, it is highly unlikely that A_{max} should decrease given the observed increases in leaf N since leaf N is known to be positively related to assimilation rates.

When applying the Scheidegger model, several points need to be taken into account for the interpretation of the results. (i) The general assumption of the Scheidegger model is that the source water isotopic signature is comparable among the different plant individuals tested (e.g., Roden and Siegwolf 2012). Since we chose comparable trees for the control and removal treatment in the same stand, we consider it as a valid assumption that all trees had access to the same water resources. (ii) The Scheidegger model assumes that leaf water evaporative enrichment and consequently $\delta^{18}O$ of organic matter are mainly controlled by the water vapor pressure deficit (VPD) of the air, which in turn controls g_s. Gessler et al. (2009*a*, 2009*b*) extended the model to enable comparisons between individual trees growing under conditions with comparable VPD in the same stand, as in our study. According to this model extension, the increase in $\delta^{18}O$ of MRT in the posttreatment phase was due to a reduced leaf level transpiration rate as a result of lower g_s (at the same VPD for MIT and MRT). The reduction in transpiration causes an increase in leaf water $\delta^{18}O$ (and consequently in $\delta^{18}O$ of the assimilates produced within that water). This is due to a relatively larger contribution of evaporatively enriched water from the sites of evaporation in the leaf and a relatively reduced contribution of water from the xylem, which is not evaporatively enriched, to lamina leaf water. This mixing phenomenon, based on the advection of nonenriched xylem water as supposed by back-diffusion of enriched water from the sites of evaporation in the leaf, is referred to as the Péclet effect (cf. Farquhar and Cernusak 2005).

The mixing and thus the Péclet effect, however, are not only driven by transpiration but also by the scaled effective path length (L) for water movement within the leaf (Farquhar and Lloyd 1993, Werner et al. 2012). It is known that L is variable and is determined by leaf morphological (Wang et al. 1998) and physiological (Ferrio et al. 2009) traits. Only recently, Marias et al. (2014) determined the difference in L between mistletoe-infected and noninfected individuals of *T. heterophylla*, and observed three times higher values in infected plants. Moreover, the authors were able to derive from a sensitivity analysis that the higher $\delta^{18}O$ values in tree rings observed in the uninfected trees in their study (and thus comparable to our results) were most likely not due to differences in g_s but due to the variation in L. Even though we have no L values available for our trees, the fact that experimentally determined g_s increased after mistletoe removal (Zweifel et al. 2012) might point to the same mechanism observed by Marias et al. (2014) and Ferrio et al. (2009). We assessed how changes in L would affect $\delta^{18}O$ of leaf water and thus leaf assimilates based on a detailed dataset on Scots pine leaf water evaporative enrichment in previous year needles published by Barnard et al. (2007). According to these data, a reduction of L by a factor of less than two after mistletoe removal would explain the observed differences in $\delta^{18}O$ between MRT and MIT in the period 2005–10 (Figure 5b), thus being in line with the observation of Marias et al. (2014). Changes in needle anatomy and in the effectiveness of the needle endodermis, which surrounds the vascular strand in conifer needles and might provide hydraulic separation between the xylem and the leaf mesophyll (Zwieniecki et al. 2007), could affect the tortuosity of water flow in the leaf and thus alter L (cf. Roden et al. 2015). In fact, mistletoe infection has been shown to alter the vascular architecture and the distance between the vascular bundle and the endodermis in pine (*Pinus contorta*) needles (Chhikara and Ross Friedman 2008), which supports this mechanism.

A recent study suggested that, in a leaf with limited ^{18}O exchange, rather discrete pools of water control the oxygen isotopic composition of leaf water and that the Péclet effect might not be important (Song et al. 2015). However, these authors noted that other studies presented evidence in support of the use of the Péclet correction for modeling $\delta^{18}O$ in organic matter pools and they concluded that more work is needed to reconcile the discrepancy in results.

