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ELUCIDATING THE DROUGHT RESPONSE OF SCOTS PINE (*PINUS SYLVESTRIS* L.) USING STABLE ISOTOPES

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GALINA TIMOFEEVA

M.Sc. in Atmospheric and Climate Science, ETH Zurich

born on 03.02.1985

citizen of Russian Federation

accepted on the recommendation of

Prof. Dr. Harald Bugmann, examiner

Dr. Matthias Saurer, co-examiner

Dr. Giovanna Battipaglia, co-examiner

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For my dear parents,

Valentina and Alexander.

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Abstract

Under anthropogenic climate change, many forest ecosystems will be more and more exposed to drought, as the frequency, duration and intensity of extreme climate events are predicted to increase in the future. Drought-induced forest decline and mortality events of various tree species have already been observed in different parts of the globe. Although the mechanisms of tree response to drought have been studied intensively in recent decades, they are still not clearly understood. Therefore, a better understanding of tree vulnerability and adaptation mechanisms is crucial to support sustainable forest management under changing climate.

The main objective of this dissertation was the investigation of the drought response of an important and wide-spread coniferous species, Scots pine (*Pinus sylvestris* L.), using a combination of methods with a focus on stable isotopes, considering two study sites at the edges of the continuous range of Scots pine in Eurasia complemented by a drought experiment in the greenhouse. Specifically, the following topics were addressed: (1) the short-term responses of tree-ring growth as well as carbon and oxygen isotope variability to irrigation (chapters I & IV); (2) the long-term, century-scale drought response of radial stem growth and isotope variability at two dry sites with contrasting climate regimes (chapters I & III); (3) the adequacy of the two-pool model to explain the oxygen isotope variability in Scots pine needles and tree rings under drought (chapter II); and (4) carbon allocation strategies of Scots pine seedlings under drought and drought release (chapter IV).

To this end, a multiproxy approach combining radial stem growth as well as tree-ring carbon and oxygen stable isotopes was applied, complemented by gas exchange as well as needle morphological data. A long-term irrigation experiment in a mature Scots pine forest provided a unique possibility to investigate the effect of drought release. In addition, the mechanisms underlying the reaction of drought-exposed trees to stress release were studied on seedlings in a greenhouse using compound-specific isotope analysis.

In the first chapter, the long-term effects of drought stress on radial stem growth and carbon isotope variability in Scots pine were investigated for the period of 1900-2014 in the Pfynwald forest (Valais, Switzerland), comparing a group of still living trees with trees that had recently died. The effect of doubling precipitation was investigated for the period 2004-

2014. Tree-ring variables were correlated with climate variables to evaluate the sensitivity and responses to changing climate conditions for the period of 1960-2003. Two phases were identified, one before the beginning of and one during a distinct decline that started in the mid-1980s. The trees that eventually died behaved differently compared to the survivors in both phases. Their growth was lower during almost the entire period of analysis, adopting a more conservative water-use strategy (inferred from increased intrinsic water use efficiency (WUE_i)) during the phase before the distinct decline. This strictly isohydric behavior may have led to a deficiency in carbohydrates and thus general weakening. However, after the mid-1980s, these trees decreased their WUE_i by not being conservative in their water use. This unusual response of dying trees may have been caused by the long-term weakening, resulting in impaired stomatal regulation. The short-term responses to irrigation at the same study site confirmed the main conclusion that water availability is indeed a limiting factor for growth and functionality of Scots pine at this site, and a vegetation shift towards more drought-resistant species such as pubescent oak (*Quercus pubescens*) is to be expected.

The second chapter focuses on the verification of two models for the estimation of stable oxygen isotope variability in needles and tree-ring cellulose of Scots pine under drought. The recently proposed two-pool model was compared with the basic Craig-Gordon model for the estimation of needle water enrichment of irrigated and non-irrigated control trees in the Pfynwald irrigation experiment, based on detailed seasonal sampling of different water pools (soil, needles) over the period of 2013-2015. The basic Craig-Gordon model estimates an enriched leave/needle water pool, assuming equilibrium between water evaporating from a leave/needle and water flowing into the leave/needle from the xylem. The two-pool model considers the ratio of unenriched (xylem) water in the needles to total needle water. This ratio was higher in drought-stressed needles, which were also shorter and damaged under drought stress, thus resulting in lower overall enrichment compared to needles from irrigated trees. The same two models were also applied for the estimation of oxygen isotope variations in tree-ring cellulose for surviving and dead trees. The results were consistent with the lower $\delta^{18}O$ values in tree rings of now-dead trees compared to surviving trees, indicating that needle morpho-physiological changes occurred in dead trees already several decades ago. Such changes in physiology and morphology and the resulting oxygen isotope fingerprints can thus be considered as an early indicator of tree decline.

In the third chapter, the effects of drought on radial growth and carbon and oxygen isotope variability in Scots pine at a site in Central Yakutia, North-Eastern Siberia, Russia, were investigated for the period of 1900-2013. This study site was chosen because (1) it is extremely xeric; (2) it is located on continuous permafrost; and (3) considerable increases in air and soil temperature were observed over the past decades. Similar methods as in the first chapter were applied for this study. Unlike for the Swiss site, there was neither a remarkable growth decline nor long-term changes in stable isotope variability. A negative relationship between oxygen isotope variability and temperature, which is quite unusual, indicated a strong influence of melting permafrost with very low isotope ratios. This source of water is thus likely quite important for the survival of Scots pines here. The depletion of the water pool owing to future warming would be a serious threat for these forests. First indications of drought stress were indeed evident after the mid-1980s, based on carbon isotope variability.

The fourth chapter identified mechanisms of carbon allocation in different tree compartments of 3-year-old *Pinus sylvestris* and *Tilia platyphyllos* seedlings under drought and drought release in a greenhouse experiment after labelling the plants with $^{13}CO_2$. It was observed that growth and the accumulation of soluble sugars were independent of each other in severely drought-stressed *P. sylvestris* seedlings. This indicates that the formation of non-structural carbohydrates could be an active process under severe drought conditions. Control seedlings invested their carbon more into growth (cellulose), storage (lipids) in the stem and coarse roots. Drought-stressed *P. sylvestris* seedlings still continued allocating carbon into storage at the cost of growth during drought release. This may be explained by prioritization of carbon investment into storage by seedlings to gain enough carbon for future growth and survival for the case that drought were to continue, the so-called "drought memory effect".

Overall, this dissertation provided novel insights for the identification and better interpretation of the drought response of Scots pine at the western and eastern edges of its continuous range, i.e. under contrasting climate regimes. The combination of stable isotope variability with growth and physiological data, including isotope fractionation models, was shown to provide valuable knowledge for the early detection of tree decline that will be helpful for the understanding of drought-responses of forests beyond those studied here.

Zusammenfassung

Aufgrund des von Menschen verursachten Klimawandels werden Waldökosysteme vermehrt Trockenstress ausgesetzt, da Häufigkeit, Dauer und Intensität von extremen Klimaereignissen wie Dürre zugenommen haben und in Zukunft wahrscheinlich weiter zunehmen werden. Dürrebedingter Rückgang verschiedener Baumarten und Waldsterben wurde bereits in unterschiedlichen Weltregionen beobachtet. Obwohl die Mechanismen dieser Reaktionen auf Trockenheit in den vergangenen Jahrzenten intensiv untersucht wurden, sind sie noch immer nicht vollständig erklärt. Folglich ist ein besseres Verständnis dieser Trockenheitsanfälligkeit und der Anpassungsmechanismen von zentraler Bedeutung für eine nachhaltige Forstwirtschaft unter sich ändernden Klimabedingungen.

Das Hauptziel dieser Dissertation war die Erforschung der Auswirkungen von Trockenstress bei einer der wichtigsten und weitverbreitetsten Nadelbaumarten, der Waldkiefer (*Pinus sylvestris* L.). Mittels einer Kombination von Methoden mit Fokus auf stabile Isotope in zwei unterschiedlichen Versuchsgebieten, welche jeweils am Rand des Verbreitungsgebietes der Waldkiefer im eurasischen Raum liegen, wurde die Reaktion auf Trockenstress untersucht. Ergänzt wurde die Studie mit einem Gewächshausversuch. Konkret wurden die folgenden Themen untersucht: (1) kurzfristige Reaktionen des Jahrringwachstums sowie die Variabilität in Kohlen- und Sauerstoffisotopen auf Bewässerung (Kapitel I & IV); (2) langfristige Reaktionen auf Trockenstress beim Wachstum von Jahrringen und Isotopenvariabilität an zwei trockenen Standorten mit gegensätzlichen Klimatypen (Kapitel I & III); (3) die Eignung des Zwei-Pool Modells, um die Sauerstoffvariabilität in Nadeln und Jahrringen trockengestresster Bäume erklären zu können (Kapitel II); sowie (4) die Mechanismen der Kohlenstoffdynamik in Jungpflanzen, welche Dürre und erneuter Bewässerung ausgesetzt waren (Kapitel IV).

Zu diesem Zweck wurde ein Multiproxy-Ansatz gewählt, welcher die Analyse der Zuwachsraten mit Kohlen- und Sauerstoffisotopen in Jahrringen, Gasaustausch und morphologischen Daten der Nadeln kombiniert. Ein langfristiges Bewässerungsexperiment in einem ausgewachsenen Kieferwald ermöglichte es, die Auswirkungen einer Minderung der Trockenheit zu untersuchen. Die zugrundeliegenden Mechanismen dieser Reaktion trockengestresster Pflanzen auf die Bewässerung wurde anhand von Jungpflanzen in Wachstumskammern erforscht.

Im ersten Kapitel werden die Langzeitauswirkungen von Trockenstress auf das radiale Wachstum und die Variabilität von Kohlenstoffisotopen bei der Waldkiefer für den Zeitraum von 1900–2014 in Pfynwald (Wallis, Schweiz) untersucht, wobei eine Gruppe (noch) lebender Bäume mit bereits abgestorbenen verglichen wurde. Der Effekt einer experimentellen Verdoppelung des Niederschlags wurde für den Zeitraum von 2004–2014 erforscht. Um die Sensitivität und Reaktion gegenüber dem Klimawandel zu beurteilen, wurden Jahrringparameter mit Klimavariablen korreliert. Es wurden zwei Phasen identifiziert, nämlich vor und nach Beginn des deutlichen Rückgangs, welcher Mitte der 80er-Jahre einsetzte. In beiden Phasen verhielten sich die abgestorbenen Bäume anders als die Überlebenden. Ihr radiales Wachstum war während beinahe des gesamten analysierten Zeitraumes geringer, und sie folgten einer konservativeren Wassernutzungsstrategie. Dieses strikt isohydrische Verhalten könnte zu einem Mangel an Kohlehydraten und somit zu einer generellen Schwächung geführt haben. Nach Mitte der 80er-Jahre wurden diese Bäume allerdings weniger konservativ in ihrer Wassernutzung. Diese ungewöhnliche Reaktion der absterbenden Bäume wurde möglicherweise durch die langfristige Schwächung verursacht, welche zu einer gestörten Stomataregulierung führte. Des Weiteren bestätigten die kurzfristigen Reaktionen auf Bewässerung die zentrale Schlussfolgerung, dass Wasserverfügbarkeit tatsächlich ein limitierender Faktor für Wachstum und Funktionsfähigkeit der Waldkiefern dieses Standorts ist und deshalb eine Verschiebung der Vegetation hin zu vermehrt trockenresistenten Arten wie beispielsweise der Flaumeiche (*Quercus pubescens* Willd.) zu erwarten ist.

Das zweite Kapitel konzentriert sich auf die Überprüfung zweier Modelle zur Berechnung der Variabilität stabiler Sauerstoffisotope in Nadeln und Zellulose in Jahrringen der trockengestressten Waldkiefer. Das kürzlich veröffentlichte Zwei-Pool Modell wurde mit dem grundlegenden Craig-Gordon Modell verglichen, und zwar zur Einschätzung der Anreicherung in Nadelwasser bei bewässerten und nicht-bewässerten Kontrollbäumen im Pfynwald-Bewässerungsexperiment basierend auf detaillierten saisonalen Probennahmen unterschiedlicher Wasserquellen (Boden, Nadeln) von 2013 bis 2015. Das Craig-Gordon Modell schätzt die isotopische Nadelwasser-Anreicherung unter Annahme eines Gleichgewichts zwischen verdunstendem Wasser und Wasser, welches aus dem Xylem in die Nadel fliesst. Das Zwei-Pool Modell berücksichtigt zusätzlich das Verhältnis zwischen unangereichertem (Xylem-)Wasser in den Nadeln und dem gesamten Nadelwasser. Dieses

Verhältnis war grösser bei trockengestressten Nadeln, welche ausserdem kleiner und vom Dürrestress geschädigt waren, woraus eine geringere Gesamtanreicherung im Vergleich zu Nadeln bewässerter Bäume resultierte. Dieselben zwei Modelle wurden ebenfalls zur Einschätzung von Sauerstoffisotopenvariationen bei Zellulose in Jahrringen überlebender und abgestorbener Bäume verwendet. Die Resultate stehen im Einklang mit den tieferen δ^{18}O-Werten aus den Jahrringen nun toter Bäume verglichen mit überlebenden, was bedeutet, dass bereits vor einigen Jahrzenten in den Nadeln der abgestorbenen Bäume morpho-physiologische Änderungen aufgetreten sind. Solche Veränderungen in Physiologie und Morphologie sowie die daraus entstandenen Sauerstoffisotopen-Fingerabdrücke können somit als früher Indikator eines Kiefernsterbens betrachtet werden.

Im dritten Kapitel wurden die Auswirkungen von Dürre auf das radiale Wachstum sowie die Variabilität der Kohlenstoff- und Sauerstoffisotopen von Waldkiefern in Zentraljakutien, Nordost-Sibirien, Russland für den Zeitraum 1900-2013 untersucht. Dieser Standort wurde ausgewählt aufgrund (1) ausserordentlich arider Verhältnisse; (2) der Lage auf kontinuierlichem Permafrost; und (3) der Beobachtung beträchtlicher Anstiege der Luft- und Bodentemperatur während der letzten Jahrzehnte. Für diese Untersuchung wurden ähnliche Methoden verwendet, welche bereits im ersten Kapitel beschrieben worden waren. An diesem Standort in Sibirien waren hingegen weder ein drastischer Wachstumsrückgang noch eine nennenswerte langfristige Änderung der Isotopenvariabilität zu verzeichnen. Der ungewöhntliche negative Zusammenhang zwischen der Variabilität der Sauerstoffisotopen und der Temperatur weist auf einen starken Einfluss des schmelzenden Permafrostes (mit äusserst niedrigen Isotopenwerten) hin. Diese Wasserquelle scheint also von grosser Bedeutung zu sein für das Überleben der Waldkiefer in diesem Gebiet. Ein Rückgang dieser Wasserzufuhr wegen Austrocknung des Bodens infolge zukünftiger Klimaerwärmung stellt für diese Wälder eine ernsthafte Bedrohung dar. Erste Anzeichen von Trockenstress waren aufgrund der Variabilität in Kohlenstoffisotopen bereits seit Mitte der 80er-Jahre nachweisbar.

Das vierte Kapitel befasst sich mit der Allokation des Kohlenstoffs bei dreijährigen Waldkiefern (*Pinus sylvestris)* und Sommerlinden (*Tilia platyphyllos)*, welche in einem Gewächshausversuch Trockenheit und erneuter Bewässerung ausgesetzt wurden. Es wurde beobachtet, dass das Wachstum bei stark trockengestressten *P. sylvestris* Keimlingen und die Konzentration von wasserlöslichen Zuckern unabhängig voneinander sind. Dies könnte darauf hinweisen, dass bei dieser Baumart eine Anhäufung nicht-struktureller Kohlehydrate ein aktiver Vorgang bei starker Trockenheit ist. Kontrollpflanzen investierten vermehrt in Wachstum (Zellulose) und Speicher (Lipide) in Stamm und Grobwurzeln. Trockengestresste *P. sylvestris*-Pflanzen wiesen während der erneuten Bewässerung den Kohlenstoff weiterhin dem Speicher zu, auf Kosten des Wachstums. Erklärbar wäre dies durch einen sogenannten „Memory"-Effekt, wobei die Pflanzender Investition von Kohlenstoff in Speicher Priorität geben, um für Wachstum und Überleben im Falle einer fortgesetzten Trockenheit gerüstet zu sein.

Insgesamt vermittelt diese Dissertation neue Einblicke in die Identifikation und bessere Interpretation von trockenheitsbedingten physiologischen Reaktionen der Waldkiefer im westlichen und östlichen Teil ihres Verbreitungsgebietes, d.h. unter unterschiedlichen Klimabedingungen. Die Kombination von Isotopen mit Wachstums- und physiologischen Daten einschliesslich Modellen der Isotopenfraktionierung bietet neue, wertvolle Erkenntnisse zur frühen Erfassung von Waldschäden, welche hilfreich sein werden bei der Untersuchung von anderen trockengestressten Wäldern.

General Introduction

Background

Climate change and drought

Changes in climate from regional to global scales since the mid-19th century have been observed and confirmed by many studies worldwide. According to temperature measurements of NASA's Goddard Institute for Space Studies (GISS), the average global temperature on Earth has increased by about 0.8 °C since 1880 (Hansen *et al.*, 2010). Most of the warming has occurred since 1975, at a rate of roughly 0.15-0.20 °C per decade, and land and sea surface temperatures are likely to continue increasing. The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2013) has demonstrated continuing changes in global air and see surface temperatures and concentrations of greenhouse gas (GHG) emissions. These changes in climate affect the Arctic and Antarctic sea ice covers, ocean acidity, the global water cycle, mean sea level and vegetation (IPCC, 2013). Changes in vegetation will in turn affect the global carbon cycle. It is further important to consider that these changes are spatially heterogeneous. For example, it has been demonstrated that significant increases in annual maximum daily precipitation amount are very regional (Westra *et al.*, 2013), and that future changes in the global water cycle will not be spatially uniform (IPCC, 2013). The divergence in precipitation amounts between humid and arid regions will become more extreme, and the same goes for the difference between wet and dry seasons, although there will be regional exceptions (IPCC, 2013).

The global evapotranspiration and soil moisture variability will also be influenced by above-mentioned changes (Sheffield & Wood, 2008, Jung *et al.*, 2010, Dong & Dai, 2017, Fisher *et al.*, 2017). As a result, there will be a higher risk of more frequent, longer lasting and more severe extreme weather and climate events, particularly drought conditions (Meehl *et al.*, 2000, Ummenhofer & Meehl, 2017). Extremely high temperatures increase evaporation and thus strongly reduce soil water content, have been shown to aggravate heat waves and lead to acute drought, such as the European summer heat wave in 2003 (Schaer *et al.*, 2004, Fischer *et al.*, 2007, García-Herrera *et al.*, 2010) and in 2010 (Barriopedro *et al.*, 2011, Trenberth & Fasullo, 2012, Zveryaev & Arkhipkin, 2017). Although there may be no changes in rainfall in some regions, increasing temperatures can still raise atmospheric moisture demand and evaporation, therefore limiting water supply to plants. Repeatedly occurring drought events may become more frequent and severe in some regions of the globe (Dai, 2011, Dai, 2013, Rajczak *et al.*, 2013). Thus, drought may become one of the major stress factors of our ecosystems as a consequence of climate change (Fig. 1).

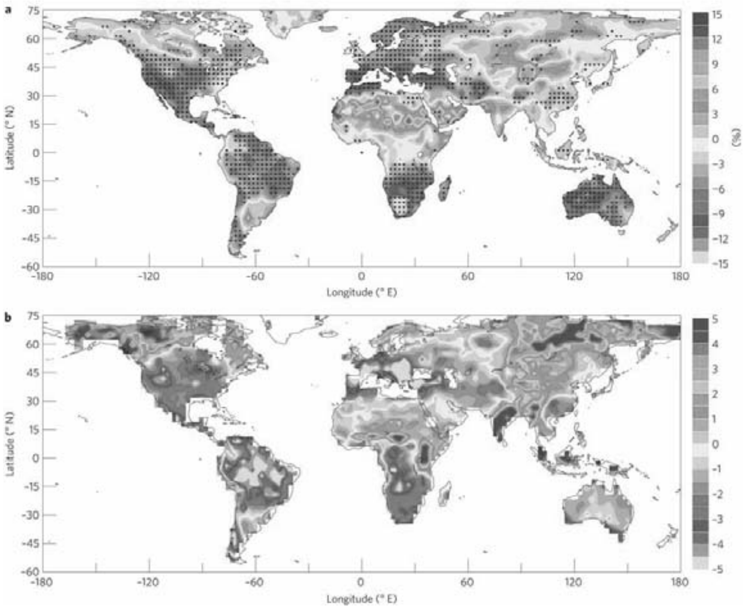

Figure 1. (a) Percentage changes from 1980–1999 to 2080–2099 in the multimodel ensemble mean soil-moisture content in the top 10 cm layer (broadly similar for the whole soil layer) simulated by 11 CMIP5 models under the RCP4.5 emissions scenario. Stippling indicates at least 82% (9 out of 11) of the models agree on the sign of change. (**b)** Mean sc_PDSI_pm averaged over 2090–2099 computed using the 14-model ensemble mean climate (including surface air temperature, precipitation, wind speed, specific humidity and net radiation) from the CMIP5 simulations under the RCP4.5 scenario. A sc_PDSI_pm value of −3.0 or below indicates severe to extreme droughts for the present climate, but its quantitative interpretation for future values in **b** may require modification. Note: the Palmer Drought Severity Index (PDSI) is a standardized index that ranges from -10 (dry) to +10 (wet) for dryness estimation based on available temperature and precipitation data. From Dai *et al*. (2013).

Drought effects on forest ecosystems and mechanisms of tree mortality

Forests cover ca. 30 % of the Earth's land area. They are habitats for many animal and plant species. Moreover, forests play a significant role in the global carbon and water cycles. They act as a carbon source via deforestation and as a sink via reforestation and forest area enhancement. They contribute to the regulation of hydrological processes particularly via transpiration. In addition, they have a socio-economic significance, providing the society wood for heating, timber for construction and furthermore opportunities for recreation and tourism. Both acute and chronic droughts affect terrestrial ecosystems, particularly they strongly affect forests (Allen *et al.*, 2010b). Therefore, a thorough investigation and better understanding of forest ecosystem response and vulnerability to ongoing climate change and to extreme weather events are crucial (Frank *et al.*, 2015). Nevertheless, the exact mechanisms underlying tree decline and mortality are still highly debated (Breshears *et al.*, 2005, Ciais *et al.*, 2005, McDowell *et al.*, 2008, Sala, 2009, Allen *et al.*, 2010b, McDowell & Sevanto, 2010, Sala *et al.*, 2010, Sala *et al.*, 2012, Anderegg *et al.*, 2013, Park Williams *et al.*, 2013, Palacio *et al.*, 2014, Allen *et al.*, 2015, Anderegg *et al.*, 2015). There are various potential abiotic and biotic factors responsible for forest decline and dieback, which are difficult to disentangle (McDowell *et al.*, 2008, Anderegg *et al.*, 2015). Air pollution, eutrophication, soil chemical stress, extreme weather and climate events, and general site conditions are known as important abiotic factors, whereas defoliation and wood decomposition after insect attack, infection by pathogenic fungi and microorganisms, and competition among the trees within a population for light and water supply are important biotic factors (Thomas *et al.*, 2002, McDowell *et al.*, 2008, Pasho *et al.*, 2012, Anderegg *et al.*, 2015, Chakraborty *et al.*, 2017). Biotic factors are common reasons of decline and consequently mortality, but often in combination with abiotic factors, particularly drought events.

The two most discussed tree-physiological mechanisms responsible for drought-induced tree decline and mortality are carbon starvation and hydraulic failure. Carbon starvation is caused by plant stomatal closure to prevent desiccation (McDowell *et al.*, 2008), which leads to a reduction of photosynthetic activity and availability of carbohydrates. The continued demand for carbohydrates to support the plant’s metabolic activity will decrease carbon reserves , leading eventually to so-called starvation, often coupled with the inability to defend against other biotic and abiotic stressors (McDowell *et al.*, 2008, McDowell & Sevanto, 2010, Hartmann, 2015, Salmon *et al.*, 2015). Hydraulic failure occurs when a plant loses a larger amount of water by transpiration than it can take up by the roots, creating high xylem water tension and resulting in progressive cavitation and conductivity loss of the xylem (Sperry *et al.*, 1998, McDowell *et al.*, 2008, Sevanto *et al.*, 2014, Salmon *et al.*, 2015). This process may be most important for very severe (short-term) droughts.

The relative importance of these two processes is not always clear in the context of tree decline and mortality. It was often found that a combination of both mechanisms was operating (McDowell *et al.*, 2008, Sala, 2009, Sala *et al.*, 2010, Gruber *et al.*, 2012, Sevanto *et* *al.*, 2014, Gaylord *et al.*, 2015, Hartmann, 2015, Rowland *et al.*, 2015, Salmon *et al.*, 2015). As more extreme and longer droughts are expected in the future (Fig. 2), quantitative knowledge of the physiological thresholds of individual tree mortality under chronic or acute drought stress is needed (McDowell *et al.*, 2008, Allen *et al.*, 2010b).

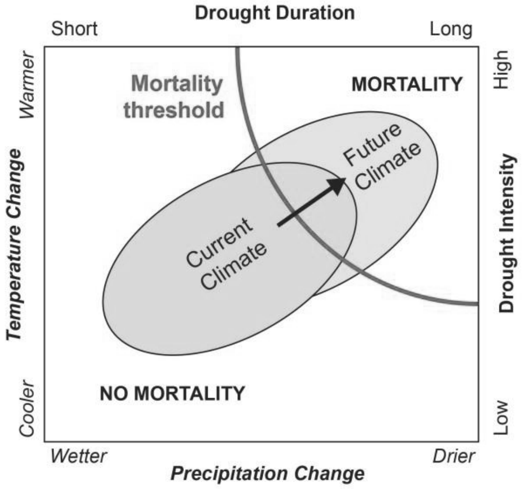

Figure 2. Conceptual diagram of forest vulnerability to a changing climate. From Allen *et al*. (2010).

In this context, species-specific plant characteristics need to be considered related to stomatal regulation, isohydric *vs.* anisohydric (Tardieu, 1993, Tardieu *et al.*, 1998, McDowell *et al.*, 2008). Isohydric species, which are closing their stomata as water is becoming scarce and thus maintain cell-internal water pressure, feature a conservative water-use strategy during drought conditions, making them prone to carbon starvation. In contrast, anisohydric species continue transpiration during drought and thus experience much decreased cell-internal water pressure, rendering them susceptible to hydraulic failure. Moreover, the role of carbon storage for adaptation and survival (O'Brien *et al.*, 2014) as well as plant regulation of allocation of freshly produced non-structural carbohydrates (NSCs) to storage pools (Sala *et al.*, 2012, Wiley & Helliker, 2012, Dietze *et al.*, 2014) under drought need to be taken into account and understood better (Galiano *et al.*, 2017). Furthermore, storage (NSCs) becomes a relevant carbon source under drought, when production of fresh carbohydrates is limited. Again these processes can be species- or even individual-specific, and not always can NSCs production under drought conditions be considered simply as a passive overflow process, as appears to be the case for some conifers in contrast to some deciduous species (Galiano *et* *al.*, 2017). Thus, to predict the survival potential of trees, detailed investigations of mechanisms underlying tree decline and mortality is needed.

Main Objectives

The general objective of this dissertation was to identify the short- and long-term growth and physiological responses of Scots pine (*Pinus sylvestris* L.) to past and current climate variability at two extremely dry study sites in canton of Valais (Switzerland) and Central Yakutia (North-Eastern Siberia, Russia). An additional goal was to test and improve existing oxygen isotope fractionation models for needles and tree-ring cellulose by a detailed assessment of isotopes in different water pools for better understanding past physiological changes under drought. Furthermore, the determination of carbon allocation strategies of *Pinus sylvestris* and *Tilia plathyphyllos* seedlings under different water regimes in a greenhouse experiment seedlings was another aim of this dissertation.

To achieve these objectives, a combination of various methods (dendrochronological and physiological analyses, including bulk and compound-specific isotope analysis (CSIA), gas-exchange and needle morphological measurements) was applied. The rationale for the selected species and sites as well as the methods is given in the following sections.

Data and Methods

Application of stable isotopes in environmental studies

Almost all elements of the periodic table have isotopes. Stable isotopes do not decay into other chemical elements, as radioactive isotopes do. Stable isotopes have almost identical chemical properties, but the additional neutrons make an element heavier than the more common and usually most abundant light stable isotope. This difference results in discrimination of the heavier against the lighter isotopes during various physical, chemical and biological processes, enabling them to be used in a broad variety of applications such as environmental and paleoclimatological studies as well as forensics (Peterson & Fry, 1987, McCarroll & Loader, 2004). Most important in such studies are the stable isotopes of carbon, oxygen, hydrogen and nitrogen. The use of stable isotopes in ecological studies based on tree rings or other organic matter has become an important complement to traditional methods (Fritts, 1976, Schweingruber, 2012, Song *et al.*, 2013, Roden *et al.*, 2015, Cernusak *et al.*, 2016).

For example, ^{13}C has been widely used for investigating the uptake of carbon dioxide (CO_2) from the atmosphere during photosynthesis through stomata and the enzymatic fixation of the carbon molecules by RuBisCO (*ribulose bisphosphate carboxylase-oxygenase*) in C_3 plants. Due to isotope fractionation processes during the diffusion of CO_2 molecules and carbon fixation, which depend on environmental conditions, there are distinct differences in the carbon isotope ratio of organic matter (Farquhar *et al.*, 1989, Ehleringer, 1993). The current value of $\delta^{13}C$ in the atmosphere is ca. -8‰ relative to Vienna Pee Dee Belemnite (VPDB). Plants, particularly those that use the C_3 type of carbon assimilation, are much depleted relative to air and have $\delta^{13}C$ values in the range of -30‰ to -20‰ (McCarroll & Loader, 2004). Carbon isotopes in plant organic matter, for instance in bulk wood or cellulose, are often used for investigating plant physiological responses (in stomatal conductance or assimilation rate) to changes in environmental conditions, and for reconstructing climate, such as drought conditions (Mazany *et al.*, 1980, Saurer *et al.*, 1997, Treydte *et al.*, 2007).

Oxygen isotopes in plant organic matter have also been applied for such studies (Sternberg, 2009). They provide valuable information on changes in source water, often precipitation and/or groundwater (Dansgaard, 1964). Although there is no fractionation during water uptake by the roots (Wershaw *et al.*, 1966), a major fractionation occurs at the leaf/needle level, where lighter isotopes are preferentially lost via transpiration, leading to an enrichment in plant tissue $\delta^{18}O$ of up to 20‰ (Craig & Gordon, 1965, Saurer *et al.*, 1998a, Saurer *et al.*, 1998b, McCarroll & Loader, 2004, Barbour, 2007, Cernusak *et al.*, 2016).

The interpretation of plant responses to varying environmental conditions based only on carbon or oxygen isotope variability in plant organic matter often is not straightforward. Therefore, the combination of both isotopes, the so-called dual-isotope approach, is increasingly applied (Scheidegger *et al.*, 2000, Roden & Farquhar, 2012). This approach has indeed been powerful for elucidating whether plant functional responses are related to the stomatal control of water losses, or to varying assimilation rates (Voltas *et al.*, 2013). However, this approach has also its disadvantages. Before using it, one has to carefully design an experiment to meet the model assumptions and avoid over-interpretation risks (Roden & Siegwolf, 2012, Roden & Farquhar, 2012). Moreover, some studies have shown that this approach is most suitable for leaf organic matter, but less acurate for tree rings (Gessler *et al.*, 2014).

In recent years, compound-specific isotope analysis (CSIA) has become possible due to new technological developments. This method allows to measure values of $\delta^{13}C$ and $\delta^{18}O$ individually in freshly produced leaf sugars (glucose, fructose, raffinose and others), transport sugars (sucrose) as well as in storage compounds (starch and lipids), providing a much more detailed picture on carbon allocation and storage (Richter *et al.*, 2009, Rinne *et al.*, 2012, Streit *et al.*, 2013, Zech *et al.*, 2013, Rinne *et al.*, 2015a). CSIA can be applied to measure seasonal changes in isotope ratios of various tree tissues (Simard *et al.*, 2013, Rinne *et al.*, 2015b).

Overall, stable isotope methods are a unique, powerful and highly valuable tool for better understanding tree responses to environmental conditions and particularly a changing climate.

Main investigated species

Scots pine (*Pinus sylvestris* L.) is native from Western Europe to Far Eastern Siberia, south to the Caucasus Mountains and Anatolia, and north to the Arctic Circle (Fig. 3). The species is well-known for its drought and frost tolerance as well as its ability to grow under extreme conditions, such as on poor, sandy soils, rocky outcrops, and peat bogs. Scots pine is widely used for heating and as construction timber and is therefore a relevant species not only for forest ecosystems, but also for human societies.

Decline and in some cases mortality of this species has already been observed in various regions, owing to a combination of factors including current climate change, particularly increasing drought conditions in different parts of its distribution (Bigler *et al.*, 2006, Allen *et al.*, 2010a, Rigling *et al.*, 2013, Vilà-Cabrera *et al.*, 2013, Voltas *et al.*, 2013, Hereş *et al.*, 2014, Matías *et al.*, 2017, Semerci *et al.*, 2017, Zadworny *et al.*, 2017). To understand and predict the future consequences of climate change on Scots pine, it is important to investigate current and ongoing responses of this species at various study sites, particularly at the edges of its distribution.

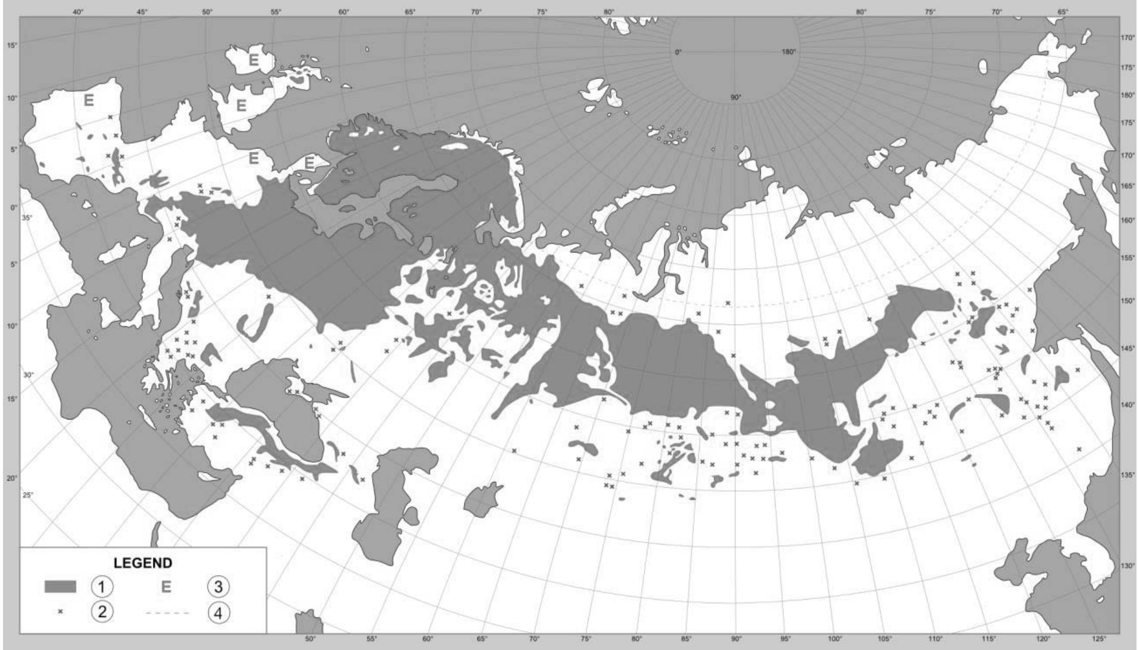

Figure 3. Map of the native range of Scots pine (*Pinus sylvestris* L.). 1 – Main range of the species; 2 – Isolated occurrences; 3 – Natural populations extinct due to human intervention (reintroduced populations established in some areas), 4 – Arctic Circle. Source: EUFORGEN 2009, www.euforgen.org.

Study sites

One main Scots pine study site investigated in this dissertation is located in an inner-Alpine valley in the canton of Valais in the southwestern part of Switzerland, named Pfynwald, where the river Rhone flows along the valley from its headwaters to Lake Geneva, separating the Pennine Alps from the Bernese Alps. This site is located in one of the driest areas not only of Switzerland, but also of the entire region of the European Alps because of the surrounding mountains that block moist oceanic air masses, resulting in low precipitation amount and low cloud cover. Furthermore, according to the current climate change scenarios for Switzerland (CH2011, 2011), temperature, particularly in summer, is expected to increase between 2.7 and 4.1 ºC above that of the 1980-2009 reference period in all regions of the country by the end of the 21st century, whereas the average summer precipitation is expected to decrease by 18-24% (Fig. 4), provided that there is a continuing increase of greenhouse gas emissions until 2100 and no specific international efforts are made to reduce their concentrations (CH2011, 2011).

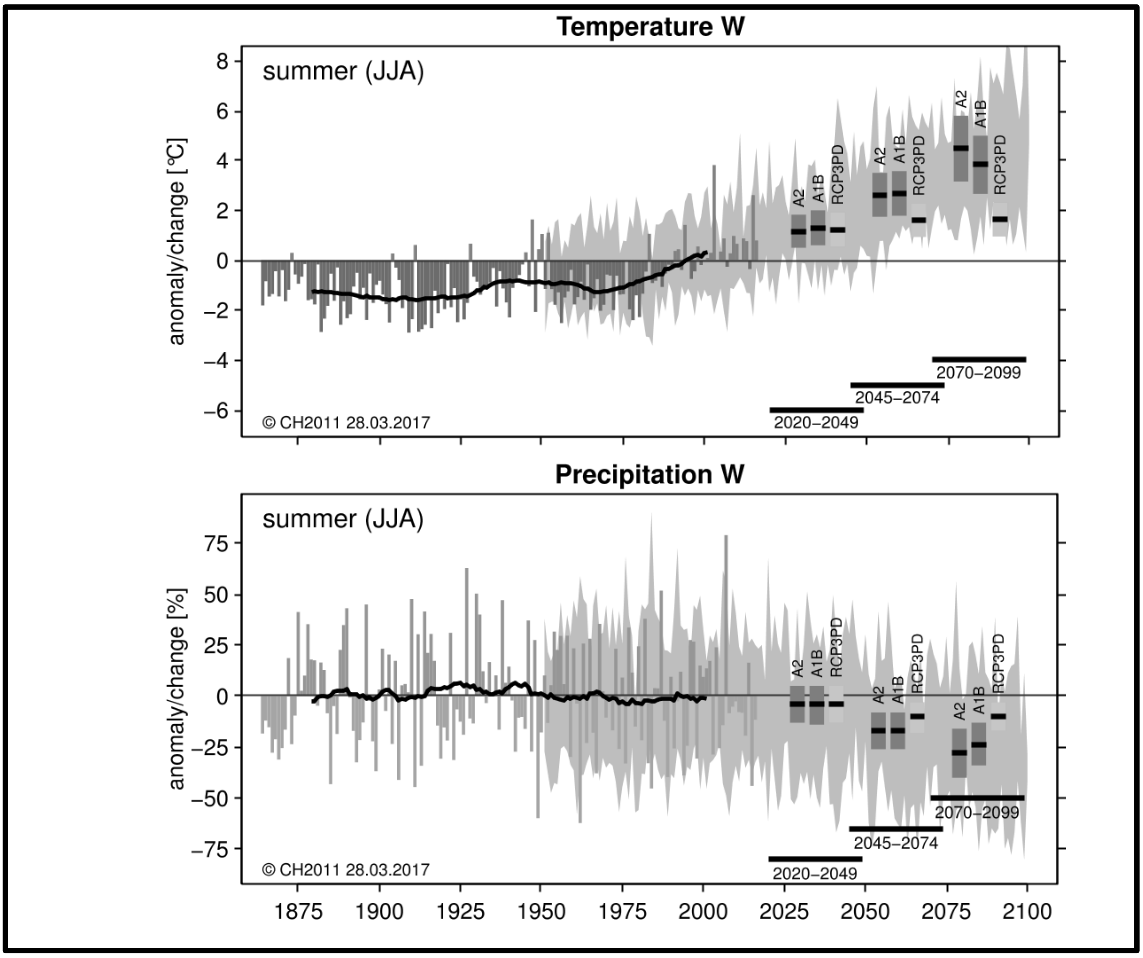

Figure 4. Past and future changes in summer temperature (°C) and precipitation (%) over western Switzerland. The changes are relative to the reference period 1980–2009. The thin colored bars display the year-to-year differences with respect to the average of observations over the reference period; the heavy black lines are the corresponding smoothed 30-year averages. The grey shading indicates the range of year-to-year differences as projected by climate models for the A1B scenario (specifically, the 5–95 percentile range for each year across the available model set). The thick colored bars show best estimates of the future projections, and the associated uncertainty ranges, for selected 30-year time-periods and for three greenhouse gas emission scenarios. Source: CH2011.

To investigate Scots pine response to drought, a long-term irrigation experiment was initiated by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL, Birmensdorf) in the Pfynwald forest (46º 18' N, 7º 36' E, 615 m a.s.l.) in the Valais (Brunner *et al.*, 2009, Eilmann *et al.*, 2009, Dobbertin *et al.*, 2010, Eilmann *et al.*, 2010, Rigling *et al.*, 2013, Herzog *et al.*, 2014). The irrigation started in summer 2003. Eight plots of 25 x 40 m each were defined, four of them are irrigated and the other four are control plots (Fig. 5). The irrigated plots receive additional water of ca. 600 mm each year during the growing season (i.e., from April to October), which is equal to doubling the annual precipitation at the experimental site.

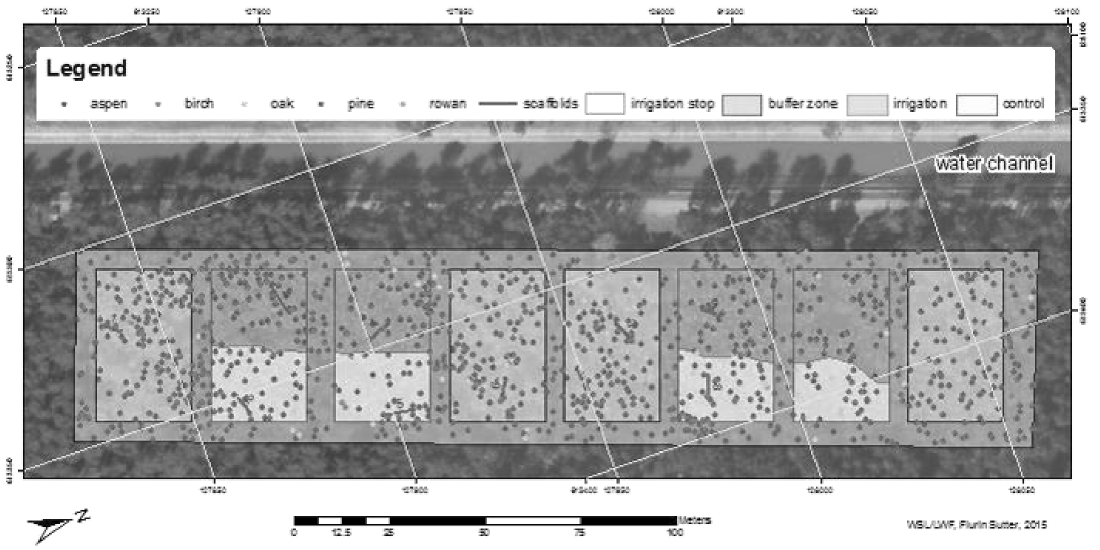

Figure 5. The irrigation experiment Pfynwald consists of four irrigated (blue) and four non-irrigated (light green) control plots (25 m x 40 m, 60-100 trees in each plot; coloured dots represent single trees). The light blue areas indicate the sub-plots where irrigation was stopped at the end of 2013. Copyright of WSL/LWF, Flurin Sutter, 2015.

A second Scots pine study site used in my dissertation is located at the Spasskaya Pad Scientific Forest Station (62º 14' N, 129º 37' E, ca. 220 m a.s.l.) in Central Yakutia (Republic of Sakha (Yakutia), North-Eastern Siberia, Russia), established and run by the Institute for Biological Problems of the Cryolithozone, Siberian Branch of the Russian Academy of Sciences. This site is located on a Pleistocene terrace at the western bank of the middle section of the river Lena, approximately 20 km north of Yakutsk and ca. 480 km north of the Arctic Circle. It is characterized by continuous permafrost with a thickness up to 150 m in some areas. Mean annual precipitation was 238 mm with most of the rainfall occurring in summer (111 mm), while mean annual temperature was -8.8 °C and 17.0 °C in summer (1971-2000). This site is located in one of the driest parts of Siberia (Fig. 6). In summer, moist southeasterly winds from the Pacific Ocean lose their moisture over the coastal mountains before reaching this region, whereas in winter there is the formation of the so-called Siberian High, an intense accumulation of very cold dry air, resulting in light snow cover (Cohen *et al.*, 2001, Gong & Ho, 2002).

Therefore, both study sites are very dry and predisposed to recurring drought events, but one is located towards the western edge of the Scots pine continuous range in Central Europe and the other one at the eastern edge in North-Eastern Siberia. These two sites are strongly contrasting in their climate regimes, temperate vs. extremely continental. Furthermore, soil conditions are different, whereby the influence of melting permafrost in Siberia plays an important role. Therefore, investigation of Scots pine response to climate variability under contrasting climate regimes will be useful for elucidating its potential acclimation to future climate conditions in different areas.

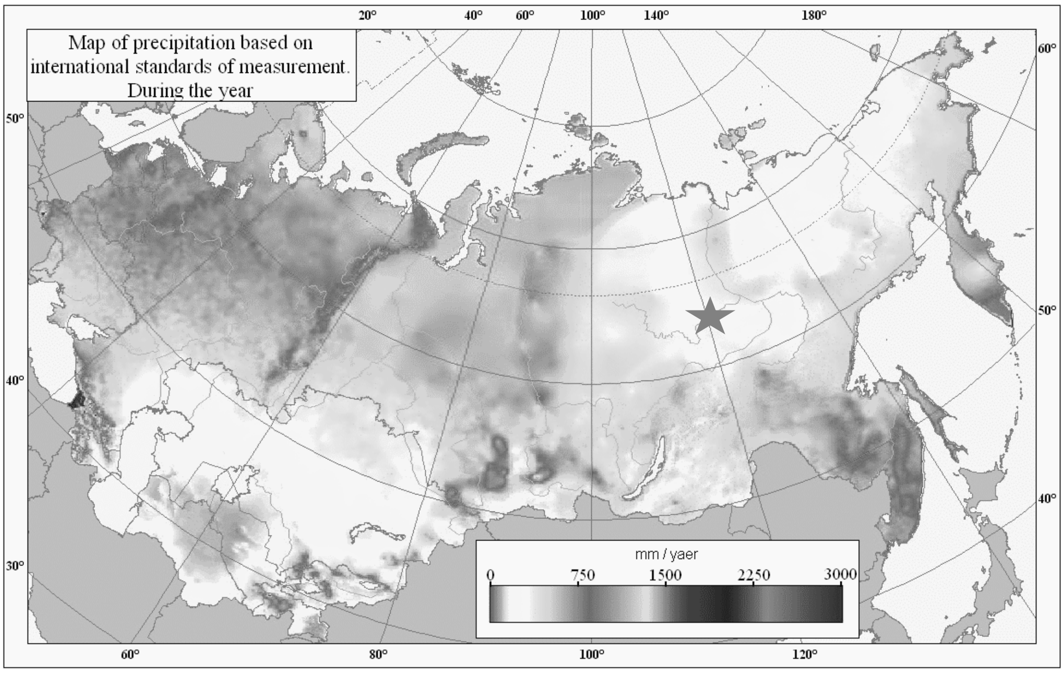

Figure 6. Annual precipitation in Russia. The map originated by A.N. Afonin and K.L. Lipiyaynen on 15.03.2008. The red star indicates the study site in Yakutia.

Greenhouse experiment with seedlings

It is relevant to study and determine tree responses to drought not only under different climate regimes, but also investigate how different tree species respond to it, for instancedeciduous vs. coniferous species. This can be helpful for estimating drought-adaptation potentials of tree species with contrasting physiology. However, it continues to be challenging to conduct drought or irrigation experiments with mature trees in a forest, owing to restrictions in time and funding. Therefore, to date greenhouse experiments under controlled conditions have often been privileged.

A drought experiment with seedlings of *Pinus sylvestris* and *Tilia platyphyllos* was conducted in a greenhouse of the WSL Birmensdorf in summer 2014. The aim of this experiment was to determine carbon allocation strategies under drought conditions as well as after drought release.

At the beginning of the experiment, 108 seedlings of each species with an age of 3 years were planted into individual pots and separated into three groups, according to treatments: control (well-watered), moderate drought, and severe drought. The drought treatment started when the seedlings had their leaves and needles fully developed after growing under favorable conditions with enough light and water available. After ca. 2 months of the treatment, the pots with the seedlings of the moderate and severe drought groups were re-watered to field capacity, and a $^{13}CO_2$-labelling was applied to trace carbon fluxes within the plants. Measurements of soil conditions in the pots and gas-exchange (stomatal conductance, assimilation rate) were performed at several occasions before, during and after the drought treatment. The seedlings (leaves/needles, stem, roots separately) were harvested for CSIA analysis and measurements of carbohydrate concentrations.