A potential alternative explanation for the increase in $\delta^{18}O$ in MRT (after the treatment) compared with MIT, which does not require the Péclet effect, is related to the timing of organic material deposited in the tree ring. It is possible that the mistletoe-removed pine trees were able to continue to fix C during periods of water stress, which are often characterized by low relative air humidity, while the MIT closed stomata and did not or only negligibly fixed C (cf. Roden and Siegwolf 2012). Such a situation might have caused the production of cellulose with increased $\delta^{18}O$ in mistletoe-removed trees. This scenario would, however, require that tree ring $\delta^{13}C$ values were not affected by mistletoe removal, since we observed a difference in $\delta^{18}O$ but not in $\delta^{13}C$.

An additional point that might need to be considered is the exchange of organic oxygen atoms with unenriched xylem water during cellulose synthesis (Barbour and Farquhar 2000). Until recently, it was assumed that the proportion of exchangeable oxygen during cellulose synthesis (p_{ex}) is constant and amounts to ~42% (Cernusak et al. 2005). Only recently, it has been shown that p_{ex} might vary during the growing season (Gessler et al. 2009*a*, 2009*b*) and can be affected by the turnover time of the NSC pool (Song et al. 2014). The latter authors assumed that a decrease in the C pool turnover time, because of a smaller pool size in relation to the fluxes into (e.g., supply via the phloem) and out of that pool (cellulose production), would give rise to lower probability for hexose molecules to undergo triose cycling (cf. Hill et al. 1995)—which allows carbonyl oxygen to be exchanged with the surrounding water—during cellulose synthesis. In our study, the removal of mistletoes increased growth and depleted the sugar pools in stems, together pointing to faster C turnover. With a simple calculation, we can estimate the change in p_{ex} required to cause the difference between MIT and MRT in the posttreatment phase (Figure 5). We assume a $\delta^{18}O$ of xylem water of −10‰ as observed for larch trees in the Valais (Treydte et al. 2014) and a p_{ex} of 0.42 for the MIT. The equilibrium fractionation factor (ε_{wc}) results in carbonyl oxygen being 27‰ more enriched than water (Sternberg and DeNiro 1983) and thus an average leaf water enrichment of 13.4‰ is needed to obtain the mean posttreatment $\delta^{18}O$ in tree rings of MIT. Keeping everything else constant, a decrease of p_{ex} to 0.38 would explain the observed increase in $\delta^{18}O$ in the MRT. We might thus speculate that such a small decrease in p_{ex} (0.42 to 0.38) (cf. Gessler et al. 2009*a*, 2009*b*) has already resulted in water enrichment in tree rings of trees without mistletoes.

In conclusion, rather than allowing us to accept or reject our initial Hypothesis II, our results clearly point to the limitation of the dual isotope approach proposed by Scheidegger et al. (2000). The dual isotope approach is not applicable if changes in the pathway of leaf water movement superimpose the effects of g_s/transpiration on $\delta^{18}O$, as recently pointed out by Gessler et al. (2014) and Barbour and Song (2014). Also, changes in p_{ex} that might be induced by faster C turnover could lead to misinterpretations. Moreover, this method is not suitable when tree rings of two treatments do not record comparable periods during the growing season.

If we assume that g_s increased after mistletoe removal (Zweifel et al. 2012), only an increase in A would explain the unchanged $\delta^{13}C$ values in the tree rings of MRT compared with MIT. Thus, exactly the opposite result as the one indicated by the dual isotope approach is the most probable.

Sala et al. (2001) investigated needle $\delta^{13}C$ values in both Douglas fir (*Pseudotsuga menziesii* Mirb.) and western larch (*Larix occidentalis* Nutt.) trees with various intensities of mistletoe infection compared with trees that were never infected and found that the uninfected trees had the highest leaf $\delta^{13}C$ values within each species, and leaf $\delta^{13}C$ values decreased (became more negative) significantly with increased levels of mistletoe infection for both species. In tree rings of *T. heterophylla*, $\delta^{13}C$ was also significantly lower in infected compared with uninfected trees (Marias et al. 2014). Logan et al. (2013) found that $\delta^{13}C$ values of needles from branches of white spruce (*Picea glauca* (Moench) Voss) infected by *Arceuthobium pusillum* Peck were significantly lower (more negative) than values of needles from uninfected branches.

From our results, we may speculate that our mistletoe removal experiment at the whole-tree level at a dry site increased g_s and assimilation rate to a similar extent. As a result, A/g_s remained constant, whereas in most other cases reported in literature, one of the two responses dominated.