Dissertation outline and specific research questions

The main part of the dissertation consists of four chapters, followed by a synthesis and outlook.

The first chapter is focused on long-term changes in increment growth and carbon isotope variability in Scots pine under drought in the Pfynwald forest in the Valais, comparing a group of declining with still healthy trees as well as short-term responses to an irrigation treatment at this site.

The second chapter presents the analysis of oxygen isotope variability between irrigated and control (healthy vs. stressed trees) after more than a decade of irrigation. This chapter includes a comparison of observed and modelled needle water enrichment data to test two modelling approaches.

The third chapter demonstrates the effects of changes in permafrost, owing to climate change, on radial growth and the variability of stable carbon and oxygen isotopes of Scots pines at an extremely dry pine forest in Central Yakutia (North-Eastern Siberia, Russia).

The fourth chapter provides insights on the responses of Scots pine and lime (*Tilia platyphyllos*) seedlings to different water regimes (severe and moderate drought, and control) as well as re-watering after ca. 10 weeks of drought experiment in a greenhouse. The main focus of this chapter is the investigation and interpretation of carbon allocation and storage mechanisms during drought and subsequent recovery.

The specific research questions of each chapter are provided below.

Chapter 1:

- What are the effects of the 10-year irrigation on tree-ring growth and tree-ring carbon isotope patterns in a Scots pine forest in Valais, Switzerland?
- How does tree-ring growth differ between still living and recently deceased trees over the past ~100 years in terms of climatic sensitivity and carbon isotope variability?
- What is the role of increasing VPD for tree decline in the study region?
- Can the combined analysis of tree-ring growth and δ^{13}C-derived water-use efficiency help to elucidate the mechanisms underlying the observed tree decline?
- Can we identify physiological key indicators that characterize trees with low *vs.* high mortality risk?

Chapter 2:

- Do δ^{18}O values predicted with the two-pool correction model adequately reflect physiological changes in Scots pines at the Swiss study site, particularly for explaining differences between the control (drought-stressed) and irrigated trees?

- Are changes in the fraction of unenriched (xylem) water in needles to total needle water (xylem + cell water) over time incorporated in the isotopic signal of the tree-ring cellulose and could this fraction be an early indicator of tree decline?

Chapter 3:

- How did air and soil temperatures change in the region during recent decades?
- Is there any growth response of Scots pine related to these changes?
- How did recent permafrost thawing affect radial stem growth and physiology of these trees?

Chapter 4:

- How do NSC concentrations of *Pinus sylvestris* and *Tilia plathyphyllos* seedlings change under drought and particularly drought release?
- In case if there was an increase in NSC concentrations, was it caused by active NSC formation (i.e. 'active' storage) or passive accumulation (e.g. through transport limitation)?
- To what extent do carbon (C) allocation strategies prioritize C storage at the expense of growth in seedlings recovering from drought, giving raise to 'drought memory effects'?

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Chapter 1

1. Long-term effects of drought on tree-ring growth and carbon isotope variability in Scots pine in a dry environment

Galina Timofeeva[1,2,3], Kerstin Treydte[3], Harald Bugmann[2], Andreas Rigling[3], Marcus Schaub[3], Rolf Siegwolf[1,3] and Matthias Saurer[1,3,4]

[1]Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland;

[2]Forest Ecology, Department of Environmental Systems Science, ETH Zurich, Universitätstrasse 16, CH-8092 Zurich, Switzerland;

[3]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstrasse 111, CH-8093 Birmensdorf, Switzerland

[4]Corresponding author (matthias.saurer@wsl.ch)

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Abstract

Drought frequency is increasing in many parts of the world and may enhance tree decline and mortality. The underlying physiological mechanisms are poorly understood, however, particularly regarding chronic effects of long-term drought and the response to increasing temperature and vapour pressure deficit (VPD). We combined analyses of radial growth and stable carbon isotope ratios ($\delta^{13}C$) in tree rings in a mature Scots pine (*Pinus sylvestris* L.) forest over the 20th century to elucidate causes of tree mortality in one of the driest parts of the European Alps (Pfynwald, Switzerland). We further compared trees that have recently died with living trees in a 10-year irrigation experiment, where annual precipitation was doubled. We found a sustained growth increase and immediate depletion of $\delta^{13}C$ values for irrigated trees, indicating higher stomatal conductance and thus indeed demonstrating that water is a key limiting factor for growth. Growth of the now-dead trees started declining in the mid-1980s, when both mean temperature and VPD increased strongly. Growth of these trees was reduced to some extent already several decades earlier, while intrinsic water-use efficiency derived from $\delta^{13}C$ values was higher. This indicates a more conservative water-use strategy compared to surviving trees, possibly at the cost of low carbon uptake and long-term reduction of the needle mass. We observed reduced climatic sensitivity of raw tree-ring $\delta^{13}C$ for the now-dead in contrast to surviving trees, possibly indicating impaired stomatal regulation, although this difference between the tree groups was smaller after detrending the data. Higher autocorrelation and a lower inter-annual $\delta^{13}C$ variability of the now-dead trees, indicating impaired stomatal regulation and further indicates a strong dependence on (low) carbon reserves. We conclude that the recent increase in atmospheric moisture demand in combination with insufficient soil water supply was the main trigger for mortality of those trees that were weakened by long-term reduced carbon uptake.

Keywords: tree decline, tree mortality, water-use efficiency, vapour pressure deficit, climate change

Introduction

Drought-related climate change is increasingly affecting many ecosystems worldwide (Allen *et al.*, 2010, Allen *et al.*, 2015). Summer 2003 was recorded as the hottest summer in Europe in the last 500 years (Schaer *et al.*, 2004), and 2015 was reported as the Earth's warmest year since start of instrumental measurements (NOAA, 2016). Moreover, model-based climate projections for the 21st century show further anticipated increases in average global temperatures, whereas precipitation is predicted to vary regionally, increasing in some parts of the globe and decreasing in others (Stott *et al.*, 2006, IPCC, 2013). Consequences of these changes include an increasing frequency and duration of extreme weather events, particularly of drought. The latter affects forest ecosystems in many climatic zones and may lead to decreased tree vigour and growth (Martínez-Vilalta *et al.*, 2002), reduction of primary productivity (Ciais *et al.*, 2005) and ultimately forest decline, mortality and vegetation shifts (Allen *et al.*, 2010, Choat *et al.*, 2012, Allen *et al.*, 2015). To better predict such changes in the future, it is crucial to deepen our understanding of the mechanisms that allow trees to survive and adapt to drought (McDowell *et al.*, 2008, Sala *et al.*, 2010, McDowell *et al.*, 2011).

Some of the key mechanisms for tree survival under drought are the capacity to accumulate sufficient carbon reserves to maintain metabolism (Breshears *et al.*, 2005, Sala, 2009, McDowell *et al.*, 2011), lower vulnerability to hydraulic failure (Bréda *et al.*, 2006, Meinzer *et al.*, 2009, Meinzer *et al.*, 2010, McDowell *et al.*, 2011), or a combination of both (Mueller *et al.*, 2005, McDowell *et al.*, 2011). Hydraulic failure occurs when plant tissues are desiccated due to cavitation, which leads to the disruption of water transport in a large number of vessel elements or tracheids. Carbon starvation develops when stomata close to prevent water loss, leading to reduced photosynthetic carbon uptake, while plant metabolism is in need of freshly produced carbohydrates, whose availability may be hindered also by phloem transport limitation (McDowell *et al.*, 2008). Many other factors such as pathogens and insect attacks may accelerate decline and possibly lead to death, particularly of weakened trees. The complex interplay of the different factors and their combined effects still remain poorly quantified, and the lack of a solid mechanistic understanding makes it difficult to predict tree mortality (McDowell *et al.*, 2011).

Several studies have found that increasing atmospheric moisture demand is becoming more important for plant performance and survival as temperature increases globally (Breshears *et al.*, 2013, Eamus *et al.*, 2013, Williams *et al.*, 2013, Allen *et al.*, 2015). Increasing trends in vapour pressure deficit (VPD) amplify evaporation and plant transpiration (McVicar *et al.*, 2012). Thus, the combination of increasing temperature and VPD is likely to cause more droughts or make them more severe and may have radical effects on plants and forest ecosystems (Eamus *et al.*, 2013). However, the role of specific climate parameters for forest decline is still not well understood (Williams *et al.*, 2013) and there is a study that has explicitly addressed the relative importance of VPD for tree decline (Eamus *et al.*, 2013).

Stable carbon isotope ratios (δ^{13}C) are very useful for unravelling the mechanistic links between growth and gas exchange, as well as determining stomatal responses to VPD. They are particularly useful for gaining insights into the ecophysiological changes associated with drought and to differentiate between the mechanisms that cause decline and mortality (Leavitt & Long, 1989, Saurer *et al.*, 2004, Moreno-Gutiérrez *et al.*, 2012, Herrero *et al.*, 2013, Gessler *et al.*, 2014, Hereş *et al.*, 2014). The carbon isotope ratio of plant tissue is mainly controlled by stomatal conductance during carbon fixation (g_s) and the rate of photosynthesis (A), both of which are driven by environmental conditions (Farquhar *et al.*, 1982, Farquhar *et al.*, 1989). When applied to tree rings, δ^{13}C in tree rings can be used to study long-term aspects of tree physiology in addition to sensitivity to climatic parameters (Saurer *et al.*, 2004, Treydte *et al.*, 2007). For instance, an increase in δ^{13}C could indicate~~s~~ stomatal closure and reduced conductance to prevent water loss during drought (Leavitt & Long, 1989, Saurer *et al.*, 1995, Treydte *et al.*, 2007, Kress *et al.*, 2010) or could be due to changes in photosynthetic rates affected by irradiance during cool and wet periods (Voelker *et al.*, 2014). One of the main drivers of stomatal regulation, which is often reflected in tree-ring δ^{13}C values in both broadleaved and conifer trees, is VPD (Treydte *et al.*, 2007, Ferrio & Voltas, 2011, Voelker *et al.*, 2014). Furthermore, intrinsic water-use efficiency (WUE_i) can be derived from δ^{13}C values in plant tissue, indicating the ratio of the photosynthesis to stomatal conductance (Seibt *et al.*, 2008, Lévesque *et al.*, 2014). Climate change and increasing atmospheric CO_2 are leading to changes in WUE_i of various tree species around the globe, observed particularly in the northern hemisphere (Keenan *et al.*, 2013, Saurer *et al.*, 2014, Frank *et al.*, 2015, van der Sleen *et al.*, 2015).

Combined studies of tree-ring growth and water-use efficiency can provide a better understanding how plants in general and trees in particular adapt their water-use strategies to secure survival. For instance, recent studies have shown that trees that died later had higher growth rates (Bigler & Veblen, 2009) as well as higher WUE_i (Levanič *et al.*, 2011) several decades prior to death compared to trees that survived. These authors concluded that dying trees were hydraulically underequipped for dry conditions (Levanič *et al.*, 2011). In this context, it would be important to have century-long, annually resolved tree-ring growth and isotope records of dying and surviving trees, which are still rare or absent in the literature. Such studies would enable analysis of the causal links between drought and tree mortality and the related physiological mechanisms in the long-term.

The goal of our study is to better understand long-term ecophysiological mechanisms that cause altered growth behaviour and sensitivity to climatic conditions, comparing Scots pine trees that died recently with still living individuals over the last ca. 100 years in a dry inner alpine valley. In this region, high Scots pine mortality rates were already reported by Dobbertin et al. (2005) and Bigler et al. (2006), similar to other dry regions in southern Europe (Galiano *et al.*, 2010, Hereş *et al.*, 2014, Aguadé *et al.*, 2015, Benavides *et al.*, 2015). Scots pine is a key species, both ecologically and commercially, due to its extended geographic range, high longevity and ability to grow in marginal conditions, whereas small changes in temperature and/or water availability can strongly affect its growth and physiology. By providing well replicated, annually resolved carbon isotope records of dying and surviving Scots pine trees, climate correlation analysis can provide insights into the relationship and sensitivity of gas-exchange to past climate changes. Furthermore, we can make use of a long-term irrigation experiment in a natural Scots pine forest (Pfynwald, Valais, Switzerland). This study was set up more than a decade ago, and provides an ideal platform to investigate the physiological effects of water availability in this ecosystem (Brunner *et al.*, 2009, Dobbertin *et al.*, 2010, Eilmann *et al.*, 2010, Herzog *et al.*, 2014) as there are still very few such controlled studies on mature trees and long time-scales (Beier *et al.*, 2012). Specifically, we wanted to address the following questions:

- What are the effects of the 10-year long irrigation on tree-ring growth and tree-ring carbon isotope patterns?

- How does tree-ring growth differ between still living and recently deceased trees over the past ca. 100 years in terms of climatic sensitivity and carbon isotope variability?

- What is the role of increasing VPD for tree decline in the study region?

- Can the combined analysis of tree-ring growth and δ^{13}C-derived water-use efficiency derived from carbon isotopes help to elucidate the mechanisms underlying the observed tree decline?

- Can we identify physiological key indicators that characterize trees with low and high mortality risk?

Data and Methods

Study site

The study site is a xeric mature Scots pine forest in the canton of Valais, Switzerland (46º 18' N, 7º 36' E, 615 m a.s.l.), where an irrigation experiment has been running since June 2003. The dominant species is Scots pine (*Pinus sylvestris* L.). The forest is characterized as uneven-aged *Erico-Pinetum sylvestris* (Dobbertin *et al.*, 2010), with an approximate average stand age of 100 years and a stand density of 730 stems ha^{-1}. The area is known to be the driest part of Switzerland, with a mean annual temperature of 10.1 °C (15.8 ºC for June-August) and mean annual precipitation of 605 mm (169 mm for June-August) for the period 1981-2010. The soil is shallow and characterized by low water retention (Brunner *et al.*, 2009). The 1.2 ha experimental area is divided into eight plots of 1,000 m^2 each. Trees on four randomly selected plots were irrigated each growing season (April-October) over the period of 2003-2014. The irrigation water was taken from a water channel next to the experimental area. The amount of irrigation water was set to roughly double the annual precipitation compared to the control plots. Trees in the other four plots are growing under naturally dry conditions and were used as controls.

Sampling and tree-ring width measurements

After the end of the growing season in October/November 2014, we sampled increment cores from a total of 75 living trees from the control and irrigated plots (2-3 cores per tree). Trees were cored below breast height (at ca. 1 m) using a 5 mm increment borer (Haglöf, Sweden) to avoid any bias of subsequent circumference measurements. Stem disks from 12 trees that had recently died were cut out at breast height in November 2013. We defined dead trees as individuals that were still standing, but with no green needles left in the crowns. Recently died, standing trees were only found in the control area (not in irrigated plots) and we sampled also some dead trees outside the experimental area (with the same conditions as in control area). All deadwood stem disks were dried for 48 h at 40 ºC. Stem disks were then sanded with progressively finer sandpaper (60-400 grit). The surface of the tree cores were cut with a core-microtome to improve ring visibility (Gärtner & Nievergelt, 2010). Tree-ring width (TRW) was measured using a Lintab system with a precision of 0.01 mm, using the TSAP-Win software V.3.5 (Rinntech, Heidelberg, Germany), and dated with existing tree-ring width chronologies from the study site (Eilmann *et al.*, 2010, Eilmann *et al.*, 2011) using the software COFECHA (Grissino-Mayer, 2001). Three tree-ring width chronologies were developed and referred to in the following as “now-dead”, “now-living” or, when referring to the irrigation experiment “control” and “irrigated”. All analyses were done with raw as well as detrended TRW data. For detrending, indices from a negative exponential curve and 30-year splines, repsectively applied to the individual tree-ring width series were calculated (Cook, 1985). The negative exponential detrending is well-known as the most classical one and retains much low-frequency variance, whereas the 30-year spline removes the low-frequency variance more rigorously (Fritts, 1976, Cook *et al.*, 1990, Schweingruber, 1996).

Carbon isotope measurements

Five trees from each group (“dead”, “control” and “irrigated”) were selected for δ^{13}C analysis of cellulose based on good agreement of their individual TRW series with the site chronologies. Individual rings were separated using a surgical scalpel under a Wild M8 stereomicroscope. In the case of the now-dead trees, isotope measurements started with the tree ring of the year 1900, and the outermost rings of the selected trees varied between 2005 and 2012 AD. Thus, the δ^{13}C chronology of the now-dead trees was established for the period 1900-2005, which is the period of common overlap of all trees. In the case of the now-living trees, the isotope analysis was done for the common overlap period 1900-2014, but for the irrigated trees for the 1980-2014 period only because in prior decades the behaviour of control and irrigated trees is expected to be indistinguishable. The separated rings were cut into small pieces and packed into teflon filter bags for subsequent chemical treatment (Ankom Technology, Macedon, NY, USA).

Cellulose extraction was performed based on Boettger et al. (2007), with an ultrasonic bath modified for larger numbers of samples that allows for the extraction of up to 400 samples in one batch (Roden *et al.*, 2009). Homogenization of the cellulose material was done following Laumer et al. (2009) using an ultrasonic treatment with a HD3100 sonotrode (Hielscher, Berlin, Germany).

For the δ^{13}C analysis, the samples from the now-dead trees were packed into tin capsules, with a sample weight ranging from 0.4 to 0.6 mg. The cellulose samples of the now-living and the irrigated tree groups were packed into silver capsules, with a sample weight ranging from 0.45 to 1.3 mg. The samples of the now-dead trees were measured using the combustion method and subsequent analysis by isotope-ratio mass spectrometry (IRMS; delta S; Thermo, Bremen, Germany; instrument precision 0.1‰). The samples of the now-living and the irrigated trees were measured using a recently developed pyrolysis method for the IRMS (delta Plus XP, Thermo, Bremen, Germany; instrument precision 0.2‰). The combustion and pyrolysis methods yield identical results for δ^{13}C within the instrument precision. The values obtained by pyrolysis needed a correction due to the addition of a small amount of carbon from the reactor to the sample gas, which results in a dampened isotope signal. We therefore measured a subset of the same samples that cover the whole range of expected δ^{13}C values using the conventional combustion method in addition to pyrolysis method to identify a linear correction curve (Woodley *et al.*, 2012, Weigt *et al.*, 2015). Carbon isotope ratios are reported against Vienna Pee Dee Belemnite (VPDB). Individual tree-ring δ^{13}C values were corrected back to pre-industrial conditions to account for the Suess effect (McCarroll & Loader, 2004). However, we did not apply an additional correction for the potential physiological response to changes in atmospheric CO_2, due to still existing uncertainties with respect to the correction procedures (McCarroll *et al.*, 2009, Treydte *et al.*, 2009).

Climate data

The meteorological station closest to the study site is Sion (distance ca. 20 km). Monthly data of mean temperature and precipitation sums were obtained for the period 1900-2014 from MeteoSwiss (Begert *et al.*, 2005). We used homogenized data, where artifacts in the series were removed, meaning that the historical measured values were adapted to current measuring conditions and non-climatic influences were excluded (Begert *et al.*, 2005).

Reliable relative humidity (RH) data are often difficult to obtain due to measurement uncertainties and small-scale heterogeneity (Fatichi *et al.*, 2015), and, hence, such measurements were available for Sion for the 1960-2014 period only. Therefore, also VPD data were calculated for this period only (1960-2014) using temperature and the reliable RH data. The VPD is the difference between the saturation vapour pressure (e_s) at air temperature, minus the actual (e_a) vapour pressure:

$$\mathrm{VPD} = e_s - e_a, \tag{1}$$

where

$$e_s = 0.611 * 10\text{^} \, (7.5 * T / (237.3 + T)) \tag{2}$$

and

$$e_a = (RH / 100) * e_s. \tag{3}$$

The T is mean temperature in degrees Celsius (ºC) and RH is relative humidity in %. Additionally, we used the global 0.5° gridded Standardized Precipitation-Evapotranspiration Index (SPEI) dataset at different time scales (from 1 to 48 months) for the 1901-2013 period based on CRU TS3.22 (Harris *et al.*, 2014). The SPEI data were originally from IPE-SCIC and obtained via the KNMI Climate Explorer (Beguería *et al.*, 2010, Vicente-Serrano *et al.*, 2010, Trouet & Van Oldenborgh, 2013).

Mean temperature for the spring (March–May) and summer (June–August) seasons have increased significantly during recent decades (*1960-2003, spring: slope = 0.04 ºC/yr, R^2 = 0.27, $P < 0.001$; summer: slope = 0.05 ºC/yr, R^2 = 0.39, $P < 0.001$, n = 44*, Fig. 1). The main increase occurred after the mid-1980s. VPD also increased significantly for both seasons (*1960-2003, spring: slope = 0.02 hPa/yr, R^2 = 0.29, $P < 0.001$; summer: slope = 0.03 hPa/yr, R^2 = 0.0.17, $P < 0.01$, n = 44*). Consistent with the VPD increase, though less pronounced (only spring, not summer), relative humidity showed a negative trend (*1960-2003, slope = -0.07 %/yr, R^2 = 0.12, $P < 0.05$, n = 44*).

In contrast, the trends of precipitation amount and the SPEI index, calculated at timescales of 3 and 6 months, had no significant trends. Considering the full period, time series of mean temperature (*1903-2003, spring: slope = 0.01 ºC/yr, R^2 = 0.~~42~~18, $P < 0.001$; summer: slope =*

0.02 ºC/yr, R^2 *= 0.0.27, P < 0.001, n = 101 yrs*), 3-month SPEI (*1903-2003, summer: slope = 0.01 1/yr,* R^2 *= 0.07, P < 0.01, n = 101 yrs*) and 6-month SPEI (*1903-2003, spring: slope = 0.01 1/yr,* R^2 *= 0.05, P < 0.05; summer: slope = 0.01 1/yr,* R^2 *= 0.08, P < 0.01, n = 101 yrs*) had significant increasing trends (Fig. S1).

Data analysis

To characterize the response of tree-ring growth and $\delta^{13}C$ values to climate, Pearson's correlation coefficients were calculated based on monthly climate data (from previous January to current December) and combinations of several months (March-May and June-August) between the mean series of each climate variable and TRW and $\delta^{13}C$ chronologies, respectively. To test the significance of the correlation coefficients, two-tailed Student's t-tests were applied. The strength and significance of two independent correlation coefficients was compared based on Fisher's Z transformation. This test provides an asymptotic confidence interval, if there are at least four complete pairs of observation. Temporal autocorrelation was taken into account by calculating the 'effective' sample size based on the sample size and first-order autocorrelation for each time series of the TRW chronologies and individual climate data (Dawdy, 1964):

$$N' = N * \frac{(1 - r_1 * r_2)}{(1 + r_1 * r_2)}, \tag{4}$$

where N is sample size; N' is effective sample size; r_1 and r_2 are the first-order sample autocorrelation of the first and second time series, respectively. Two-tailed Student's t-tests were applied to test for significant differences between the means of two groups.

WUE_i for each group of trees was derived from tree-ring $\delta^{13}C$ values. WUE_i is defined as the ratio of the rate of carbon assimilation (A, photosynthesis) and stomatal conductance (g_s); it can be calculated as:

$$WUE_i = A/g_s = c_a * \frac{[b - (\delta^{13}C_{atm} - \delta^{13}C_{plant})]}{[(b - a) * 1.6]}, \tag{5}$$

where c_a is atmospheric CO_2-concentration; $\delta^{13}C_{atm}$ is the isotopic ratio of atmospheric CO_2; $\delta^{13}C_{plant}$ is the isotopic ratio of the plant; a (4.4‰) is the fractionation due to diffusion, and b (27‰) is the biochemical fractionation (Farquhar *et al.*, 1982, Farquhar *et al.*, 1989).

Average Pearson's correlation coefficients between individual series for each group (*rbar*) and the expressed population signal (*EPS*) were calculated to quantify the strength of common variation within a group. This common variation is considered to be strong when EPS is equal or above 0.85, which usually indicates strong climate forcing (Wigley *et al.*, 1984). In addition, the 95% confidence limits ($\bar{x} \pm 1.96*SE$, SE – standard error) were calculated for both groups. The F-test for equality of variance was used to identify if the true ratios of variances are equal or not to one between the control and dead groups for two periods (1900-1979 and 1980-2005). Mann-Whitney U-test was additionally applied as a nonparametric test for comparing two groups of values. To test the temporal stability of the climate-growth and isotope relationships, 30-year moving correlations between mean temperature, precipitation and TRW and δ^{13}C chronologies on a seasonal basis (spring and summer) were calculated for the 1903-2003 period.