Conclusion

Tree growth is a consequence of many complex physiological processes, among which C fixation is considered the most important factor (Samuelson et al. 1998). Under the dry conditions prevailing at our experimental site, mistletoe removal clearly increased shoot and stem growth of host trees. This growth increase was related to an increase in needle N concentration found in the present study and to an increase in needle g_s found in the same tree species at the same site (Zweifel et al. 2012), which points to increased photosynthetic capacity after mistletoe removal. The NSC concentrations in tree tissues partially support this assumption but preclude straightforward interpretations because of increased biomass production and the related dilution effects in MRT, as well as differences in the water status between MRT and MIT. The dual isotope approach, from which we aimed to derive retrospective information on assimilation rate and g_s, did not produce results that could be interpreted in this way. Our results strongly point to the need for further research on the effects of biotic and abiotic conditions on the scaled effective path length for water movement in the leaf, which can affect $\delta^{18}O$ in tree rings but is not accounted for in the Scheidegger model (Scheidegger et al. 2000). Moreover, a deeper understanding of the variability of p_{ex} and thus the relative contribution of leaf water enrichment to the tree ring $\delta^{18}O$ signal is necessary. However, combining the tree ring $\delta^{13}C$ data of MRT and MIT with the observations of Zweifel et al. (2012) that needles from uninfected branches of Scots pine showed higher g_s than infected ones indicate that an increase in assimilation rate as a consequence of the removal treatment is most likely.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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References

Bannister P, Strong GL (2001) Carbon and nitrogen isotope ratios, nitrogen content and heterotrophy in New Zealand mistletoes. Oecologia 126:10–20.

Barbour MM, Farquhar GD (2000) Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. Plant Cell Environ 23:473–485.

Barbour MM, Song X (2014) Do tree-ring stable isotope compositions faithfully record tree carbon/water dynamics? Tree Physiol 34:792–795.

Barnard R, Salmon Y, Kodama N, Sörgel K, Holst J, Rennenberg H, Gessler A, Buchmann N (2007) Evaporative enrichment and time lags between delta $\delta^{18}O$ of leaf water and organic pools in a pine stand. Plant Cell Environ 30:539–550.

Bell TL, Adams MA (2011) Attack on all fronts: functional relationships between aerial and root parasitic plants and their woody hosts and consequences for ecosystems. Tree Physiol 31:3–15.

Bickford CP, Kolb TE, Geils BW (2005) Host physiological condition regulates parasitic plant performance: *Arceuthobium vaginatum* subsp. *cryptopodum* on *Pinus ponderosa*. Oecologia 146:179–189.

Brendel O, Iannetta PPM, Stewart D (2000) A rapid and simple method to isolate pure alpha-cellulose. Phytochem Anal 11:7–10.

Brugnoli E, Farquhar GD (2000) Photosynthetic fractionation of carbon isotopes. Photosynthesis 9:399–434.

Cechin I, Press MC (1993) Nitrogen relations of the *Sorghum-sfriga-hermonthica* hostparasite association: growth and photosynthesis. Plant Cell Environ 16:237–247.

Cernusak LA, Farquhar GD, Pate JS (2005) Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. Tree Physiol 25:129–146.

Chapin FS, Schule ED, Mooney HA (1990) The ecology and economic of storage in plants. Annu Reve Ecol Syst 21:423–447.

Chhikara A, Ross Friedman CM (2008) The effects of male and female *Arceuthobium americanum* (lodgepole pine dwarf mistletoe) infection on the relative positioning of vascular bundles, starch distribution, and starch content in *Pinus contorta* var. *latifolia* (lodgepole pine) needles. Botany 86:539–543.

Clark J, Bonga JM (1970) Photosynthesis and respiration in black spruce (*Picea mariana*) parasitized by eastern dwarf mistletoe (*Arceuthobium pusillum*). Can J Bot 48:2029–2031.

Coplen TB (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun Mass Spectrom 25:2538–2560.

Dobbertin M, Rigling A (2006) Pine mistletoe (*Viscum album* ssp. *Austriacum*) contributes to Scots pine (*Pinus sylvestris*) mortality in the Rhone valley of Switzerland. Forest Pathol 36:309–322.

Dobbertin M, Hilker N, Rebetez M, Zimmermann NE, Wohlgemuth T, Rigling A (2005*a*) The upward shift in altitude of pine mistletoe (*Viscum album ssp. austriacum*) in Switzerland—the result of climate warming? Int J Biometeorol 50:40–47.

Dobbertin M, Mayer P, Wohlgemuth T, Feldmeyer-Christe E, Graf U, Zimmermann N, Rigling A (2005*b*) The decline of *Pinus sylvestris* L. forests in the Swiss Rhone Valley: a result of drought stress? Phyton Horn 45:146–153.

Dobbertin M, Eilmann B, Bleuler P, Giuggiola A, Pannatier EG, 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 Physiol 30:346–360.

Ehleringer JR, Schulze ED, Ziegler H, Lange OL, Farquhar GD, Cowan IR (1985) Xylem-tapping mistletoes: water or nutrient parasites? Science 227:1479–1481.

Ehleringer JR, Field CB, Lin Z-f, Kuo C-y (1986*a*) Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. Oecologia 70:520–526.

Ehleringer JR, Cook CS, Tieszen LL (1986*b*) Comparative water use and nitrogen relationships in a mistletoe and its host. Oecologia 68:279–284.

Escher P, Eiblmeier M, Hetzger I, Rennenberg H (2004) Seasonal and spatial variation of carbohydrates in mistletoes (*Viscum album*) and the xylem sap of its hosts (*Populus* × *euamericana* and *Abies alba*). Physiol Plant 120:212–219.

Escher P, Peuke AD, Bannister P, Fink S, Hartung W, Jiang F, Rennenberg H (2008) Transpiration, CO_2 assimilation, WUE, and stomatal aperture in leaves of *Viscum album* (L.): effect of abscisic acid (ABA) in the xylem sap of its host (*Populus* × *euamericana*). Plant Physiol Biochem 46:64–70.

Farquhar GD, Cernusak LA (2005) On the isotopic composition of leaf water in the non-steady state. Funct Plant Biol 32:293–303.

Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego, CA, pp 47–70.

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137.

Ferrio JP, Cuntz M, Offermann C, Siegwolf R, Saurer M, Gessler A (2009) Effect of water availability on leaf water isotopic enrichment in beech seedlings shows limitations of current fractionation models. Plant Cell Environ 32:1285–1296.

Flanagan LB, Marshall JD, Ehleringer JR (1993) Photosynthetic gas exchange and the stable isotope composition of leaf water: comparison of a xylem-tapping mistletoe and its host. Plant Cell Environ 16:623–631.

Gessler A, Brandes E, Buchmann N, Helle G, Rennenberg H, Barnard R (2009*a*) Tracing carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree-ring archive. Plant Cell Environ 32:780–795.

Gessler A, Löw M, Heerdt C et al. (2009*b*) Within-canopy and ozone fumigation effects on $\delta^{13}C$ and $\delta^{18}O$ in adult beech (*Fagus sylvatica*)