Results

Effects of irrigation on tree-ring width and δ^{13}C

We determined averages of the main parameters investigated over the period 2003-2014, when the irrigation experiment was carried out. Irrigation resulted in an approximate doubling of available water from 543 ± 87 mm (mean ± SD) to 1139 ± 197 mm (Fig. 2a; difference significant at $P < 0.001$). Mean TRW increased up to 1.11 ± 0.28 mm for the irrigated trees, while it was only 0.69 ± 0.15 mm for the control trees (Fig. 2b; $P < 0.001$).

The mean δ^{13}C values of the irrigated trees were significantly lower (-23.67 ± 0.65 ‰) than those of the control trees (-21.87 ± 0.48 ‰), with depletion starting already in the first growing season after the beginning of irrigation in 2004 (Figs. 2c, 3b). WUE_i calculated by Eq. 5 was strongly reduced from 143.67 ± 4.80 µmol mol^{-1} to 124.38 ± 6.31 µmol mol^{-1} (Fig. 2d).

Individual tree-ring δ^{13}C series of the control trees did not show any trend after the Suess-correction, while the values of the irrigated trees showed a step-like change with persistently low values from 2004 on and a stronger common variability compared to the control trees (Fig. 3, *control trees, 1980-2014: rbar = 0.63, P < 0.001; irrigated trees, 1980-2014: rbar = 0.73, P < 0.001*). A relatively high δ^{13}C value during the irrigation period was observed during a year with a dry summer (2011). A step-like increase in TRW was observed for the same trees after irrigation, with a stronger common variability compared to control trees (Fig. S2, *control trees, 1980-2014: rbar = 0.62, P < 0.001; irrigated trees, 1980-2014: rbar = 0.70, P < 0.001*).

Long-term tree-ring width and tree-ring $\delta^{13}C$ data

Although relative variations in TRW of the now-living and the now-dead trees were quite similar during their early life phase until mid-1980s, absolute growth rates of the now-dead trees were significantly lower than those of the now-living trees already during that period (Fig. 4, Student's t-test for differences between groups, 1900-1979: *-0.158 mm ± 0.047 mm (mean difference ± SD), P < 0.01*). Afterwards, the growth rates of the now-dead trees started to deviate more strongly from the now-living trees and continuously declined until the trees eventually died (Fig. 4, Student's t-test for differences between groups, 1980-2005: *-0.334 mm ± 0.066 mm, P < 0.001*). The growth decline started approximately at the same time as the increasing trends in mean temperatures and VPD (Fig. 1a, d) were recorded. The detrended TRW indices mainly keep the high-frequency, thus reducing the offset between the now-living and the now-dead trees (Figs. 4, S3).

Individual $\delta^{13}C$ series of the now-dead trees had lower variability, expressed as average standard deviation between trees compared to the now-living individuals, particularly during the period of decline (Fig. 5, *Control trees: SD = 0.68 (1900-1979), SD = 0.73 (1980-2005); Dead trees: SD = 0.60 (1900-1979), SD = 0.55 (1980-2005)* and this difference in variability was significant (1900-1979: *F = 1.23, P < 0.05*; 1980-2005: *F = 1.74, P < 0.01*). Furthermore, the first-order autocorrelation was much higher for the now-dead compared to the now-living trees (Fig. S5). The different trees showed a rather coherent behavior with the 95% confidence limits for the dead and control groups below 0.95 ‰ and 1.04 ‰, respectively. The average correlation between all trees and the EPS was within the same range (Fig. 5). For TRW data, the now-dead trees had also slightly lower SD values compared to the now-living trees (Fig. S6, *Control trees: SD = 0.63 (1900-1979), SD = 0.47 (1980-2005); Dead trees: SD = 0.58 (1900-1979), SD = 0.43 (1980-2005)*).

The average tree-ring $\delta^{13}C$ series of both groups varied within a similar range, but in fact they were significantly higher for the now-dead trees before the onset of growth decline in the mid-1980s (Fig. 6a, Student's t-test for differences between groups, 1900-1979: *0.269 ± 0.078, P < 0.001)*. This difference disappeared afterwards (1980-2005: *-0.104 ± 0.112, n.s.*). Comparison of the two groups for each individual year indicated that the difference between groups was significant for a few years only due to the tree-to-tree variability (Mann-Whitney U-test). A strong increase in WUE$_i$ over the 20th century derived from δ^{13}C series was observed for both groups (Fig. 6b). Again, WUE$_i$ of the now-dead trees was higher from the 1930s until the mid-1980s, but then fell below the values of now-living trees later (Fig. 6c, Student's t-test for differences between groups, 1900-1979: *2.329 ± 1.252, P < 0.001;* 1980-2005: *-1.117 ± 2.106, n.s.*).

Tree-ring widths and δ^{13}C response to climate

Raw tree-ring width chronologies were significantly and negatively correlated with summer temperatures and VPD (Fig. 7c, summer: *T, dead trees: r = -0.66, P < 0.001, control trees: r = -0.37, P < 0.05; VPD, dead trees: r = -0.51, P < 0.001, control trees: -0.47, P < 0.01*). Correlation coeeficients of the dead trees with these same variables were even more pronounced and significant in spring compared to the control trees (Fig. 7a, spring: *T, dead trees: r = -0.62, P < 0.001, control trees: -0.03, P > 0.05; VPD, dead trees: r = -0.70, P < 0.001, control trees: r = -0.27, P > 0.05*). However, the now-dead trees did not show any significant correlation with precipitation and SPEI (Fig. 7a, c). Furthermore, the now-dead trees reacted more sensitively to temperature in the previous year than the now-living trees (Fig. S7). Correlation results of the TRW chronologies detrended with negative exponential curve did not substantially differ from the results derived from raw TRW chronologies (Fig. S8). However, the difference in the correlation between the control and dead trees was much weaker, when all the climate and TRW data were more rigorously detrended with 30-year splines (Fig. S9).

Carbon isotope variations of the now-living trees were highly significantly negatively (*P < 0.01*) correlated with precipitation, SPEI, and RH (Fig. 7b, d), and positively with temperature and significantly positively (*P < 0.05*) with VPD (Fig. 7b, d) for both, spring and summer. For the now-dead trees, correlations were generally weaker and less significant (Fig. 7b, d) and for temperature and VPD even in opposite direction compared to living trees. These differences in correlation between the groups were mainly due to low-frequency trends in the data, as these differences also were much smaller, when all the climate and δ^{13}C data were detrended with 30-year splines (Fig. S9). A weaker δ^{13}C-response of the now-dead trees to precipitation and drought was still present. Moving correlations in 30-year windows between growth and temperature as well as δ^{13}C and temperature showed similar values for the now-dead trees and the survivors for the first part of the record, but values for the now-dead trees started diverging after the mid-1970s, for both, spring and summer (Fig. 8). Due to the availability of VPD data only for 1960-2014, this analysis was restricted to temperature.

Relationships between tree-ring widths and δ^{13}C values

For the irrigated trees tree-ring widths and δ^{13}C values were strongly negatively correlated ($R^2 = 0.42, P < 0.001, n = 35$), for the control group they were still significantly correlated ($R^2 = 0.16, P < 0.001$, $n = 115$), but for the now-dead trees there was no relationship at all (Fig. 9). Results of correlation analysis between groups over the various time periods showed that the now-dead trees did not exhibit any relationship between tree-ring growth and tree-ring δ^{13}C values before (1900-1979, Table 1) and after the growth decline (1980-2005, Table 1). During the common period of analysis (1980-2005), the relationship between growth and tree-ring δ^{13}C values was stronger for the now-living trees compared to the other two groups (Table 1). However, in this period, there are only three years of irrigation (2003-2005). During the entire period of irrigation (2003-2014), the strongest relationship was found for the irrigated trees (Table 1).

Discussion

Effects of irrigation

Water supply is typically assumed to be a key limiting factor for Scots pine in dry inner alpine valleys (Dobbertin *et al.*, 2005, Bigler *et al.*, 2006, Rigling *et al.*, 2013). This hypothesis can be tested directly using the results from the long-term irrigation experiment (Dobbertin *et al.*, 2010) and was indeed confirmed at our study site. Irrigation almost doubled tree-ring width compared to the control trees. A strong response was also observed by the depletion of tree-ring δ^{13}C values of the irrigated trees, which occurred immediately after the start of irrigation. This recovery was not only a short-term response, but was sustained throughout the period of irrigation, reflected also in the absence of standing recently deceased trees in the irrigation area. The fast and distinct growth and isotope response to the irrigation is consistent with results of Eilmann et al. (2010). They observed a decrease in δ^{13}C of the irrigated trees independent of crown transparency in the year after the irrigation start, for both early- and latewood as well as for intra-annual δ^{13}C patterns. They interpreted this decrease in δ^{13}C as increased stomatal conductance due to improved water availability. The fast isotope response in their and our cases indicates that freshly synthesized assimilates with relatively low isotope values are immediately and directly transported to the sites of xylem cell production showing a strong carbon demand in the stem. This indicates either a low availability of long-term stored carbohydrates or that the trees are not able to access stored carbon under drought (McDowell & Sevanto, 2010).

Relationship between tree-ring width and $\delta^{13}C$

Increasing growth along with decreasing carbon isotope values were often reported for sites under water-limiting conditions (McDowell *et al.*, 2010, Hereş *et al.*, 2014). As observed also in our irrigation experiment for Scots pine, an inverse relationship indicates that trees take advantage of the favorable conditions, reduce the water-use efficiency and greatly enhance stomatal conductance. Similar relationships were observed for various tree species in the Mediterranean region and in North America, particularly during drought periods (McDowell *et al.*, 2010, Voltas *et al.*, 2013, Hereş *et al.*, 2014, Voelker *et al.*, 2014). In our study, however, this relationship was weaker for the control and absent for the now-dead trees. This may indicate a decoupling between gas-exchange and growth for severely damaged trees. Comparing co-habiting now-dead with now-living Scots pines in northeastern Spain, Hereş et al. (2014) also found that the WUE_i response of the now-dead trees was weaker than of the now-living ones and it was not associated with a growth increase. In contrast, the growth-WUE_i relationship was significantly stronger for now-dead ponderosa pines (*Pinus ponderosa*) compared to surviving trees along an elevational transect in northern New Mexico (McDowell *et al.*, 2010). Such apparent contradictions could be related to species-specific differences. Our findings could also be related to recent observations, where under severe drought conditions the isotope signal generated in the leaves is no longer represented in the tree-rings, because growth is halted under driest summer conditions (Sarris *et al.*, 2013, Pflug *et al.*, 2015). Such a weakened response of the tree-ring isotopic variation to environmental conditions has been also observed in slow-growing trees in unthinned stands of ponderosa pines (*Pinus ponderosa*) compared to fast-growing thinned stands in Arizona, USA (Sohn *et al.*, 2014). The authors of this study assumed that such response of control trees and their extremely low growth throughout the entire experimental period are most likely due to chronic water stress. The latter might have suppressed within-tree carbon sink activity rather than source activity (Hartmann, 2015).

The lack of a relationship between growth and WUE_i in any case seems to be an indication of severe drought stress.

Long-term weakening of growth and physiology

Tree-ring studies are particularly useful for providing a retrospective view on growth performance and physiological characteristics long before an actual tree decline may have started. This can help us understanding why some trees succumb to drought, while others do not (McDowell et al 2008). We considered different phases of tree life, particularly an early phase, when growth of the now-dead and the now-living trees differed only to a small extent (1900-1979), and a later or a “final” phase, when differences augmented. Our results show that the now-dead trees had slightly lower growth and higher WUE_i already early in their life, i.e. several decades before the distinct decline, as found also for pinyon (*Pinus edulis*) and Scots pine (Macalady & Bugmann, 2014, Camarero *et al.*, 2015). In Macalady and Bugmann (2014), the dying pinyon pines also appeared to have generally lower average growth rates than the surviving trees, but growth prior to drought-induced mortality was rather variable depending on site conditions (Macalady & Bugmann, 2014). Our findings indicate that the now-dead trees were exerting strong stomatal control and were more conservative in their water-use strategy over many decades, showing stronger isohydric behavior compared to the now-living trees. Stomatal closure then probably led to lower photosynthesis and reduced production of fresh carbohydrates. The effect of lower conductance and photosynthesis in the long-term can as a consequence result in multiple other physiological changes. In fact, not only stomatal conductance, but total needle mass and needle specific area were reduced due to water limitation at this site (Dobbertin *et al.*, 2010). Further, lower growth could result in smaller tree height a competitive disadvantage compared to neighbouring trees. Overall, the distinct decline that started in the mid-1980s was most likely a result of the previous long-term weakening process due to reduced carbon uptake.

Carbon starvation or hydraulic failure?

Our results point to an important role of storage depletion in dying trees caused by a long-term water-conservation strategy, although additional factors in the final decline phase when trees are already weak may be important (McDowell *et al.*, 2011). However, actual reduction of carbon reserves (non-structural carbohydrates; NSC) in all parts of a tree (branch, stem, roots) is rarely observed (Gruber *et al.*, 2012) and the role of carbon starvation therefore has been debated for over a decade now (Körner, 2003, Hartmann, 2015, Körner, 2015). Several studies of Scots pine and also for other genera indicated a different water-use strategy compared to our site, as dying/declining trees grew significantly better and had higher WUE_i in the earlier life phase than living/surviving trees (Levanič *et al.*, 2011, Morán-López *et al.*, 2013, Hentschel *et al.*, 2014). These studies hypothesized that the divergent patterns of survival and mortality within a stand were mainly due to hydraulic acclimation prior to drought. The faster growth in the earlier phase predisposed trees to become more vulnerable to drought. These studies indicated that trees that grow fast and build large xylem vessels are not able to adapt their water-use strategy during drought conditions, thus rendering them more vulnerable to severe drought and predisposing them to cavitation and hydraulic failure, rather than carbon starvation. Such patterns were observed for Scots pines in Switzerland as well in the eastern Pyrenees (Morán-López *et al.*, 2013). On the other hand, carbon limitation may be more frequent than assumed from stem NSC measurements, because stored C may not be available for growth anymore (Sala 2012). Results from the literature, including our study, therefore demonstrate a varying, but non-negligible role of carbon depletion for tree mortality.

Final phase and role of VPD

Remarkably, the final tree-ring decline after the mid-1980s occurred when mean temperatures and VPD started to increase, although there were no significant trends in precipitation and drought index at that time. Furthermore, the weakened trees that died later did not maintain their conservative water-use strategy by increasing WUE_i further but reduced it, in contradiction with expected behavior during drought conditions. This unusual behavior of growth and WUE_i also resulted in the absence of a correlation between both, reflecting the de-coupling between gas-exchange and growth mentioned above. Our findings indicate that VPD may be a critical factor, triggering decline and mortality at our site. Higher temperatures and increased VPD have been suggested as possible drivers of the recent large-scale die-off event in the southwestern USA (Breshears *et al.*, 2005, Williams *et al.*, 2010, Williams *et al.*, 2013). Higher rates of potential water loss from soils substantially reduce water supply for plants due to rising temperatures and associated increases in VPD (Breshears *et al.*, 2013, Eamus *et al.*, 2013). Increased VPD leads to stomatal closure and reduced carbon metabolism as well as transpiration, the net result of which is likely a further intensification of plant water stress (Breshears *et al.*, 2013, Eamus *et al.*, 2013). Studies analyzing variations in $\delta^{13}C$ and TRW may be particularly helpful in this respect, as shown also for bur oaks in North America (Voelker *et al.*, 2014). Studies explicitly using VPD are still rare (Eamus *et al.*, 2013). Reliable local relative humidity data may further be needed rather than large-scale extrapolated data due to the high spatial variability of this variable (Fatichi *et al.*, 2015). Our results indicate that the recent increase in VPD for trees growing under chronically low water supply may be detrimental for the most weakened individuals.

Climate sensitivity

For a detailed analysis of the influence of different climate variables on tree physiology and for understanding the sensitivity to drought, a correlation analysis is very useful (McDowell *et al.*, 2010, Levanič *et al.*, 2011, Voltas *et al.*, 2013). We found that the growth of both tree groups was strongly associated with spring and summer temperatures as well as VPD. Correlations were significant for the now-dead trees only, similar as reported earlier (McDowell *et al.*, 2010, Voltas *et al.*, 2013, Macalady & Bugmann, 2014), but in contrast the influence of precipitation and drought (SPEI) was less important for the now-dead trees compared to the control trees. The results of this analysis, however, depended quite strongly on the statistical treatment of the data, i.e. detrending. When removing only the age-related TRW trends by negative exponential fitting, the correlation results were still similar, but differences between groups largely disappeared after more rigorously detrending TRW as well as climate data. This shows that now-dead trees still responded similarly to year-to-year climate fluctuations as control trees. Nevertheless, we consider the complete removal of low-frequency signals not adequate for investigating the causes of tree decline, where actually the low-frequency trends are most important to understand. Regarding carbon isotopes, we observed a diverging response between mean temperatures, VPD and tree-ring $\delta^{13}C$ values between the two groups of trees: tree-ring $\delta^{13}C$ of the now-dead trees was negatively correlated with spring and summer temperatures, as well as with VPD, but it was positively correlated with both temperature variables and VPD for the control trees. As mentioned above, this divergence was mainly observed for analysis with raw data. A positive relationship between tree-ring $\delta^{13}C$ values and increasing temperatures as well as VPD, as observed for the control trees, is an expected response during drought conditions (Treydte *et al.*, 2007, Ferrio & Voltas, 2011, Saurer *et al.*, 2014, Voelker *et al.*, 2014). The divergent isotope response to temperature and VPD of the now-dead trees may be explained once more by impaired stomatal regulation of the weakened trees (McDowell *et al.*, 2010). Growth of the now-dead trees was more strongly coupled to relative humidity, VPD, maximum temperatures and PDSI as observed also in other studies (McDowell *et al.*, 2010, Macalady & Bugmann, 2014). These findings may indicate that mortality is associated with greater climate sensitivity of stem growth, rather than leaf gas exchange (Seibt *et al.*, 2008), i.e. a de-coupling of leaf- and stem-level processes under drought (Sala *et al.*, 2012, Fatichi *et al.*, 2014, Palacio *et al.*, 2014, Gessler & Treydte, 2016).

Indicators of tree decline

Some characteristics of tree-ring data may be indicative for trees that later die and therefore signify “early-warnings of tree-dieback” (Camarero *et al.*, 2015). Possibly related to the lack of stomatal response to climate variations, we found that the first-order temporal autocorrelations were positive and high for the now-dead trees in both TRW and $\delta^{13}C$, but very low for the now-living trees. This is consistent with the fact that the climate-growth relationships of the months of previous years were significant for dying trees only (Fig. S5). Furthermore, the variance of tree-ring $\delta^{13}C$ values was lower for the now-dead trees during the period of distinct decline (1980-2005). This may be explained as follows: during decline, the trees shed part of the needles and with a reduced needle mass the potential difference between years becomes down-scaled and the variance smaller. The low year-to-year variability also indicates the influence of old, well mixed reserves. These findings therefore suggest that the growth and physiology of the now-dead trees were dependent strongly on the environmental conditions of the previous growing season, and these trees relied mainly on carbon reserves, although they were already reduced (Eilmann *et al.*, 2010). Focusing on growth alone, Camarero et al. (2015) observed a loss of synchronicity and decreases in the autocorrelation and variance for declining Scots pine in northeastern Spain, but opposite patterns in other species at the same site. Consideration of autocorrelation and variability prior to death may improve our knowledge of tree vitality at least for some species (Fritts, 1976, Camarero *et al.*, 2015). Our results indicate that several factors in combination may help to identify trees that are potentially threatened and to elucidate the role of different physiological processes under long-term, chronic drought. Specifically, we suggest as indicators of impending mortality the lack of correlation between growth and isotope data, a high autocorrelation and low variance as well as a reduced gas-exchange response to high T/VPD of the now-dead trees.

Conclusions

A multitude of inter-related factors influence tree decline, which are difficult to disentangle. The irrigation experiment in a mature Scots pine forest therefore was very useful to quantify the isolated effect of water on tree physiology. The growth response as well as the strong relationship to water-use efficiency has demonstrated the limiting effect of water for non-irrigated trees. The long-term perspective provided by the tree-ring analysis indicates that trees were subject to a weakening process over several decades, which slowly reduced their vigor and led to the mortality of the most isohydric individuals. Lower stomatal conductance and reduced photosynthetic activity most likely resulted in insufficient carbon reserves of now dead trees compared to the still living trees, visible also in reduced needle length and specific needle area. Freshly produced assimilates may not have been sufficient to support metabolic processes. Although there is a general lack of water at the site, we could show that recent increases in temperature and elevated atmospheric moisture demand (increased VPD) are most likely the triggers that are responsible for the final decline. This is concerning as climate models predict further increases in both temperature and VPD in many parts of the world. In our study area it was already proposed that due to the mechanisms outlined above, pine forests might be eventually substituted by oak forests, particularly at low elevations (Rigling *et al.*, 2013). Therefore, possible improvement of the water balance by thinning has been suggested (Giuggiola *et al.*, 2016). Our analysis also showed that high-resolution growth and carbon isotope data are very useful for extracting indicators that characterize weakened trees, such as the lack of correlation between growth and isotope data and a high autocorrelation. Such growth-carbon isotope-related indicators may help to identify trees predisposed for decline and could be useful for detecting threatened trees also for other species and sites.

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Figures and Tables

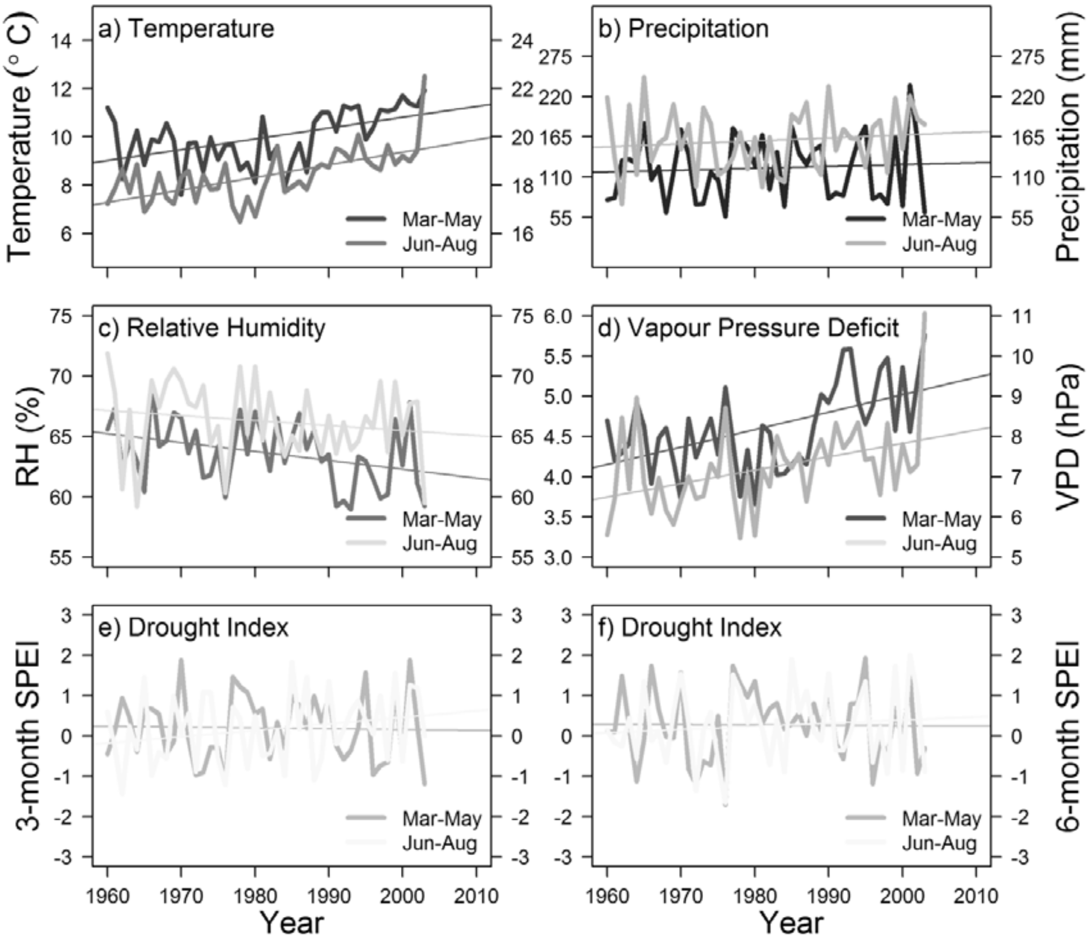

Figure 1. Spring and summer meteorological variables at the Sion meteorological station for 1960-2003: (a) mean surface temperature; (b) precipitation sum; (c) relative humidity; (d) vapor pressure deficit; (e) 3-month SPEI drought index; (f) 6-month SPEI drought index. Dark colors are for spring (Mar-May; y-axis to the left) and light colors for summer data (Jun-Aug; y-axis to the right). Linear regression lines are also indicated.

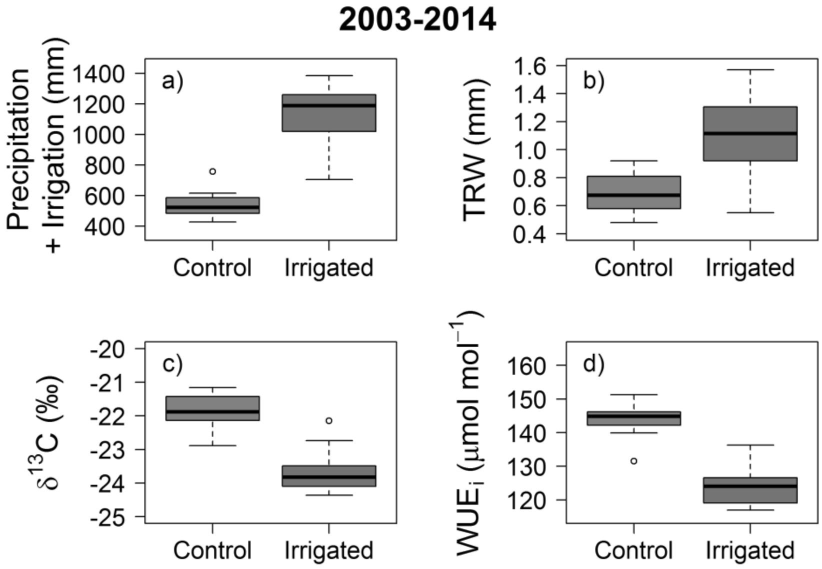

Figure 2. Effect of irrigation on mean tree-ring width (TRW) and δ^{13}C values for the period 2003-2014. All differences between control and irrigated tree groups are significant at *P* < 0.0001.

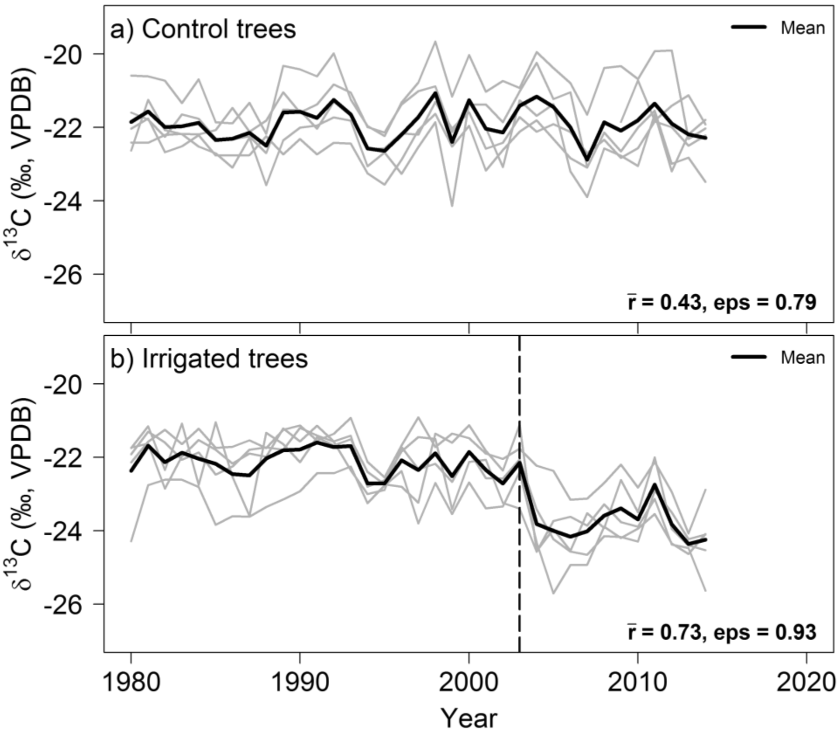

Figure 3. Individual series (grey lines) and mean curves of tree-ring δ^{13}C: (a) control trees; (b) irrigated trees. The dashed vertical line indicates the start of irrigation in 2003.

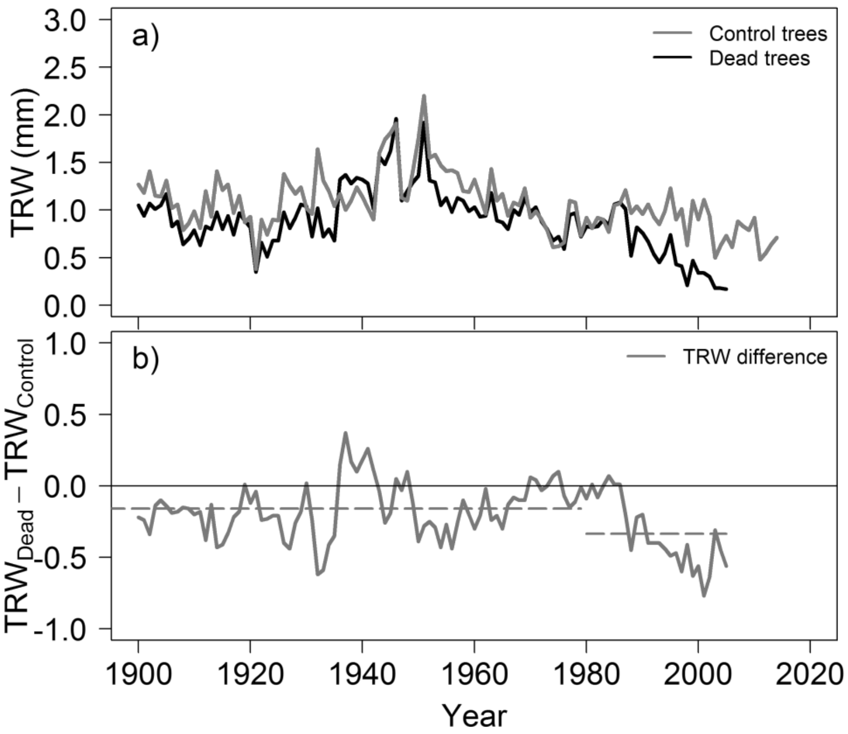

Figure 4. Tree-ring width chronologies of now-living and now-dead trees (a) and their differences (b) for the period 1900-2014. The dashed lines indicate the mean of the differences between now-dead and now-living trees for 1900-1979 and 1980-2005, respectively. Note: the chronology of the now-dead trees covers the period 1900-2005.

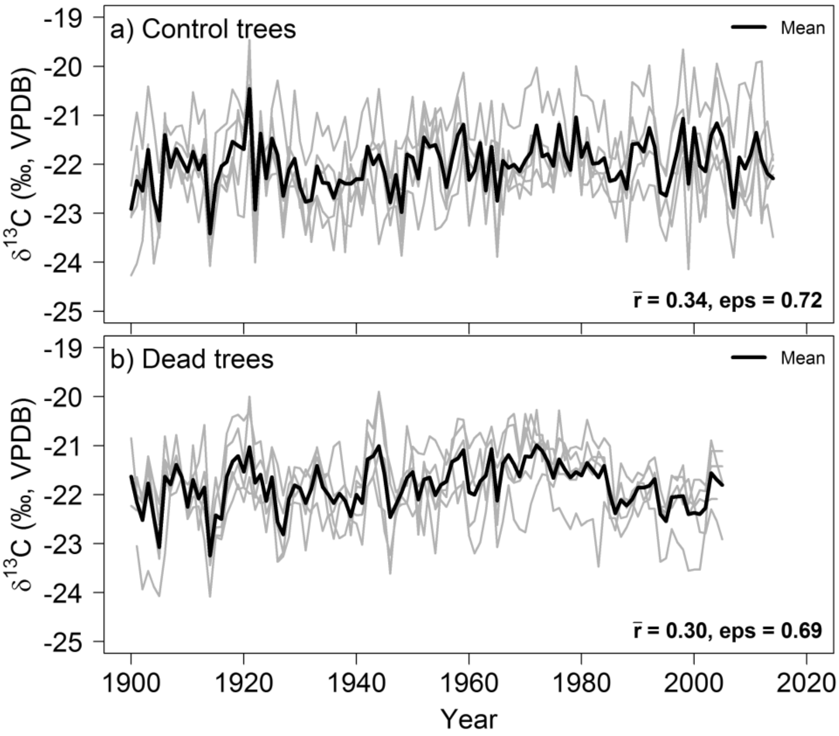

Figure 5. Individual series (grey lines) and mean curves of tree-ring δ^{13}C: (a) control trees; (b) now-dead trees. Note: chronology of now-dead trees covers the period 1900-2005.

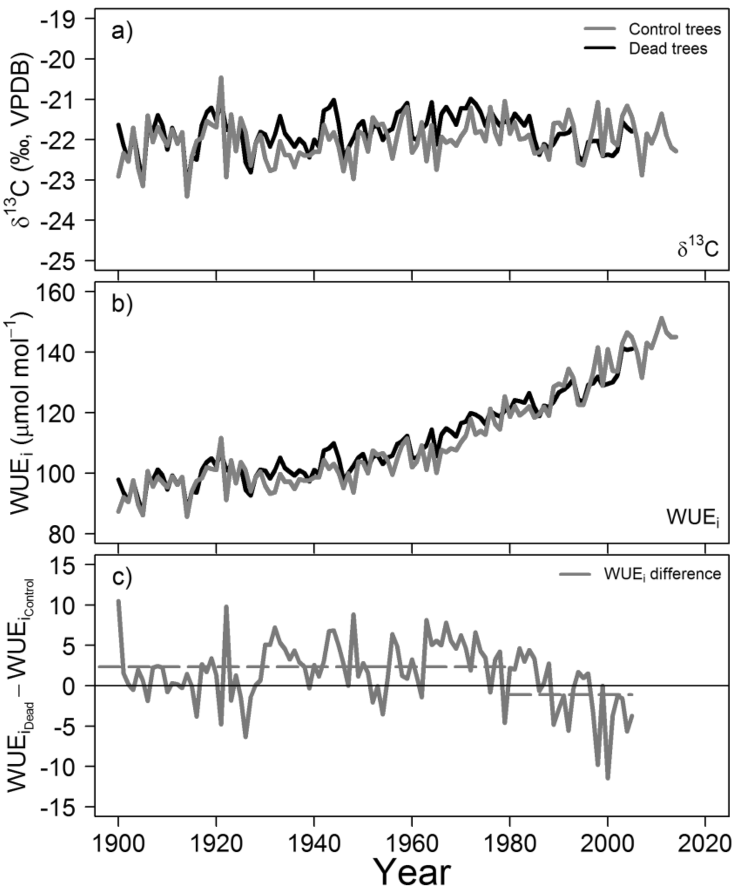

Figure 6. Tree-ring $\delta^{13}C$ chronologies of now-living and now-dead trees (a), WUE_i chronologies (b) and WUE_i differences (c). The dashed lines indicate the mean of the differences between now-dead and now-living for 1900-1979 and 1980-2005, respectively. Note: the chronology of the now-dead trees covers the period 1900-2005.

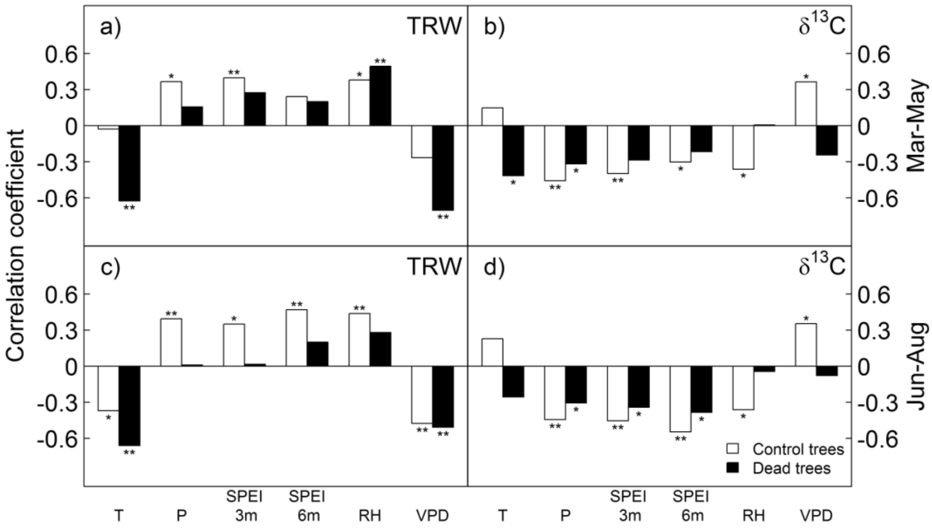

Figure 7. Pearson's correlation coefficients between tree-ring width and $\delta^{13}C$ chronologies and climate variables for the period of 1960-2003 for the spring (March-May; upper panel) and summer (June to August; lower panel). Black bars refer to the now-dead trees and white bars to the control trees. Significant correlations are marked by * (P < 0.05) and ** (P < 0.01).

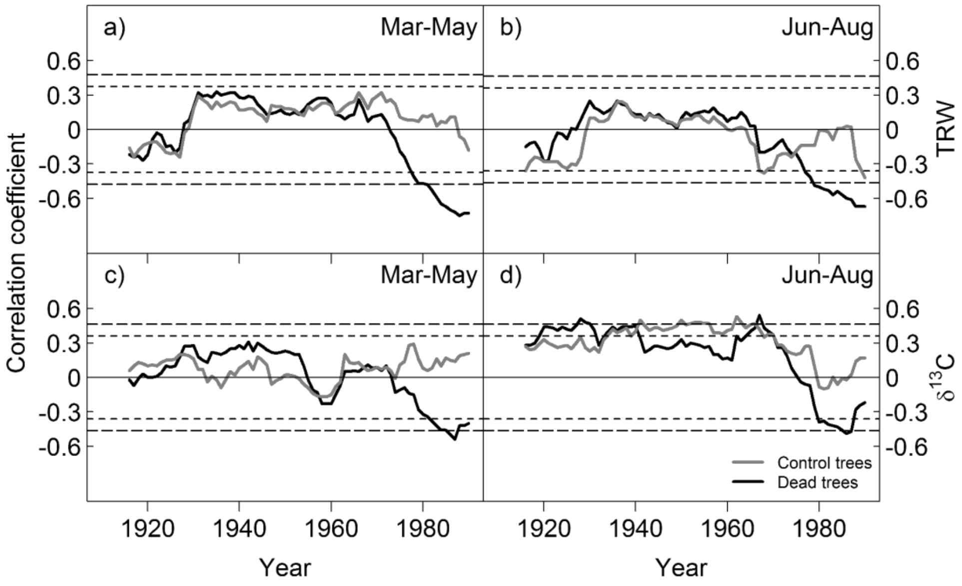

Figure 8. 30-year moving correlation coefficients between tree-ring width and temperature (a, b) and $\delta^{13}C$ chronologies and temperature (c, d) for the period of 1902-2005. The short dashed line indicates the significance level at 95 % and the long dashed line at 99 %.

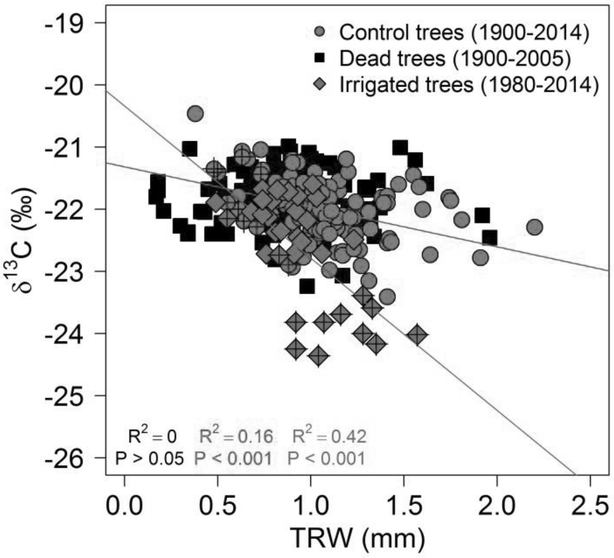

Figure 9. Relationships between tree-ring growth and $\delta^{13}C$ for now-dead, control and irrigated trees. Symbols marked by crosses indicate the values for the irrigation period (2003-2014).

Table 1. Results of correlations between raw tree-ring and $\delta^{13}C$ data for all groups over the various time periods.

Time period	Now-dead trees	Now-living trees (control)	Irrigated trees
1900-1979	r = -0.18, $P > 0.05$	r = -0.36, $P < 0.001$	NA
1980-2005	r = 0.10, $P > 0.05$	r = -0.68, $P < 0.001$	r = -0.53, $P < 0.01$
2003-2014	NA	r = -0.52, $P > 0.05$	r = -0.60, $P < 0.05$

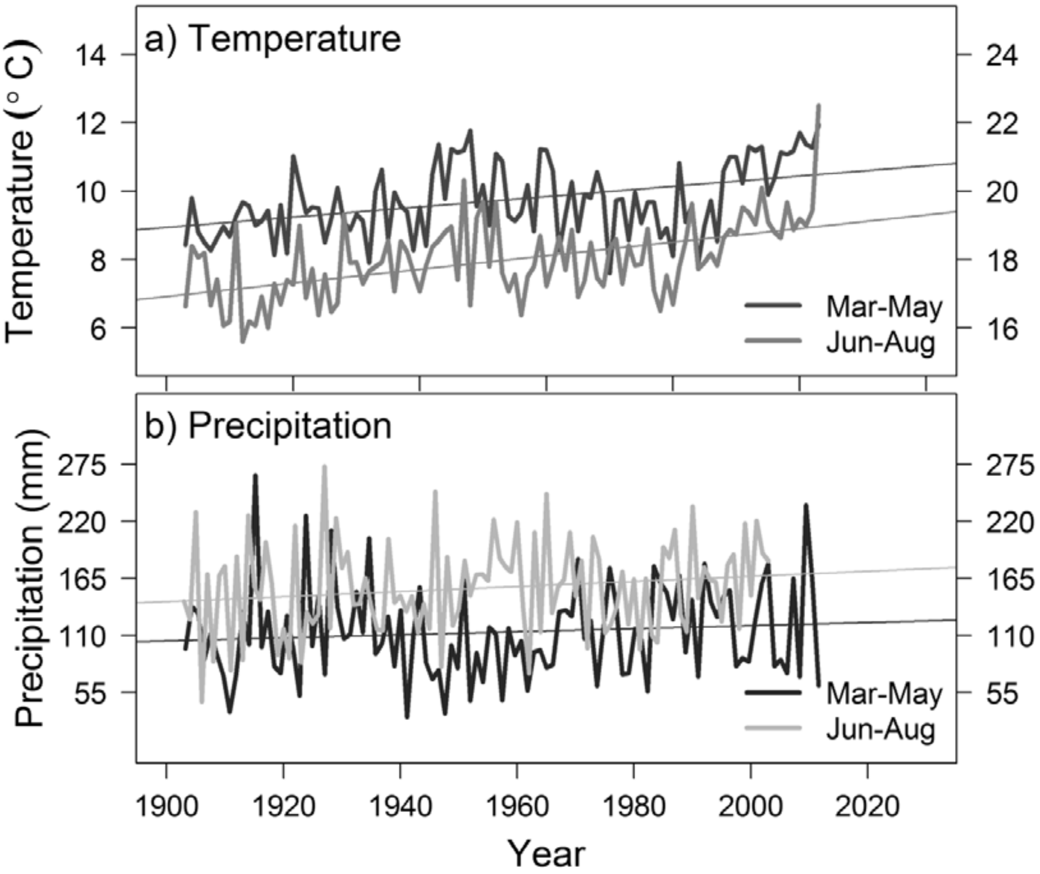

Figure S1. Mean surface temperatures (a) and precipitation amount (b) of spring (Mar-May = March, April and May) and summer (Jun-Aug = June, July and August) from the meteorological station of Sion for 1903-2003.

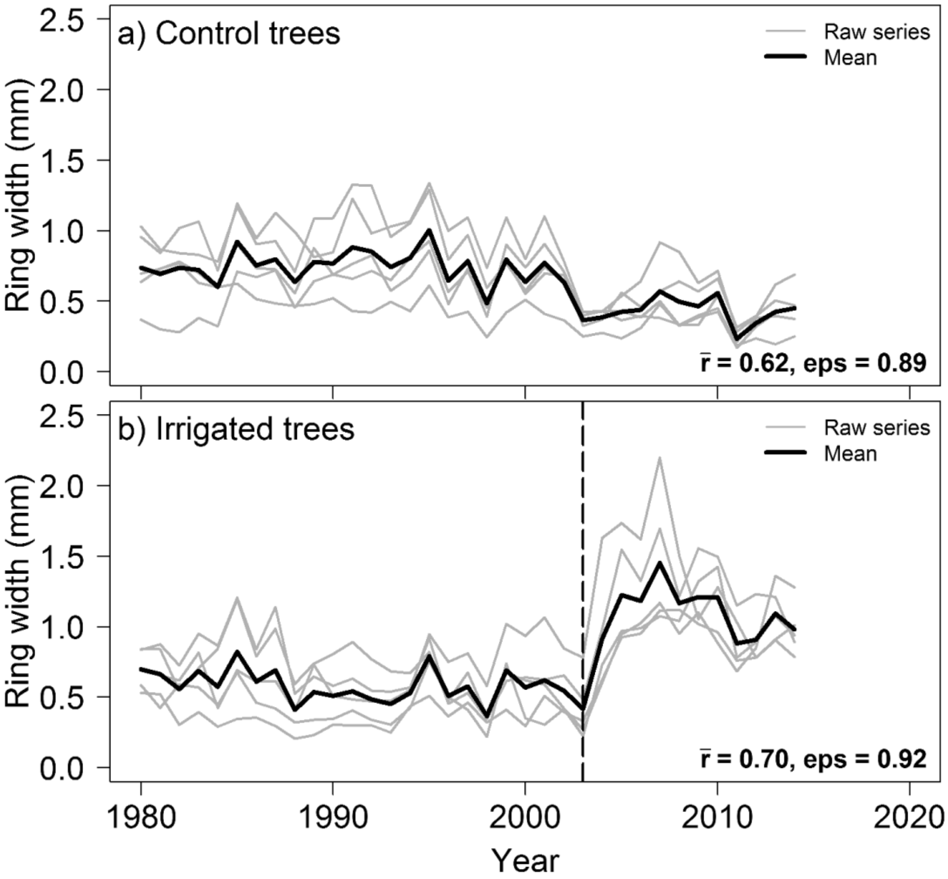

Figure S2. Individual raw tree-ring width series of trees used for isotope analysis (grey lines) and their means (black lines): (a) control trees; (b) irrigated trees. The dashed vertical line indicates the start of irrigation in 2003.

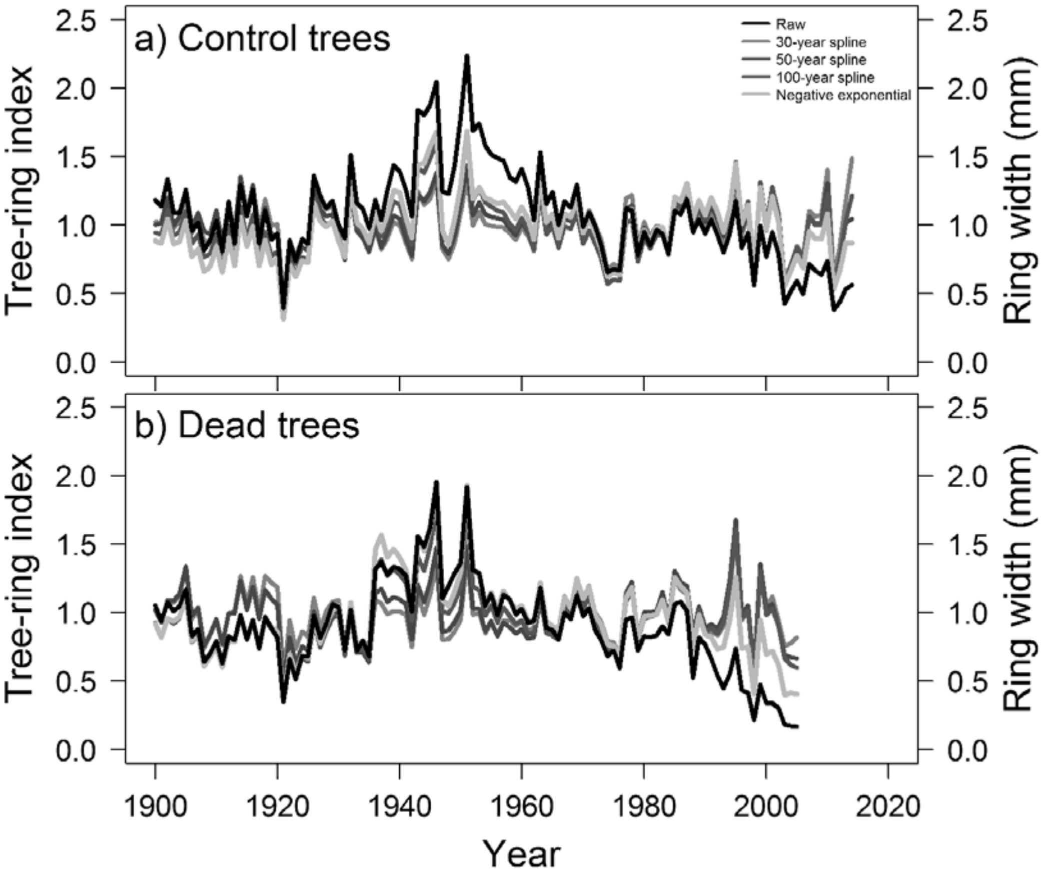

Figure S3. Raw and detrended tree-ring width chronologies of control (a) and dead (b) trees over the period 1900-2014. Note: chronology of dead trees covers the period 1900-2005.