trees: relation to meteorological and gas exchange parameters. Tree Physiol 29:1349–1365.
Gessler A, Ferrio JP, Hommel R, Treydte K, Werner RA, Monson RK (2014) Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood. Tree Physiol 34:796–818.
Gibson I (1967) The influence of disease factors on forest production in Africa. Sect.24:14# Congress of IUFRO, Munich, pp 327–360.
Glatzel G (1983) Mineral nutrition and water relations of hemiparasitic mistletoes: a question of partitioning. Experiments with *Loranthus europaeus* on *Quercus petraea* and *Quercus robur*. Oecologia 56: 193–201.
Gruber N, Galloway JN (2008) An earth-system perspective of the global nitrogen cycle. Nature 451:293–296.
Heizmann U, Kreuzwieser J, Schnitzler J-P, Brüggemann N, Rennenberg H (2001) Assimilate transport in the xylem sap of pedunculate oak (*Quercus robur*) saplings. Plant Biol 3:132–138.
Hill SA, Waterhouse JS, Field EM, Switsur VR, Aprees T (1995) Rapid recycling of triose phosphates in oak stem tissue. Plant Cell Environ 18:931–936.
Hosseini SM, Kartoolinejad D, Mirnia SK, Tabibzadeh Z, Akbarinia M, Shayanmehr F (2007) The effects of *Viscum album* L. on foliar weight and nutrients content of host trees in Caspian forests (Iran). Pol J Ecol 55:579–583.
Johnson JM, Choinski JS Jr (1993) Photosynthesis in the *Tapinanthus-Diplorhynchus* mistletoe-host relationship. Ann Bot 72:117–122.
Kozlowski TT (1971) Growth and development of trees. Academic Press Inc., London, New York, p 443.
Küppers M, Küppers BIL, Neales TF, Swan AG (1992) Leaf gas exchange characteristics, daily carbon and water balances of the host/mistletoe pair *Eucalyptus behriana* F. Muell. and *Amyema miquelii* (Lehm. ex Miq.) Tiegh. at permanently low plant water status in the field. Trees 7:1–7.
Küppers M, Küppers BIL, Swan AG (1993) Leaf conductances and xylem pressures of the host/mistletoe pair *Eucalyptus behriana* F. Muell. and *Amyema miquelii* (Lehm. ex Miq.) Tiegh. at permanently low plant water status in the field. Trees Struct Funct 8:110–114.
Lamont B (1983) Mineral nutrition of mistletoes. The biology of mistletoes. Academic Press, San Diego, pp 129–143.
Li M, Hoch G, Körner C (2002) Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. Trees 16:331–337.
Li MH, Hoch G, Körner C (2001) Spatial variability of mobile carbohydrates within *Pinus cembra* trees at the alpine treeline. Phyton Ann Rei Bot 41:203–213.
Li M-H, Kräuchi N, Dobbertin M (2006) Biomass distribution of different-aged needles in young and old *Pinus cembra* trees at highland and lowland sites. Trees 20:611–618.
Li M-H, Xiao WF, Shi PL, Wang SG, Zhong YD, Liu XL, Wang XD, Cai XH, Shi ZM (2008*a*) Nitrogen and carbon source–sink relationships in trees at the Himalayan treelines compared with lower elevations. Plant Cell Environ 31:1377–1387.
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*b*) Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain limitation but not for growth limitation. Tree Physiol 28:1287–1296.
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 Biol 15:177–184.
Logan BA, Demmig-Adams B, Rosenstiel TN, Adams WW III (1999) Effect of nitrogen limitation on foliar antioxidants in relationship to other metabolic characteristics. Planta 209:213–220.
Logan BA, Reblin JS, Zonana DM et al. (2013) Impact of eastern dwarf mistletoe (*Arceuthobium pusillum*) on host white spruce (*Picea glauca*) development, growth and performance across multiple scales. Physiol Plant 147:502–513.