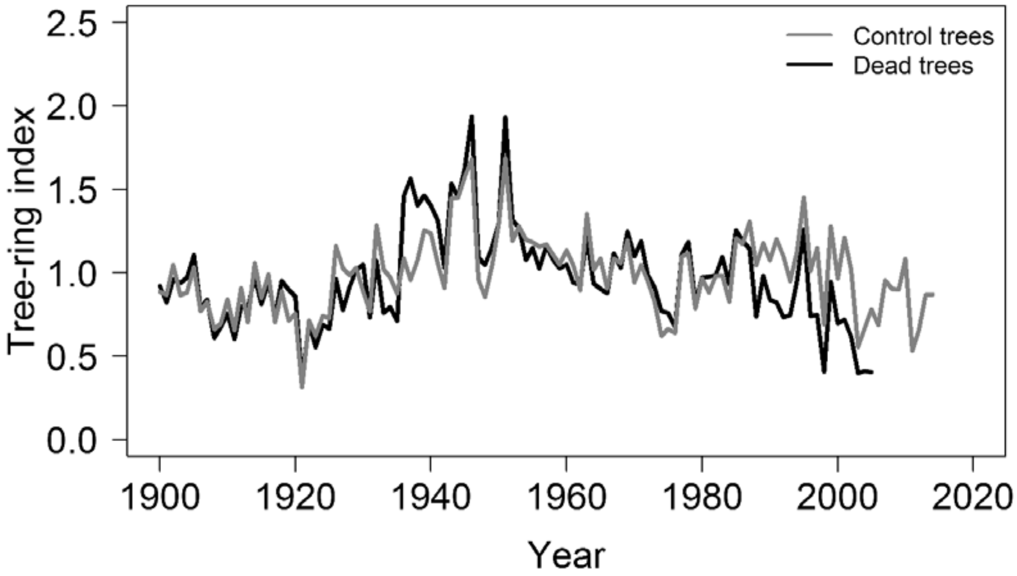

Figure S4. Detrended tree-ring width chronologies of control and dead trees over the period 1900-2014, detrended with a negative exponential curve. Note: chronology of dead trees covers the period 1900-2005.

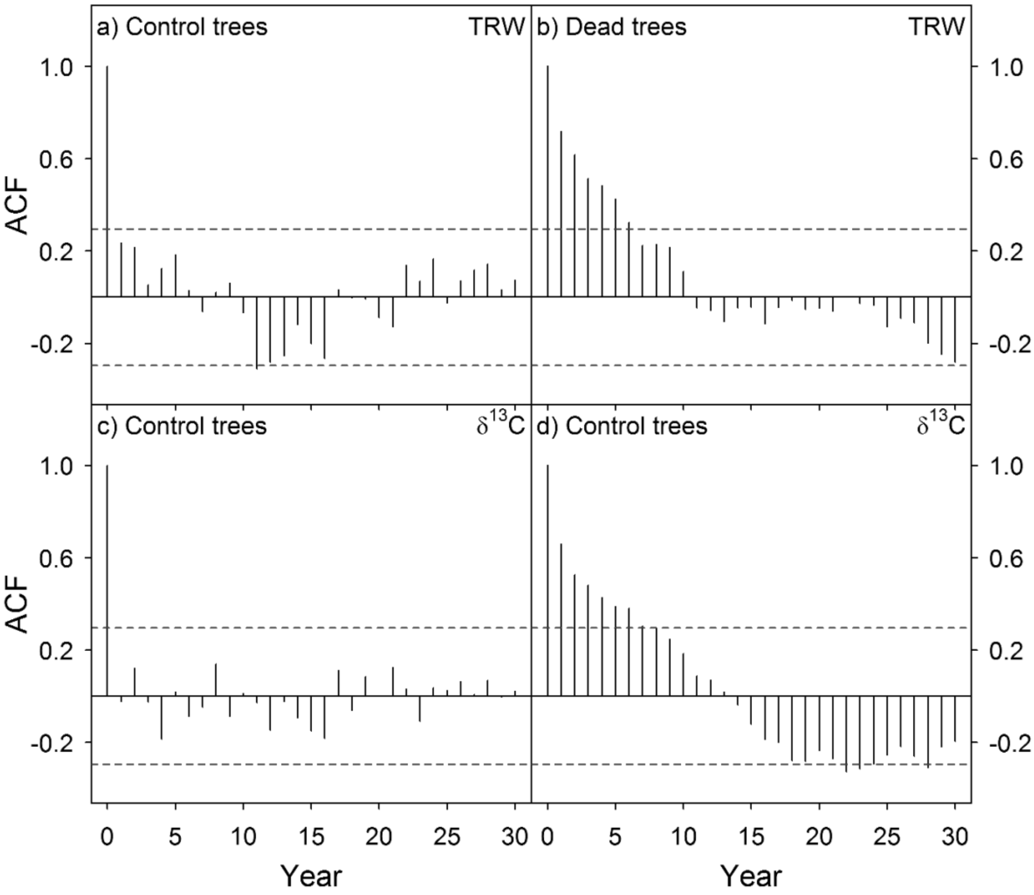

Figure S5. First to 30th-order autocorrelations for raw tree-ring width (a, b) and δ^{13}C (c, d) chronologies.

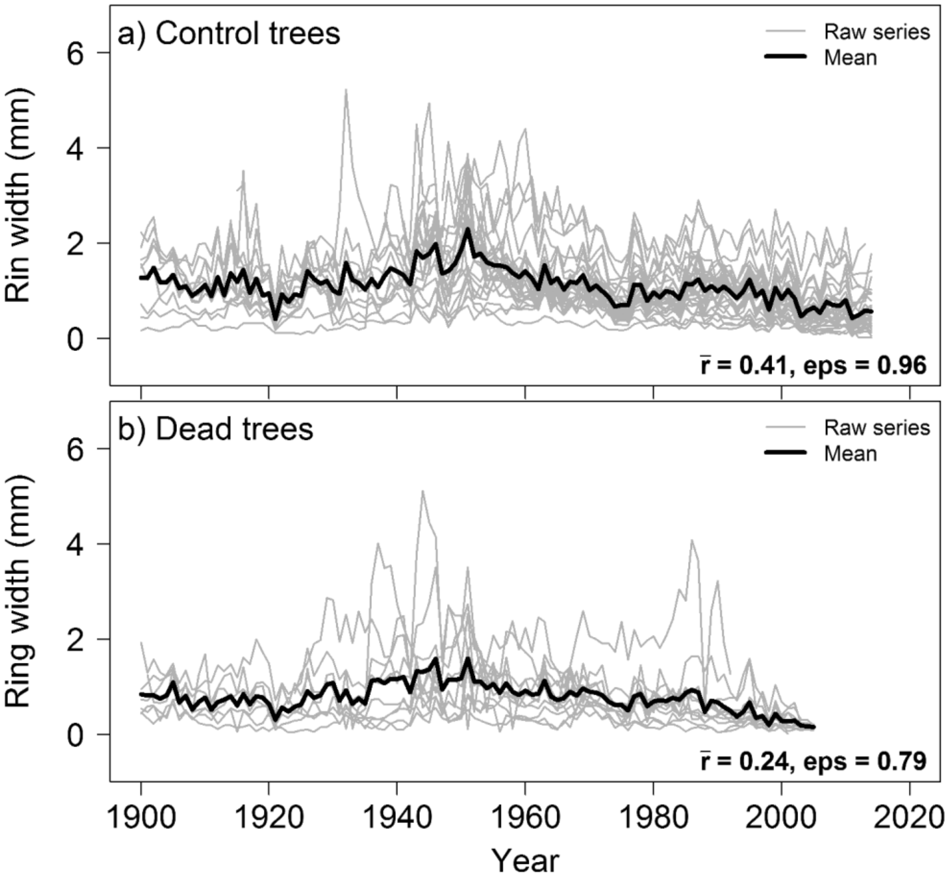

Figure S6. Individual raw tree-ring width series (grey lines) and their means (black lines) of control (a) and dead (b) trees. Note: chronology of dead trees covers the period 1900-2005.

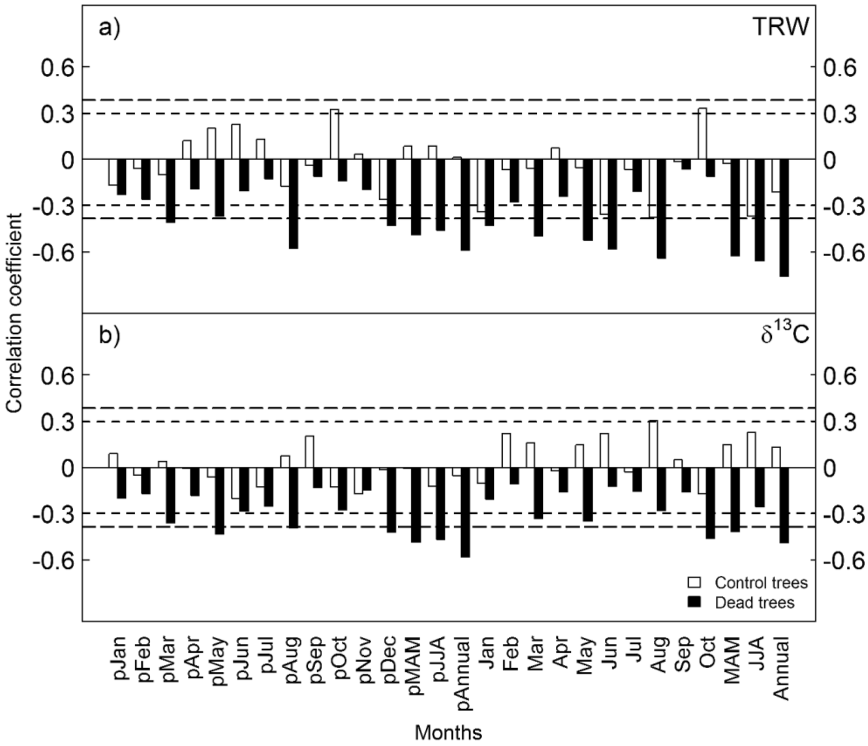

Figure S7. Pearson's correlation coefficients between raw tree-ring width (a) and $\delta^{13}C$ (b) chronologies, respectively, and monthly (previous year January to current year October) and seasonal (MAM = March, April and May; JJA = June, July and August) mean temperatures over the period 1960-2003. White bars represent the control trees and black bars the dead trees. The short dashed line indicates the significance level at 95 % and the long dashed line at 99 %.

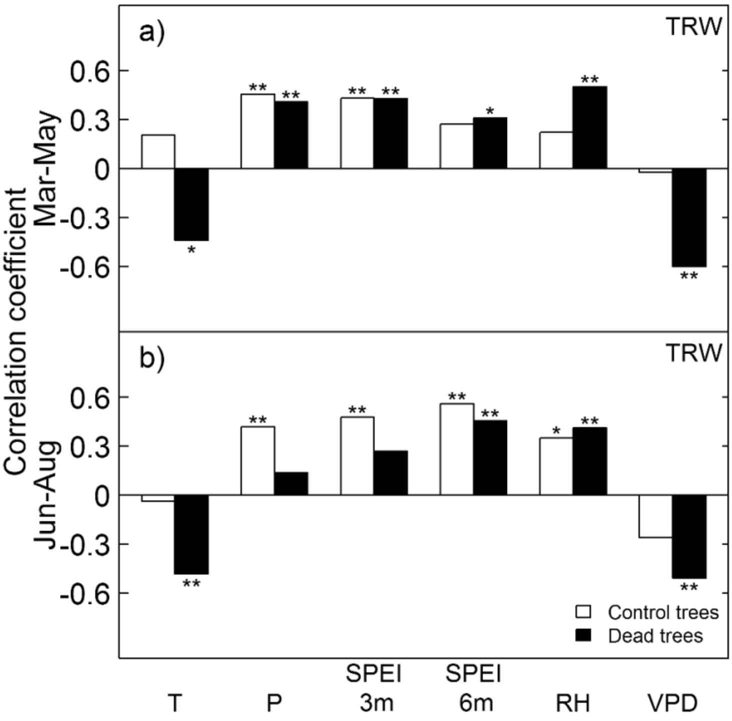

Figure S8. Pearson's correlation coefficients between tree-ring width chronologies after detrending with a negative exponential curve and raw climate variables for the period of 1960-2003 for spring (March to May; upper panel) and summer (June to August, lower panel). White bars represent the control trees and black bars the dead trees. Significant correlations are marked by * ($P < 0.05$) and ** ($P < 0.01$).

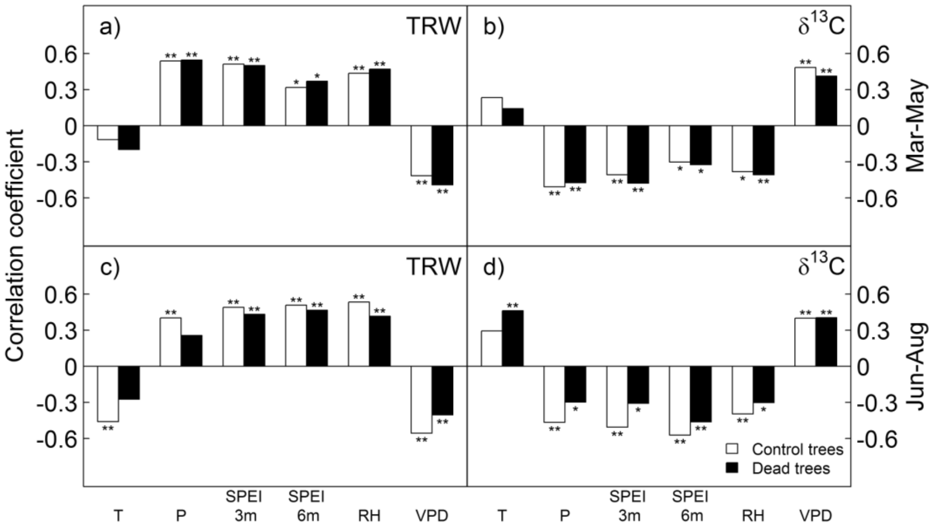

Figure S9. Pearson's correlation coefficients between tree-ring width (a, c) and $\delta^{13}C$ (b, d) chronologies, respectively, and climate variables after detrending all datasets with 30-yr splines for the period of 1960-2003 for spring (March to May, upper panel) and summer (June to August, lower panel). White bars represent the control trees and black bars the dead trees. Significant correlations are marked by * ($P < 0.05$) and ** ($P < 0.01$).

Chapter 2

2. Can the two-pool model explain oxygen isotope variations in needles and tree rings of Scots pine exposed to varying water supply?

Galina Timofeeva[1,2,3], Kerstin Treydte[2], Harald Bugmann[3], Yann Salmon[4,5], Andreas Rigling[2], Marcus Schaub[2], Rolf Siegwolf[1,2] and Matthias Saurer[1,2,6]

[1]Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland;

[2]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstrasse 111, CH-8093 Birmensdorf, Switzerland;

[3]Forest Ecology, Department of Environmental Systems Science, ETH Zurich, Universitätstrasse 16, CH-8092 Zurich, Switzerland;

[4]Department of Physics, University of Helsinki, Helsinki 00014, Finland;

[5]School of GeoSciences, University of Edinburgh, EH9 3FF Edinburgh, UK

[6]Corresponding author (matthias.saurer@wsl.ch)

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Abstract

In many regions, drought is a suspected to be a cause for Scots pine decline and mortality, but the underlying physiological mechanisms remain unclear. Because of their relationship to ecohydrological processes, δ^{18}O ratios in tree rings are potentially useful for deciphering long-term physiological responses and tree adaptation to increasing drought. We investigated 20th century δ^{18}O variations in tree rings of Scots pine in a dry inner-Alpine valley in Switzerland, comparing stressed vs. healthy trees (irrigated for >10 yrs), including a group of trees that had recently died. We further investigated seasonal δ^{18}O variations in soil and needle water. We applied a recently proposed two-pool correction approach that considers the ratio of unenriched water in the needles to total needle water. We found this ratio to be higher in drought-stressed needles, which also were smaller, resulting in lower overall δ^{18}O enrichment compared to needles from irrigated trees, which could be accurately modelled with the two-pool approach. This is consistent with observed lower δ^{18}O values in tree rings of trees that recently died compared to surviving individuals. This may indicate that the needle anatomical changes in the now-dead trees already occurred several decades ago. Such changes can thus be considered as an early indication of tree decline.

Keywords: drought, tree rings, oxygen isotopes, source water, needle water enrichment

Introduction

Understanding the plants' response to drought, particularly trees, is a pressing issue in view of climate change, having triggered many studies (Chaves *et al.*, 2003, Xu *et al.*, 2010, Ryan & Way, 2011). Drought-induced forest decline and dieback have already been reported for various regions (Allen *et al.*, 2010, Choat *et al.*, 2012, Anderegg *et al.*, 2013, Hicke & Zeppel, 2013, Allen *et al.*, 2015). However, most drought experiments were performed with seedlings and saplings (Chhin & Wang, 2008, Galle *et al.*, 2010, Pearson *et al.*, 2013, Duan *et al.*, 2015, Taeger *et al.*, 2015, Cocozza *et al.*, 2016), and only rarely with mature trees (Galiano *et al.*, 2010, Poyatos *et al.*, 2013, Aguadé *et al.*, 2015, Gaylord *et al.*, 2015, Salmon *et al.*, 2015). Therefore, drought manipulation studies in natural environments are crucial for increasing our understanding of physiological responses of mature living trees to drought as well as causes of tree decline (Herzog *et al.*, 2014, Rowland *et al.*, 2015).

The values of stable oxygen isotopes ($\delta^{18}O$) in plant organic matter have already been used to investigate ecophysiological and -hydrological responses during drought (Moreno-Gutiérrez *et al.*, 2012, Altieri *et al.*, 2015). It is becoming common to measure $\delta^{18}O$ values of organic compounds in different parts of plants (e.g., leaves/needles, stem, roots) and substances such as carbohydrates, cellulose or bulk wood, although the interpretation of the results may sometimes still be challenging (Weigt *et al.*, 2015, Lehmann *et al.*, 2017). It is known that $\delta^{18}O$ values in meteoric water are variable due to large-scale hydrological processes and temperature effects (Dansgaard, 1964, Craig & Gordon, 1965). Furthermore, they are modified in the soil owing to evaporation. Soil water is then taken up by plants without fractionation (Ehleringer & Dawson, 1992), but the oxygen isotope composition is later modified, owing to several equilibrium and kinetic fractionation processes during transpiration in the leaves (Dongmann *et al.*, 1974, Farquhar *et al.*, 2007) and carbohydrate production (Roden & Ehleringer, 1999, Lehmann *et al.*, 2017). Therefore, $\delta^{18}O$ variations in plant organic matter such as tree rings are characterized by a complex, mixed isotope signal arising from the source water, the evaporative leaf water enrichment, and biochemical fractionations.

Analyses of $\delta^{18}O$ in tree rings have often been used to reconstruct past environmental conditions (Libby *et al.*, 1976, Masson-Delmotte *et al.*, 2005, Danis *et al.*, 2006, Treydte *et al.*, 2006, Edwards *et al.*, 2008, Rinne *et al.*, 2013, Labuhn *et al.*, 2016). However, in these studies climate reconstruction procedures mainly relied on correlation analysis. Thus, such reconstruction approaches may only explain a small part of the isotopic variability and need improvements (McCarroll & Loader, 2004, Treydte *et al.*, 2014). It is therefore important to apply mechanistic, process-based models for better understanding the controlling factors of isotope fractionation in leaves and tree rings related to various environmental conditions. The mechanistic Craig-Gordon model has often been applied to this end (Craig & Gordon, 1965, Farquhar & Lloyd, 1993). However, it has been critically discussed that this approach often overestimates leaf water enrichment and thus needs a correction for improved accuracy (Barbour, 2007, Ogée *et al.*, 2007). To date, there is no general correction approach suitable across different species. In many studies, the so-called Péclet correction was applied, requiring sometimes rather unrealistic adjustments of its parameters (Ferrio *et al.*, 2012, Song *et al.*, 2013, Cernusak *et al.*, 2016, Holloway-Phillips *et al.*, 2016). Therefore, it has been suggested that the so-called two-pool correction could be more adequate, particularly for conifers (Roden *et al.*, 2015, Song *et al.*, 2015, Bögelein *et al.*, 2017). Additionally, current mechanistic models have been challenged by the limited understanding of post-photosynthetic isotope fractionation and the biochemical processes responsible for the incorporation of the cellulose oxygen isotopic signature into tree rings (Waterhouse *et al.*, 2002, Gessler *et al.*, 2009, O'Reilly Sternberg Lda, 2009, Ogée *et al.*, 2009, Gessler *et al.*, 2014). The multitude of factors involved, including climate, source water variability and physiological properties like stomatal conductance, make it still difficult to apply such models for tree rings. For understanding long-term drought responses of trees by using stable oxygen isotopes, it is therefore required to conduct studies that investigate various isotope fractionation processes in the soil and leaves and link those to the observed tree-ring variations.

In this study, we focused on a drought-stressed Scots pine population from one of the driest parts of the European Alps (Valais, Switzerland) that has been subjected to a long-term irrigation experiment since 2003. We measured δ^{18}O values in water extracted from different soil depths and needles of the control and irrigated trees sampled multiple times between 2013 and 2015 to identify the factors, controlling the isotope fractionation, owing to environmental and physiological changes in the trees, like the evaporative leaf water enrichment and needle morphological properties. Particularly, the basic Craig-Gordon model and the two-pool correction were tested to better understand the cause for δ^{18}O variations in needles. At the same site, we analyzed the $\delta^{18}O$ values in tree-ring cellulose from Scots pine trees that had recently died and compared it with living trees over the period 1900-2014. We compared measured tree-ring $\delta^{18}O$ values with predicted ones using a fractionation model corrected with a similar approach as for needles to explore long-term differences in tree physiology and to shed light on drought-induced decline. Overall, our main hypotheses are the following:

- $\delta^{18}O$ values predicted with the two-pool correction model adequately reflect physiological changes in Scots pines at our study site, particularly for the control (drought-stressed) trees vs. irrigated ones;

- changes in the fraction of unenriched (xylem) water in needles to total needle water (xylem + cell water) over time are incorporated into the isotopic signal of the tree-ring cellulose and could be an early indicator of tree decline and mortality.

Data and Methods

Study site

The study was carried out in the Pfynwald forest (46º 18' N, 7º 36' E, 615 m a.s.l.) in Valais, Switzerland. The site is a natural Scots pine (*Pinus sylvestris* L.) forest located in one of the driest inner-Alpine valleys of the European Alps, where Scots pine decline and mortality have already been observed (Bigler *et al.*, 2006, Rigling *et al.*, 2013). Precipitation distribution in this area is uneven because of the surrounding mountains that obstruct the moist oceanic masses to reach this area. For 1981-2010, mean annual precipitation was 605 mm, of which only 169 mm occurred during summer, while the mean annual temperature was 10.1 °C (19.1 °C for summer). In 2003, an irrigation experiment was initiated at this site (Dobbertin *et al.*, 2010, Eilmann *et al.*, 2010, Herzog *et al.*, 2014), consisting of four control and four irrigated plots of 1000 m^2 each. The irrigation water was taken from a river channel near the experimental area, and irrigation took place every growing season between April and October. The irrigated trees received ca. twice the amount of total annual precipitation compared to the control trees, i.e. approximately 1300 mm (Dobbertin *et al.*, 2010, Eilmann *et al.*, 2010, Herzog *et al.*, 2014).

Sampling, sample preparation and measurements

To measure seasonal $\delta^{18}O$ variations in needle water, one tree per plot (i.e. four control trees and four irrigated trees) were selected for multiple samplings between August 2013 and August 2015. Sampling was conducted every month from June to August and ca. every second month for the remaining periods. Several twigs were cut using a pruner mounted on a telescopic pole. Current year needles were collected and immediately separated from twigs and stored in air-tight glass vials with screw caps, and placed in a cooling box with ice packs at temperatures around 0 ºC to minimize evaporative water loss.

At the same sampling dates and using a soil sampler, soil samples were collected at two sampling depths (0-10 and 10-20 cm) and stored in air-tight glass vials to monitor seasonal $\delta^{18}O$ variations of the source water. Deeper sampling depths were generally not accessible due to the rocky soil. However, there was one soil profile down to 80 cm available, where soil samples were excavated on April 9th, 2014. Water samples from the channel (irrigation source) were collected at each sampling date as well. Additionally, at two sampling dates in August and October 2014 short increment cores from the four control and four irrigated trees mentioned above were also sampled for stem water extraction.

Water from soil, needles and tree increment cores was obtained using a cryogenic vacuum extraction system (West *et al.*, 2006, Saurer *et al.*, 2016). In brief, glass vials with samples are placed in a water bath at 80 °C. The evaporated water from samples is collected into U-shaped glass tubes that are cooled down with liquid nitrogen (N_2). Both glass vials with samples and tubes are connected to a vacuum system at ca. 4×10^{-2} mbar. Subsequently, the extracted water samples were transferred into 2 ml sealed glass vials. The $\delta^{18}O$ values of these water samples were measured by injecting 0.6 µl of the sample into a Thermal Conversion Elemental Analyzer (TC/EA; Thermo Finnigan, Bremen, Germany), where the water was pyrolysed in a glassy carbon reactor at 1450 °C to hydrogen (H_2) and carbon monoxide (CO). These gases were carried in a helium stream to an isotope ratio mass spectrometer (IRMS) Delta plus XP (Thermo Finnigan, Bremen, Germany) for $\delta^{18}O$ analysis. The results were reported in the standard δ-notation as per mil (‰) relative to the Vienna Standard Mean Ocean Water (VSMOW), with a precision of < 0.2 ‰.

Twelve stem disks of standing dead trees (subsequently called “now-dead/dead trees”) and increment cores from 32 control trees (“now-living/living trees”) were sampled within the non-irrigated area. Tree-ring widths (TRW) were measured at the tree-ring laboratory of the Swiss Federal Institute for Forest, Snow and Landscape Research WSL in Birmensdorf, Switzerland. The chronologies for the dead and living trees cover the period of 1900-2005 and 1900-2014, respectively. More detailed information on sampling, preparation and measurements of TRW can be found in Timofeeva *et al.* (2017). To measure $\delta^{18}O$ values in tree rings, they were separated according to calendar year for cellulose extraction and homogenization (Boettger *et al.*, 2007, Laumer *et al.*, 2009, Roden *et al.*, 2009), and *aliquots* were packed into silver capsules for isotope analyses, which were conducted using a pyrolysis method at 1420 °C in an elemental analyzer (PYRO-cube, Elementar, Hanau, Germany) connected to an Isotope Ratio Mass Spectrometer (IRMS, Delta Plus XP) via a Conflo III interface (Thermo Fischer Scientific, Bremen, Germany) (Weigt *et al.*, 2015). The measurement precision was typically lower than 0.2 ‰.

Needle gas exchange and anatomical measurements

Leaf gas exchange, including net-photosynthesis (A_N) and transpiration (E) of five sun-exposed twigs per tree (4 irrigated trees, 4 control (ambient) trees) were measured in May and June of 2013 and 2014. Measurements were performed using a portable photosynthesis system equipped with a 6400-22L lighted conifer chamber (LI-COR 6400Xt; LI-COR, Lincoln, NE, USA). Conditions in the cuvette were kept constant during the measurements at 400 ppm [CO_2] and a photon flux density of 1,000 μmol m^{-2} s^{-1}, while the temperature was adjusted close to ambient conditions. Initial leaf-gas exchange values were corrected *a posteriori* for the exact projected leaf area according to Fleck *et al.* (2016).

To measure needle length, 10 trees at the control and 12 trees at the irrigated plots were selected. Measurements were performed repeatedly every year in summer on the same trees for the period of 2013-2015.

Oxygen isotope theory

The needle water isotope ratio ($\delta^{18}O_N$) in the steady-state can as a first approximation be calculated by the Craig & Gordon (1965) equation modified by Dongmann *et al*. (1974):

$$\delta^{18}O_N = \delta^{18}O_{SW} + \varepsilon^{+} + \varepsilon_k + (\delta^{18}O_V - \delta^{18}O_{SW} - \varepsilon_k)\cdot e_a/e_i, \qquad (1)$$

where $\delta^{18}O_{SW}$ is the isotope value of the source water (xylem), ε^{+} is the equilibrium fractionation related to the phase change from liquid to vapour, ε_k is the kinetic fractionation related to the diffusion in air, $\delta^{18}O_V$ is the atmospheric water vapour isotope value, and e_a/e_i is the ratio of needle external (ambient) to internal water vapour pressures. To exclusively characterize the isotope fractionation within the leaf without influences of the source water isotope variation, the needle water enrichment term $\Delta^{18}O_N$ is often used instead of $\delta^{18}O_N$ and is approximately given as follows:

$$\Delta^{18}O_N = \delta^{18}O_N - \delta^{18}O_{SW}. \qquad (2)$$

Therefore, equation 1 can be re-written as

$$\Delta^{18}O_N = \varepsilon^+ + \varepsilon_k + (\delta^{18}O_V - \delta^{18}O_{SW} - \varepsilon_k)\cdot e_a/e_i. \qquad (3)$$

The equilibrium fractionation (ε^+) depends on temperature (Bottinga & Craig, (1968). The kinetic fractionation (ε_k) has an approximate value of 28 ‰ (Cernusak *et al.*, 2016). The ratio e_a/e_i can be simplified by using relative humidity of the atmosphere (RH) for the calculations, assuming that the leaf and air temperatures are equal, although this may not always be the case, particularly for the sun-exposed needles or leaves. Part of equations 1 and 3, i.e. $\delta^{18}O_V - \delta^{18}O_{SW}$, can be replaced by $-\varepsilon^+$, assuming isotopic equilibrium between soil (source) water and water vapour (Foerstel & Huetzen, 1983). This assumption may be appropriate in particular for humid conditions, but some deviation could be expected in dry situations, owing to soil evaporation effects (Ueta *et al.*, 2013, Bögelein *et al.*, 2017).

Equations 1 and 3 were found to predict reasonably well variations in observed needle water enrichment (Farquhar & Lloyd, 1993, Roden & Ehleringer, 1999, Cernusak *et al.*, 2016), but they often overestimate actual values. Therefore, a model correction based on the Péclet effect was proposed (Farquhar & Lloyd, 1993, Barbour, 2007, Cernusak *et al.*, 2016). This effect can be quantified by the Péclet number $\wp$ and considers an isotopic gradient between xylem and evaporative sites, mainly driven by changes in transpiration (E) and a so-called effective path length (L):

$$\wp = L\cdot E/(C\cdot D), \qquad (4)$$

where L (m) describes the tortuous path that water travels from leaf veins to sites of evaporation, E is the transpiration rate (mmol m^{-2} s^{-1}), C is the molar density of water (55.56 × 10^3 mol m^{-3}), and D is the diffusivity of $H_2{}^{18}O$ in water, which depends on temperature (Cuntz *et al.*, 2007). However, the effective path length (L) is not a directly measurable parameter and difficult to quantify. Song *et al.* (2013) identified relationships between effective path length (L) and leaf transpiration rate (E) for various species and across all sampling periods, which we used in our study. They established a phenomenological relationship both for deciduous and coniferous species (chestnut oak, red maple, black oak, pitch pine, white cedar, Monterey pine) and all sampling periods as follows:

$$L = 2.36 \times 10^{-5}\, E^{-1.20}. \qquad (5)$$

Alternatively, a two-pool model was suggested as a correction for equations 1 and 3 (Yakir *et al.*, 1990, Song *et al.*, 2015, Cernusak *et al.*, 2016). It assumes that total leaf water is a mixture of two discrete pools of water, i.e. unenriched xylem water (the central part of the needle) and ^{18}O enriched water at the evaporative sites (mesophyll tissue), as follows:

$$\Delta^{18}O_N{}^{*} = \Delta^{18}O_N \cdot (1 - \phi), \qquad (6)$$

where ϕ is the ratio of unenriched xylem (stem) water to total needle water (Song *et al.*, 2015, Cernusak *et al.*, 2016). This ratio ϕ can have a value between 0 (infinitely small xylem contribution) to 1 (xylem water fully dominates needle water). ϕ is higher for old or stressed leaves/needles because of the higher degree of compartmentalization, where the water volume of the xylem is relatively big compared to the remaining needle water due to suberization and tissue shrinkage (Parker, 1952, Roden *et al.*, 2015), which results in a reduction of total needle water enrichment (Fig. 1).

A modified version of equation 1 can be used to predict ^{18}O enrichment in tree rings:

$$\begin{aligned}\delta^{18}O_{TR} &= \delta^{18}O_{SW} + f \cdot (\varepsilon_e + \varepsilon_k + (\delta^{18}O_V - \delta^{18}O_{SW} - \varepsilon_k)\cdot e_a/e_i) + \varepsilon_{wc} \\ &= \delta^{18}O_{SW} + f \cdot \Delta^{18}O_N + \varepsilon_{wc}, \qquad (7)\end{aligned}$$

where $\delta^{18}O_{TR}$ is the predicted tree-ring isotopic ratio, ε_{wc} is the biochemical exchange between the oxygen atoms of xylem water and those of the cellulose carbonyl groups, equaling 27 ‰ (DeNiro & Epstein, 1981, Sternberg *et al.*, 1986), and *f* is a dampening or exchange factor ($0 < f < 1$) associated mainly with the exchange of oxygen atoms of carbonyl groups with xylem (stem) water during cellulose formation (Saurer *et al.*, 1998, Roden *et al.*, 2000, Treydte *et al.*, 2014). *f* must be calibrated and should reflect the combined effects of isotopic variation of both metabolites and the source water. As a surrogate for source water ($\delta^{18}O_{sw}$) variations over the investigated time period, the averages of summer months of

⍰^{18}O of precipitation can be used. We used a modification of equation 7 that considers an adjusted dampening of the source water:

$$\delta^{18}O_{TR} = f \cdot (\delta^{18}O_{SW} + \Delta^{18}O_{N}) + \varepsilon_{wc}. \qquad (8)$$

Because we cannot separate the damping factors for the source water and the O-exchange processes between xylem water and the sucrose during cellulose formation, the dampening factor as applied in Eq. 8 accounts for both dampenings.

To correct for the overestimation of the predicted leaf values, the equation can be further modified by incorporating the two-pool model correction:

$$\delta^{18}O_{TR}^{*} = f \cdot (\delta^{18}O_{SW} + \Delta^{18}O_{N} \cdot (1 - \phi)) + \varepsilon_{wc}, \qquad (9)$$

where the proportion of unenriched xylem water ϕ in leaf/needle water is considered as defined above.

Climate data

For modeling of needle water enrichment, we used temperature and relative humidity data from Pfynwald with a resolution of ca. 10 min for the period of 2013-2015. These measurements were maintained by Decentlab GmbH (Dübendorf, Switzerland). To measure temperature and relative humidity, Sensirion SHT-21 sensors with a multiple-plate radiation shield (±0.3 °C and ±2% RH accuracy) were used. Monthly climate data for 1900-2014 were obtained from the Sion meteorological station (MeteoSwiss archives). Moreover, a multiscalar drought index (Standardized Precipitation Evapotranspiration Index, SPEI) data was used for correlation analysis with tree-ring parameters, for more details see Timofeeva *et al*. (2017). Monthly δ^{18}O data of precipitation were obtained from the Global Network of Isotopes in Precipitation (GNIP) and from the Federal Office of Water and Geology, Bern, Switzerland (FOWG). The GNIP data from the Grimsel station (46.57 N, 8.33 E, 1950 m a.s.l.) for the period 1972-2014 were used for tree-ring δ^{18}O modeling. They were corrected to the elevational offset, using data from the Sion station available for the period 1994-2014 as follows. We first calculated averages for each month for this period at both stations and used their differences for the correction. The raw data from the Sion station were not used for tree-ring modelling due to the short observation period.

Data analysis

We determined the response of $\delta^{18}O$ variations to climate by calculating Pearson's correlation coefficients for individual months and seasons such as spring (March-May) and summer (June-August) between the mean series of each climate variable (temperature, precipitation, relative humidity, drought index and VPD) and $\delta^{18}O$. We tested the significance of the correlation coefficients by applying two-tailed Student's t-tests.

Temporal autocorrelation was taken into account by calculating the 'effective' sample size, which is based on sample size and the first-order autocorrelation for each time series and climate data (Dawdy, 1964):

$$N' = N \cdot \frac{(1 - r_1 \cdot r_2)}{(1 + r_1 \cdot r_2)}, \qquad (10)$$

where N is sample size; N' is effective sample size; and r_1 and r_2 are the first-order sample autocorrelation of the first and second time series, respectively.

Two-tailed Student's t-tests were applied to test for significant differences between groups.

Results

Seasonal variations in $\delta^{18}O$ of needle and soil water

The $\delta^{18}O$ values of needle water strongly fluctuated between sampling points, with values up to ca. 10 ‰ during spring and summer, and negative values during winter (Fig 2a). Soil water $\delta^{18}O$ also featured a seasonal pattern for both soil depths, but with values always below zero (Fig. 2b,c). However, the $\delta^{18}O$ values of the channel water (source of irrigation) did not show significant seasonal changes and showed on average a low value of -15.59 ‰ ± 0.73 ‰ (mean ± SD, Fig. 2b,c). Treatment differences between control and irrigated trees were not significant for $\delta^{18}O$ of needles, but clearly visible for soil water isotope values most likely due to the addition of the depleted channel water for the irrigated trees (significant for August 2013 and April 2014, $P < 0.05$, t-test). Isotope values at soil depths from 0 to 10 cm were mostly enriched compared to those at soil depths from 10 to 20 cm during spring and summer (except for summer 2014), particularly for the control treatment (Table 1). This strong isotope enrichment near the soil surface was also apparent from the results of the deep soil profile, but below a depth of 20 cm there was much less variation in the isotopic composition (Fig. S1). The lower soil depth 10-20 cm was therefore considered representative for the depth from where the source water was mainly taken up, also because stem water collected in August and October 2014 showed values that were more similar to the soil water values from this depth rather than from top soil. We calculated the needle water enrichment Δ^{18}O above source water based on the difference between δ^{18}O of needles and water of soil depths 10-20 cm (Eq. 2). Irrigated trees showed higher needle water isotope enrichment compared to the control trees at almost all sampling points (Fig. 3). The divergence was stronger during spring and summer than during winter. However, significant treatment effects ($P < 0.05$, t-test) were observed for August 2013 and April 2014 only, but not for summers 2014 and 2015. For comparison, when we used as the source water the data from 0-10 cm depth, the irrigated trees still had higher needle water enrichment, but the amplitude of Δ^{18}O was lower for both treatments (Fig. S2).

Gas exchange and needle properties

Transpiration rate was on average higher for the irrigated compared to the control trees, except for the sampling in June 2013 (Table 2). However, differences between treatments were only marginally significant ($p < 0.1$, t-test) for the averaged values indicated in the table, but they were significant using all the measured values of each tree in the treatment for both samplings in 2014 ($P < 0.01$, t-test) and for the combined sampling dates over the period of 2013-2014 ($P < 0.05$, t-test). Variability was also relatively high for the irrigated trees. Needle length was lower for the control trees, but with a similar and high variability for both treatments (Table 3). Differences between treatments were significant both for the averaged and using all the measured values for 2013 ($P < 0.01$, t-test), 2014 ($P < 0.05$, t-test) and over the period of 2013-2014 ($P < 0.001$, t-test).

Modeling needle water isotope enrichment

A highly significant correlation was observed between measured and Craig-Gordon (CG)-modelled needle water ^{18}O enrichment values using the basic equation (Eq. 3, Fig. 4). However, the modeled values were only accurate for winter conditions, when enrichment was low, but values were strongly overestimated for both treatments during summer months. Accordingly, slopes of the linear regression strongly deviated from 1 (Fig. 4a, *control trees: slope = 0.39, $R^2 = 0.66$, $P < 0.01$; irrigated trees: slope = 0.52, $R^2 = 0.62$, $P < 0.01$*). Furthermore, the basic CG model could not explain the observed difference in enrichment between the treatments. For further correction, we initially estimated effective path length values for needles of the control (1196 mm) and irrigated (611 mm) trees, using Eq. 5. As they did not appear realistic for applying the correction based on the Péclet effect, we focused on the two-pool correction (Eq. 6) to improve our model. Indeed, the modeled values were significantly better, as the linear regression slopes were closer to 1 (Fig. 4b, *control trees: slope = 0.79*, $R^2 = 0.66$, $P < 0.01$; *irrigated trees: slope = 0.79*, $R^2 = 0.62$, $P < 0.01$). The best agreement between measured and estimated data in this case was found by varying ϕ. The obtained correction factor ϕ was higher for the control than the irrigated trees ($\varphi_{control} = 0.443$; $\varphi_{irrigated} = 0.328$), indicating a higher proportion of xylem to mesophyll water for the control trees (Fig. 1). This model also explained the observed treatment difference well (Fig. 3).

Tree-ring $\delta^{18}O$ values and their relationship to climate

The mean tree-ring $\delta^{18}O$ values calculated over the five control (living) and five now-dead trees, respectively, were highly correlated when comparing the entire common period (Fig. 5a, 1900-2004, $r = 0.76$, $P < 0.001$), but also between individual tree series for both groups (Fig. S3 & S4). Values of tree-ring $\delta^{18}O$ did not show any significant differences between the control and the now-dead trees until the 1970s. After the 1970s, however, the records started to deviate significantly (Fig. 5) and remained more depleted for the now-dead until their death compared to the control trees (Fig. 5b, Student's t-test for differences between groups, 1900-1959: *0.130 ± 0.118 (mean difference ± SD), n.s.;* 1960-2005: *-0.786 ± 0.171*, $P < 0.001$). Correlation analysis between monthly climate variables and tree-ring $\delta^{18}O$ chronologies showed that the spring (Mar-May) and summer (Jun-Aug) seasons were most important for explaining our results, and we thus focus on the correlations for these periods. Tree-ring $\delta^{18}O$ values of the control trees were positively and significantly correlated with spring temperatures and VPD as well as RH, and also with summer temperature, precipitation amount and the drought index (Fig. 6). For the now-dead trees, correlations were similar and in some cases also significant (spring VPD, summer precipitation amount and drought index). Generally, trees that died later showed a weaker response in spring, but a stronger response in summer compared to the control trees (Fig. 6).

Modeling of $\delta^{18}O$ in tree rings

The correlation between measured and modeled $\delta^{18}O$ tree ring values was significant when using the basic CG model (Eq. 7) for both tree groups (Fig. 7a, *control trees: slope = 0.25*, $R^2 = 0.29$, $P < 0.001$; *dead trees: slope = 0.28*, $R^2 = 0.37$, $P < 0.001$), although the regression slopes deviated significantly from 1. However, the model variability was much too high (Fig. 8a). The modeled period covers the time period where oxygen isotope data of precipitation are available (starting in 1972). Modeling results applying Eq. 8 showed values with appropriate variation, but the absolute values were too high (Fig. S5). However, after applying the modified Eq. 9, modeled values were in a better agreement with measurements regarding both, their absolute level and their variability (Fig. 7b, *control trees: slope = 0.42,* $R^2 = 0.29$, $P < 0.001$*; dead trees: slope = 0.45,* $R^2 = 0.36$, $P < 0.001$). According to this model, the correction factor ϕ was higher for the more stressed (now-dead) compared to the less stressed (living) trees ($\varphi_{dead} = 0.37$; $\varphi_{control} = 0.30$). However, deviations still remained between the measurements and modeling results, even after improvement of the model, i.e. applying a common dampening factor and an optimized ϕ for each treatment, implying that ϕ was assumed to be constant over the entire study period (Fig. 8b). Alternatively, ϕ may be varied from year to year to obtain the best correlation between data and model based on Eq. 9. Thus, this physiological parameter can be reconstructed over the modeled period (Fig. 9).

Discussion

Our measurements and modeling results show that δ^{18}O variations in tree rings of Scots pine reflect not only climate signals of the past, as previously shown (Libby *et al.*, 1976, Masson-Delmotte *et al.*, 2005, Danis *et al.*, 2006, Treydte *et al.*, 2006, Edwards *et al.*, 2008, Hilasvuori *et al.*, 2009, Rinne *et al.*, 2013, Labuhn *et al.*, 2016), but that they can potentially be used to retrospectively investigate the health status of trees and related needle physiological changes. We observed a deviation in tree-ring δ^{18}O starting in the 1970s between surviving trees and trees that died later, which may be an early indication of tree decline. Due to the detailed seasonal dataset of needle water and soil water δ^{18}O, we were able to identify the most important driving factors of the oxygen isotope variations in this stand as discussed in the following.

Oxygen isotope variations in needles and soil water

Confirming our first hypothesis, agreement between data and modeled values significantly improved when applying the two-pool correction on the basic CG model of needle water enrichment. Values estimated by the basic, purely physical CG model were too high for both control and irrigated trees compared to observations of Δ^{18}O, showing the need for a physiological correction. Furthermore, we observed lower needle water enrichment for the control compared to the irrigated trees, which could not be explained by the basic model, but by the two-pool model correction provided that a different ϕ value was used for the two tree groups. The two-pool model was originally proposed more than two decades ago (Yakir *et al.*, 1990, Gat & Bowser, 1991), but only recently has it been revived with the expectation that it may be superior to the Péclet model (Song *et al.*, 2015, Bögelein *et al.*, 2017). When the proportion of the transfusion tissue surrounding the needle xylem is relatively small (high ϕ value), needle water will be strongly dominated by the unenriched xylem water, resulting in low Δ^{18}O according to the two-pool model. This was observed for the needles of stressed trees in our study and is likely caused by changes in needle properties under drought. Discoloration, decrease in length and thickness are typical examples of changes of needle properties of Scots pine under drought in this region (Dobbertin *et al.*, 2010). Indeed, needle lengths of the control trees at our study site were significantly lower than for the irrigated ones. Such behavior was experimentally tested in needles of white (*Pinus strobus* L.) and Austrian (*P. nigra* Arn.) pine by Parker (1952). He showed that drying of needles was accompanied by shrinkage of transfusion tissue, mainly endodermis and mesophyll cells, while the xylem was less affected. However, this was a short-term experiment and it has to be taken into account that the effects of a short- and long-term drought on needle morphology may be different. Relatively high ϕ was also observed for older needles compared to newer needles for various species, including pine, verified by anatomical measurements such as mean mesophyll thickness (Roden *et al.*, 2015). This indicates that needle properties and the resulting isotope enrichment change not only under stress, but also with age.

In contrast, our results could not be explained with a model based on the Péclet effect. The Péclet number $\wp$ is driven primarily by transpiration (E) and effective path length (L). Under drought stress, transpiration often decreases, which reduces $\wp$ (Eq. 4). Therefore, needle water is less diluted by re-filling with xylem water, and higher needle Δ^{18}O values should be observed under drought or, on the contrary, more depleted needle Δ^{18}O values as transpiration increases (no drought stress/low VPD). According to this theory, therefore, more stressed plants should have higher isotope enrichment, as was indeed observed in some studies (Yakir *et al.*, 1990, Roden & Ehleringer, 1999), but not at our site. Additionally, $\wp$ depends on the effective path length describing the water path within the leaf or needle, which can be affected by leaf/needle morpho-physiological changes as well (Kahmen *et al.*, 2008, Ferrio *et al.*, 2012). For deciduous plants, L can be estimated reasonably well (Kahmen *et al.*, 2009), but for conifers, it is often challenging to estimate L, mostly due to the different structure of a needle, its low conductance, and generally low transpiration. Our estimates of L were much greater (ca. 60-120 cm) compared to measured needle length. Such unrealistic estimates of L have previously been reported for various deciduous and coniferous species, including pine (Song *et al.*, 2013). To rectify such problems, more complex models such as non-steady state approaches were proposed, particularly for modelling diurnal changes in $\delta^{18}O$ variability (Barbour, 2007, Ogée *et al.*, 2007, Cernusak *et al.*, 2016). However, a relatively simple steady-state two-pool model was sufficient to describe seasonal $\delta^{18}O$ variability in drought-prone Scots pines at our study site.