Lo Gullo MA, Glatzel G, Devkota M, Raimondo F, Trifilò P, Richter H (2012) Mistletoes and mutant albino shoots on woody plants as mineral nutrient traps. Ann Bot 109:1101–1109.
March WA, Watson DM (2007) Parasites boost productivity: effects of mistletoe on litterfall dynamics in a temperate Australian forest. Oecologia 154:339–347.
Marias DE, Meinzer FC, Woodruff DR, Shaw DC, Voelker SL, Brooks JR, Lachenbruch B, Falk K, McCay J (2014) Impacts of dwarf mistletoe on the physiology of host *Tsuga heterophylla* trees as recorded in tree-ring C and O stable isotopes. Tree Physiol 34:595–607.
Marshall JD, Dawson TE, Ehleringer JR (1994) Integrated nitrogen, carbon, and water relations of a xylem-tapping mistletoe following nitrogen fertilization of the host. Oecologia 100:430–438.
McCarthy MC, Enquist BJ (2007) Consistency between an allometric approach and optimal partitioning theory in global patterns of plant biomass allocation. Funct Ecol 21:713–720.
Mcdowell N, Pockman WT, Allen CD et al. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol 178:719–739.
Meinzer FC, Woodruff DR, Shaw DC (2004) Integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection. Plant Cell Environ 27:937–946.
Miller AC, Watling JR, Overton IC, Sinclair R (2003) Does water status of *Eucalyptus largiflorenss* (Myrtaceae) affect infection by the mistletoe *Amyema miquelii* (Loranthaceae)? Funct Plant Biol 30:1239–1247.
Noetzli KP, Müller B, Sieber TN (2003) Impact of population dynamics of white mistletoe (*Viscum album* ssp. *abietis*) on European silver fir (*Abies alba*). Ann For Sci 60:773–779.
Norton DA, Carpenter MA (1998) Mistletoes as parasites: host specificity and speciation. Trends Ecol Evol 13:101–105.
Orozco A, Rada F, Azocar A, Goldstein G (1990) How does a mistletoe affect the water, nitrogen and carbon balance of two mangrove ecosystem species? Plant Cell Environ 13:941–947.
Panvini AD, Eickmeier WG (1993) Nutrient and water relations of the mistletoe *Phoradendron leucarpum* (Viscaceae): how tightly are they integrated? Am J Bot 80:872–878.
Pate JS, Davidson NJ, Kuo J, Milburn JA (1990) Water relations of the root hemiparasite *Olax phyllanthi* (Labill) R.Br. (Olacaceae) and its multiple hosts. Oecologia 84:186–193.
Polle A, McKee I, Blaschke L (2001) Altered physiological and growth responses to elevated [CO_2] in offspring from holm oak (*Quercus ilex* L.) mother trees with lifetime exposure to naturally elevated [CO_2]. Plant Cell Environ 24:1075–1083.
Popp M, Richter A (1998) Ecophysiology of xylem-tapping mistletoes. Prog Bot 56:659–674.
Reblin JS, Logan BA, Tissue DT (2006) Impact of eastern dwarf mistletoe (*Arceuthobium pusillum*) infection on the needles of red spruce (*Picea rubens*) and white spruce (*Picea glauca*): oxygen exchange, morphology and composition. Tree Physiol 26:1325–1332.
Reid N, Yan ZG, Fittler J (1994) Impact of mistletoes (*Amyema miquelii*) on host (*Eucalyptus blakelyi* and *Eucalyptus melliodora*) survival and growth in temperate Australia. Forest Ecol Manag 70:55–65.
Rigling A, Bräker O, Schneiter G, Schweingruber F (2002) Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within the Erico-Pinion in the Valais (Switzerland). Plant Ecol 163:105–121.
Rigling A, Eilmann B, Koechli R, Dobbertin M (2010) Mistletoe-induced crown degradation in Scots pine in a xeric environment. Tree Physiol 30:845–852.
Ripullone F, Grassi G, Lauteri M, Borghetti M (2003) Photosynthesis–nitrogen relationships: interpretation of different patterns between

Pseudotsuga menziesii and *Populus* × *euroamericana* in a mini-stand experiment. Tree Physiol 23:137–144.
Robinson CT, Gessner MO (2000) Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia 122:258–263.
Roden J, Siegwolf R (2012) Is the dual-isotope conceptual model fully operational? Tree Physiol 32:1179–1182.
Roden J, Kahmen A, Buchmann N, Siegwolf R (2015) The enigma of effective path length for ^{18}O enrichment in leaf water of conifers. Plant Cell Environ 38:2551–2565.
Sala A, Carey EV, Callaway RM (2001) Dwarf mistletoe affects whole-tree water relations of Douglas fir and western larch primarily through changes in leaf to sapwood ratios. Oecologia 126:42–52.
Samuelson LA, Anagnostopoulos A, Alva KS, Kumar J, Tripathy SK (1998) Biologically derived conducting and water soluble polyaniline. Macromolecules 31:4376–4378.
Sangüesa-Barreda G, Linares JC, Camarero JJ (2012) Mistletoe effects on Scots pine decline following drought events: insights from within-tree spatial patterns, growth and carbohydrates. Tree Physiol 32:585–598.
Scharpf RF, Smith RS, Vogler D (1988) Management of western dwarf mistletoe in ponderosa and Jeffrey pines in forest recreation areas. General Technical Report, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA.
Scheidegger Y, Saurer M, Bahn M, Siegwolf R (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. Oecologia 125:350–357.
Schulze E-D, Ehleringer JR (1984) The effect of nitrogen supply on growth and water-use efficiency of xylem-tapping mistletoes. Planta 162:268–275.
Schulze E, Kelliher FM, Korner C, Lloyd J, Leuning R (1994) Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: a global ecology scaling exercise. Annu Rev Ecol Syst 25:629–662.
Schulze E-D, Lange OL, Ziegler H, Gebauer G (1991) Carbon and nitrogen isotope ratios of mistletoes growing on nitrogen and non-nitrogen fixing hosts and on CAM plants in the Namib desert confirm partial heterotrophy. Oecologia 88:457–462.
Seibt U, Griffiths H, Berry JA (2008) Carbon isotopes and water use efficiency: sense and sensitivity. Oecologia 155:441–454.
Shaw DC, Huso M, Bruner H (2008) Basal area growth impacts of dwarf mistletoe on western hemlock in an old-growth forest. Can J For Res 38:576–583.
Song X, Farquhar GD, Gessler A, Barbour MM (2014) Turnover time of the non-structural carbohydrate pool influences $\delta^{18}O$ of leaf cellulose. Plant Cell Environ 37:2500–2507.
Song X, Loucos KE, Simonin KA, Farquhar GD, Barbour MM (2015) Measurements of transpiration isotopologues and leaf water to assess enrichment models in cotton. New Phytol 206:637–646.
Stanton S (2006) The differential effects of dwarf mistletoe infection and broom abundance on the radial growth of managed ponderosa pine. Forest Ecol Manag 223:318–326.
Sternberg L, DeNiro MJD (1983) Biogeochemical implications of the isotopic equilibrium fractionation factor between the oxygen atoms of acetone and water. Geochim Cosmochim Acta 47:2271–2274.
Sullivan PF, Sommerkorn M, Rueth HM, Nadelhoffer KJ, Shaver GR, Welker JM (2007) Climate and species affect fine root production with long-term fertilization in acidic tussock tundra near Toolik Lake, Alaska. Oecologia 153:643–652.
Tennakoon KU, Pate JS (1996) Effects of parasitism by a mistletoe on the structure and functioning of branches of its host. Plant Cell Environ 19:517–528.
Tennakoon KU, Chak WH, Bolin JF (2011) Nutritional and isotopic relationships of selected Bornean tropical mistletoe–host associations in Brunei Darussalam. Funct Plant Biol 38:505–513.
Treydte K, Boda S, Graf-Pannatier E et al. (2014) Seasonal transfer of oxygen isotopes from precipitation and soil to the tree ring: source water versus needle water enrichment. New Phytol 202:772–783.
Wang L, Kgope B, D'Odorico P, Macko SA (2008) Carbon and nitrogen parasitism by a xylem-tapping mistletoe (*Tapinanthus oleifolius*) along the Kalahari Transect: a stable isotope study. Afr J Ecol 46:540–546.
Wang X-F, Yakir D, Avishai M (1998) Non-climatic variations in the oxygen isotopic compositions of plants. Glob Change Biol 4: 835–849.
Watling JR, Press MC (2001) Impacts of infection by parasitic angiosperms on host photosynthesis. Plant Biol 3:244–250.
Werner C, Schnyder H, Cuntz M et al. (2012) Progress and challenges in using stable isotopes to trace plant carbon and water relations across scales. Biogeosciences 9:3083–3111.
Xia B, Tian C-M, Luo Y-Q, Liu L-Y, Cairang D-Z, Ma J-H, Han F-Z (2012) The effects of *Arceuthobium sichuanense* infection on needles and current-year shoots of mature and young Qinghai spruce (*Picea crassifolia*) trees. For Pathol 42:330–337.
Xia J, Wan S (2008) Global response patterns of terrestrial plant species to nitrogen addition. New Phytol 179:428–439.
Zuber D (2004) Biological flora of Central Europe: *Viscum album* L. flora-morphology, distribution. Flora 199:181–203.
Zweifel R, Rigling A, Dobbertin M (2009) Species-specific stomatal response of trees to drought—a link to vegetation dynamics? J Veget Sci 20:442–454.
Zweifel R, Bangerter S, Rigling A, Sterck FJ (2012) Pine and mistletoes: how to live with a leak in the water flow and storage system? J Exp Bot 63:2565–2578.
Zwieniecki MA, Brodribb TJ, Holbrook NM (2007) Hydraulic design of leaves: insights from rehydration kinetics. Plant Cell Environ 30:910–921.