On the one hand, the accuracy of leaf water isotope modeling depends strongly on the model parameters, as discussed above. On the other hand, it also critically depends on the source water value. We must therefore take into account that the estimated needle water isotope enrichment strongly depends on the difference between needle and soil water $\delta^{18}O$. It is crucial to use most representative source water $\delta^{18}O$ values for modeling, particularly considering root distribution and isotopic gradients at different depths (Treydte *et al.*, 2014, Saurer *et al.*, 2016). Uncertainties induced by a lack of knowledge on the depth from which trees actually take up water may be quite large. For our model, we used the values of $\delta^{18}O$ from the lower soil depth (10-20 cm), rather than values from the top layer, which were enriched due to evaporation from the soil surface. However, even when using the topsoil water values as the source, calculated needle water enrichment of the control trees was still lower than of the irrigated trees. In addition, the $\delta^{18}O$ soil water values down to 80 cm determined from soil excavation did not show strong changes below 20 cm, suggesting that the source water values we used are likely to be appropriate. Overall, we conclude that the irrigation experiment enabled us to disentangle the effects of drought on needle-level oxygen isotope fractionation in mature trees, and we captured the important seasonal driving factors of needle water isotopic fractionation at our study site. This is a prerequisite for understanding the tree-ring isotope signal.

Tree-ring oxygen isotope variations

The tree-ring δ^{18}O values of the individual control and now-dead trees as well as the means of the two groups had a very strong common variability. This demonstrates that these trees were strongly affected by similar (external) environmental factors. Our correlation analysis indeed showed that mainly spring mean temperature and VPD were responsible for the common δ^{18}O variations of both groups, consistent with earlier studies (Treydte *et al.*, 2007, Treydte *et al.*, 2014, Giuggiola *et al.*, 2016). This can be explained by the effect of high temperatures on the isotope ratios of precipitation and source water (Dansgaard, 1964), and by the higher leaf water enrichment under dry conditions (Roden & Ehleringer, 1999). It is also consistent with the occurrence of frequent droughts in the studied region (Bigler *et al.*, 2006, Rigling *et al.*, 2013). However, the dead trees were more depleted in δ^{18}O compared to the control trees after the 1970s. This indicates that climate alone cannot explain the isotope variability. The results from our seasonal water samples showed that drought-stressed trees generally showed lower oxygen isotope enrichment, owing to changes in needle morphology. This fits very well to the lower tree-ring isotope values of the now-dead (i.e., more stressed) trees compared to the survivors, thereby providing evidence for our second hypothesis of an isotopic signal transfer from needles to tree-rings.

Lower δ^{18}O of tree-rings was also shown to be related to seasonal differences in photosynthetic activity (Sarris *et al.*, 2013). Drought-stressed trees may be strongly influenced by favorable spring and early summer environmental conditions. This signal would be influenced by isotopically depleted source water from winter and spring. The tree-rings could therefore reflect the isotopic signature of carbohydrates produced during the earlier part of growing season rather than during very dry conditions in summer, when trees might even completely stop investing in their growth and using freshly produced carbohydrates only for maintenance (Sarris *et al.*, 2013, Pflug *et al.*, 2015). Such extreme drought may, however, not be occurring at our site. A previous study based on carbon isotopes and tree-ring growth at the Pfynwald site indicated that tree individuals with the most isohydric strategy were prone to suffer due to weakening as a result of long-term reduced carbon uptake (Timofeeva *et al.*, 2017). As atmospheric moisture demand was increasing across recent decades due to higher temperatures and frequent drought years (Rebetez & Dobbertin, 2004, Rebetez & Reinhard, 2008), Scots pine trees with this strategy tended to close their stomata strongly and therefore likely had lower transpiration. The stressed Scots pines at our study site therefore probably transpired less for many decades already, although on the verge of death this does not always need to be the case as Scots pines in Spain showed higher transpiration compared to the healthier individuals (Salmon *et al.*, 2015). According to the Péclet theory, the tree-ring $\delta^{18}O$ values of more stressed trees would be more enriched due to lower transpiration at our site. As this was not the case, our results suggest that for tree rings, similar as shown above for the needle values, the two-pool model is appropriate.

To confirm this finding, we further applied isotope fractionation models, although this was only possible over the period where isotope data of precipitation were available. Modeling of $\delta^{18}O$ variations in tree-ring cellulose has generally been more challenging (Roden *et al.*, 2000, Saurer *et al.*, 2012, Treydte *et al.*, 2014) than modeling of changes in leaf/needle water $\delta^{18}O$ values (Barbour, 2007, Cernusak *et al.*, 2016). The transfer of the isotope signal from leaf water to organic compounds in leaves such as sucrose and later to cellulose is complex and involves isotope fractionation on various occasions (Gessler *et al.*, 2014, Treydte *et al.*, 2014). Potential fractionations may occur because of the use of stored carbohydrates or during phloem loading and transport, but the strongest modifications are observed during cellulose formation (Lehmann *et al.*, 2017). The latter occurs because of exchange of carbonyl groups with xylem water, thus diluting the leaf isotope signal. Therefore, previous studies had difficulties in distinguishing the leaf physiological from the source water signal in tree-ring cellulose/wood $\delta^{18}O$ variations (Ogée *et al.*, 2009, Treydte *et al.*, 2014). Modeled tree-ring cellulose $\delta^{18}O$ values in our study were also overestimated according to the basic CG model (Eq. 5). However, applying a common dampening factor and the two-pool correction with an optimized ϕ value for each group of trees (Eq. 9) strongly improved the agreement between data and model. The ϕ values obtained were reasonable compared to those obtained from modeling the seasonal needle data, showing higher values for the more stressed trees. Additionally, we showed that it is possible to reconstruct ϕ values over the last few decades (Fig. 9). These data provide valuable information on past tree physiological and needle morphological changes at our site, indicating shrinkage of the transfusion tissue around the xylem for the dead trees after the 1970s.

Interestingly, lower $\delta^{18}O$ values were also observed for declining Norway spruce (*Picea abies* L. Karst.) at two sites in Norway (Hentschel *et al.*, 2014). Although the authors did not model the oxygen enrichment, they concluded that such behavior may be due to changes in anatomical and physiological traits of trees under drought conditions, which could be examined to infer a risk for future tree decline (Hentschel *et al.*, 2014). Therefore, such changes and related isotope traces under drought stress could be common for conifers, and the identification of ϕ values may be a powerful tool for estimating the 'health status' of trees. Our results are among the first that show that anatomical and morphological needle traits of trees are reflected in the ⍰^{18}O-variations of tree rings. This method can potentially be applied either for retrospectively analyzing past tree-physiological changes, or to predict decline and/or mortality of vulnerable trees in the future.

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Conflict of interest

None declared.

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Figures and Tables

Table 1. Differences between soil water δ^{18}O values at 0-10 cm and at 10-20 cm soil depth.

Difference (‰)	Aug-2013	Oct-2013	Dec-2013	Feb-2014	Apr-2013	Jun-2014	Jul-2014	Aug-2014	Oct-2014	Jun-2015	Jul-2015	Aug-2015
Control	2.92	0.81	-0.87	-0.19	4.39	4.19	2.72	2.10	1.96	5.36	4.38	2.22
Irrigated	1.19	1.03	-0.67	0.24	4.59	3.22	3.27	2.18	2.15	2.97	4.02	0.76

Table 2. Transpiration rates for the period of 2013-2014. Numbers are mean values of four trees ± standard deviation. NA – not available.

Date	Transpiration rate (mmol m^{-2} s^{-1})	
	Control	Irrigated
18.06. 2013	0.35 ± 0.08	0.36 ± 0.17
06.05.2014	0.22 ± 0.07	1.01 ± NA
03.06.2014	0.12 ± 0.05	0.21 ± 0.05
2013-2014	0.24 ± 0.12	0.43 ± 0.34

Table 3. Needle length data determined during summer 2013 and 2014. Numbers are mean values (± standard deviation) of 10 trees for control and 12 trees for irrigated treatments.

Date	Needle length (mm)	
	Control	Irrigated
2013	38.77 ± 7.61	48.24 ± 7.27
2014	30.57 ± 8.57	40.38 ± 8.45
2013-2014	34.67 ± 8.94	44.31 ± 8.69

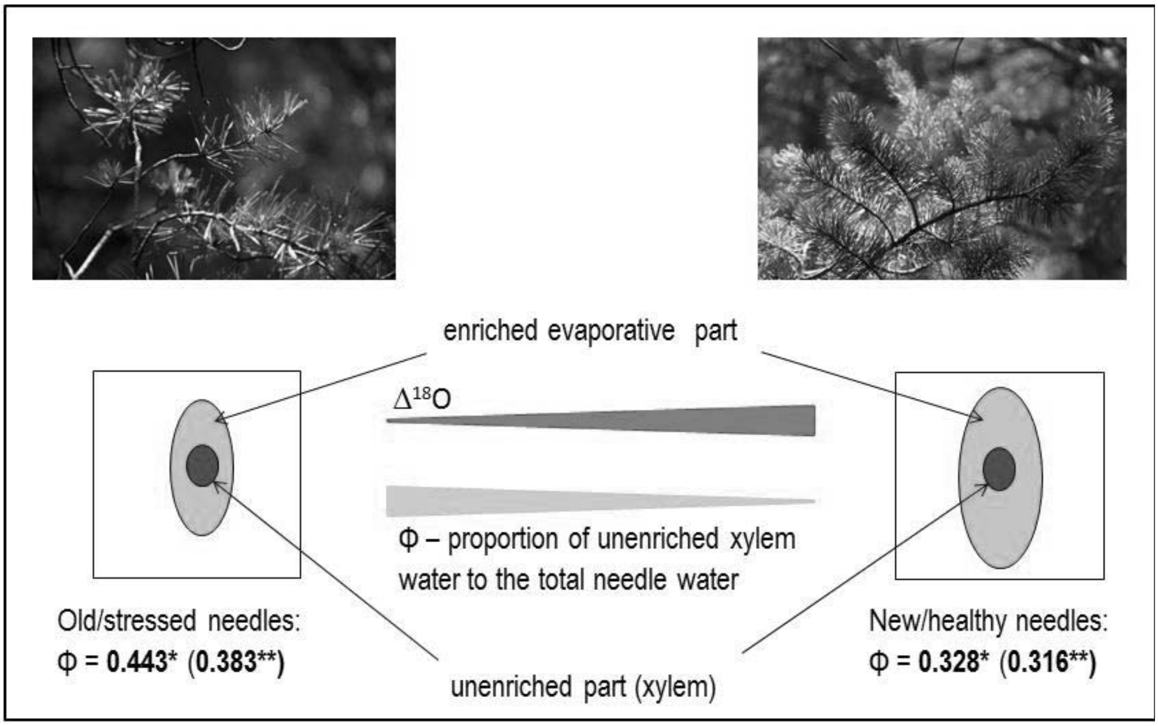

Figure 1. Schematic representation of the two-pool model showing needles with differing fraction of xylem water to total needle water ϕ: either high for stressed or old needles (left side) or low for healthy or new needles (right side). The red and yellow arrows show that needle water enrichment is expected to increase with decreasing ϕ. *Values derived in this study; ** values for Scots pine from Roden et al., 2015. Pictures from Pfynwald pine trees by Marcus Schaub, WSL (left picture: control trees; right picture: irrigated trees).

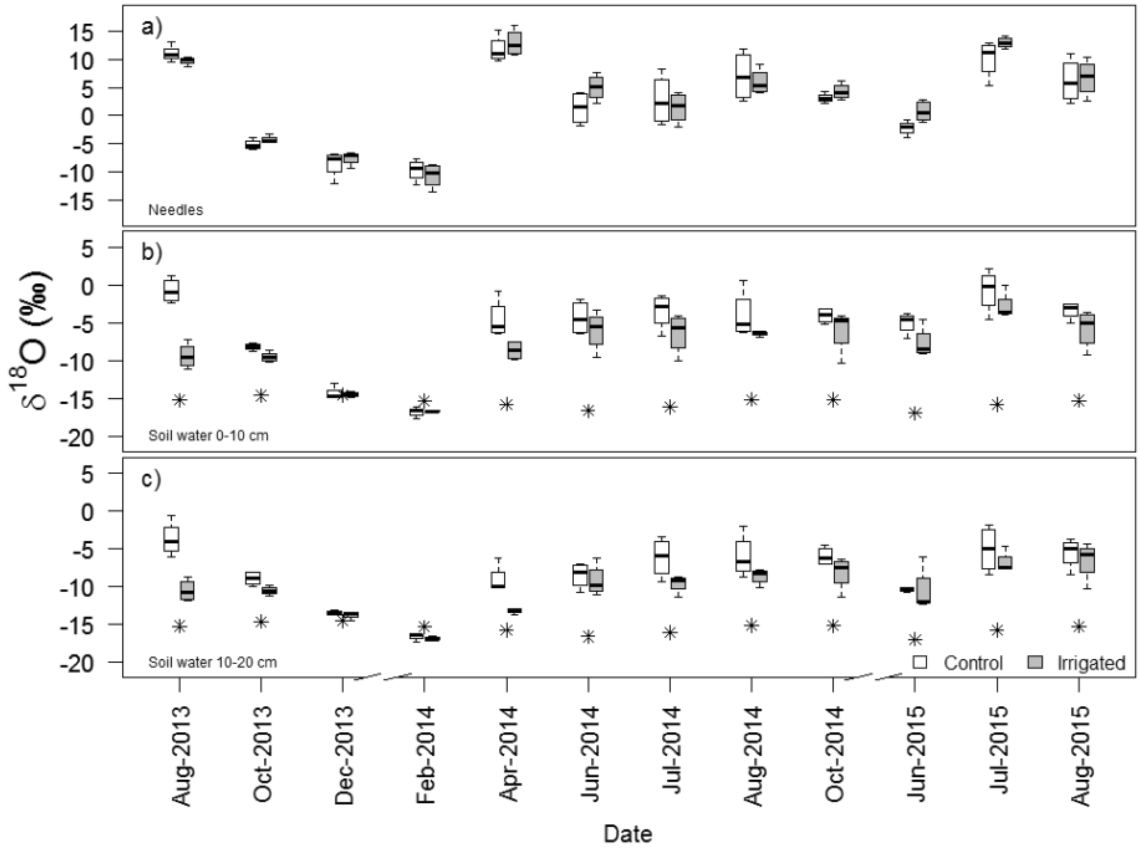

Figure 2. Seasonal variations in needle water $\delta^{18}O$ (a), soil water (0-10 and 10-20 cm, b, c) and channel water used for irrigation (asterisks, b, c).

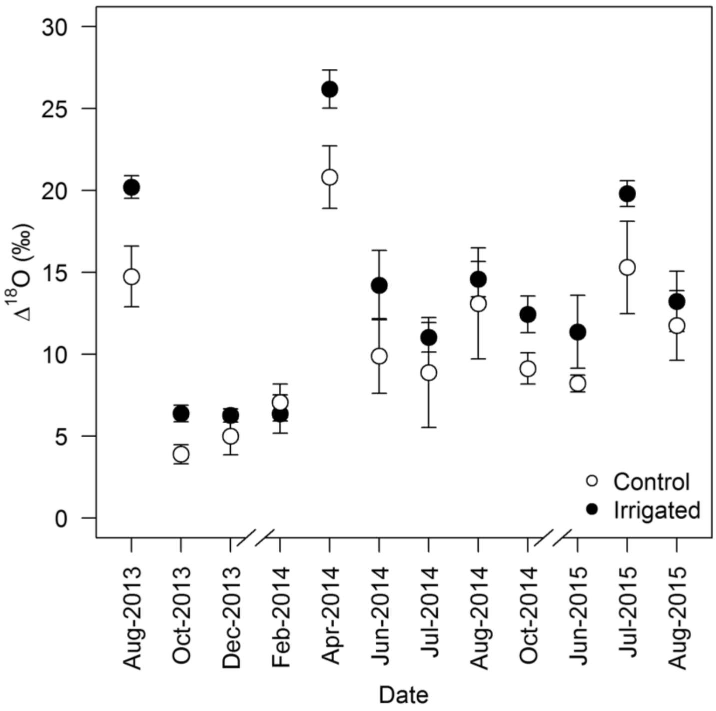

Figure 3. Seasonal changes in needle water enrichment.

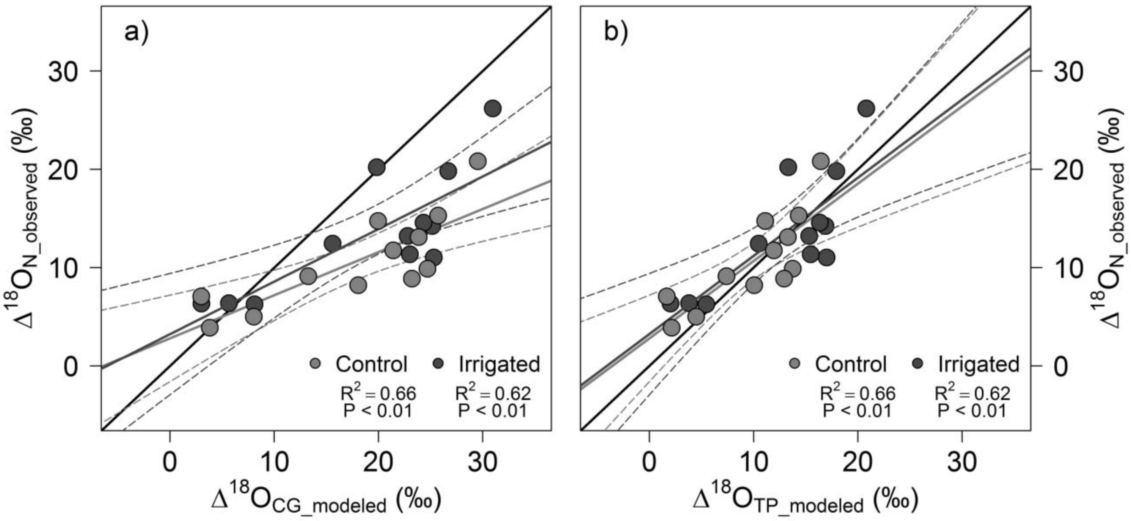

Figure 4. Relationship between observed and predicted needle water ^{18}O enrichment using the basic Craig-Gordon model (Eq. 3, a) and the modified model (Eq. 6) with the two-pool correction (b). The dashed lines indicate 95% confidence intervals.

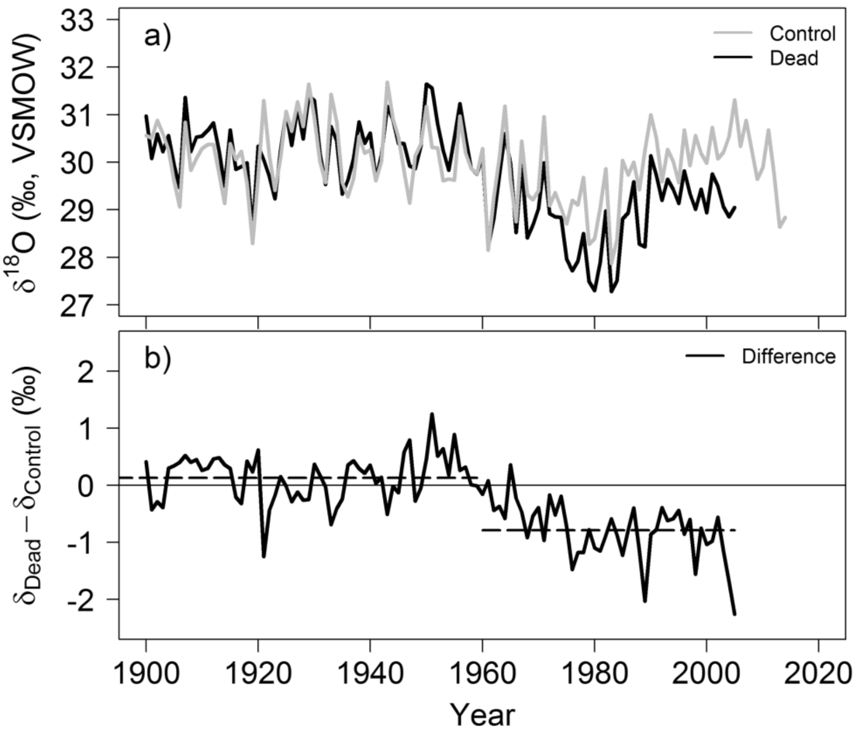

Figure 5. Tree-ring δ^{18}O chronologies of control and now-dead trees (a) and their differences (b). The dashed lines indicate the mean of the differences between dead and control for 1900-1959 and 1960-2005, respectively. Note: the chronology of the dead trees covers the period 1900-2005.

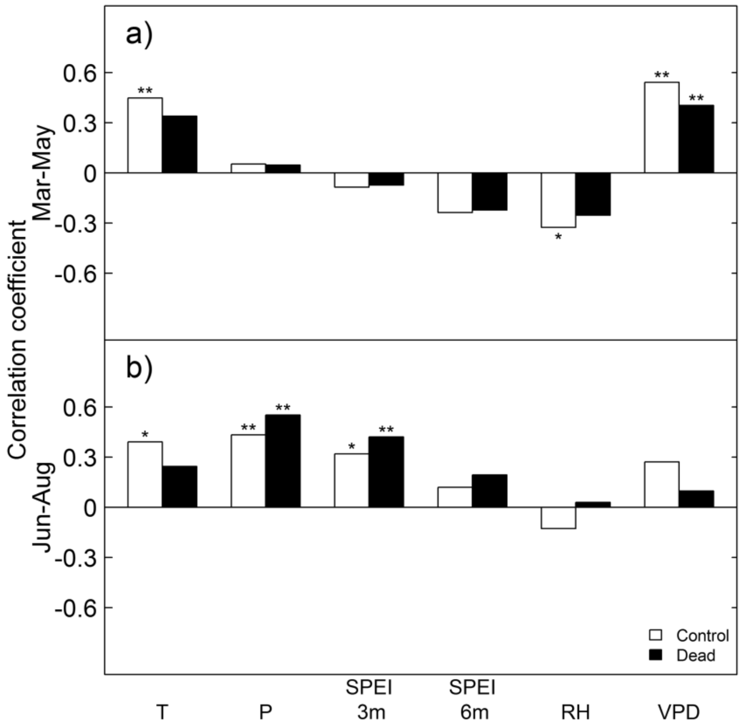

Figure 6. Pearson's correlation coefficients between δ^{18}O chronologies and climate variables (T – mean temperature, P – precipitation amount, SPEI – standardized precipitation-evapotranspiration index, RH – relative humidity, VPD – vapour pressure deficit) for the period of 1960-2003 for spring (March-May; a) and summer (June to August; b) White bars refer to control trees and black bars to dead trees. Significant correlations are marked by * (P < 0.05) and ** (P < 0.01).

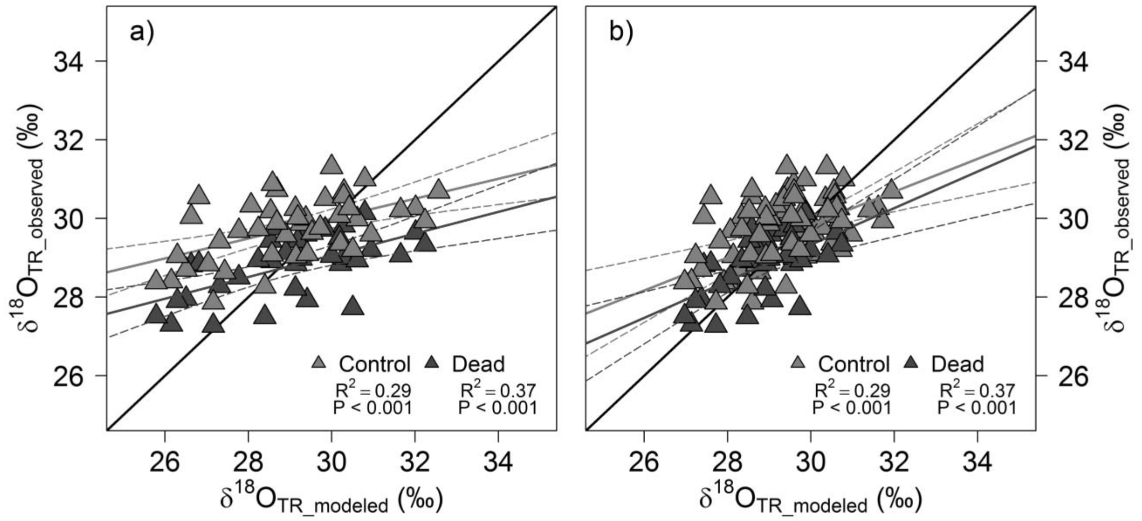

Figure 7. Relationship between observed and predicted tree-ring cellulose δ^{18}O values using the basic model (Eq. 7, a) and the modified model (Eq. 9) with the two-pool correction (b). The dashed lines indicate 95% confidence intervals.

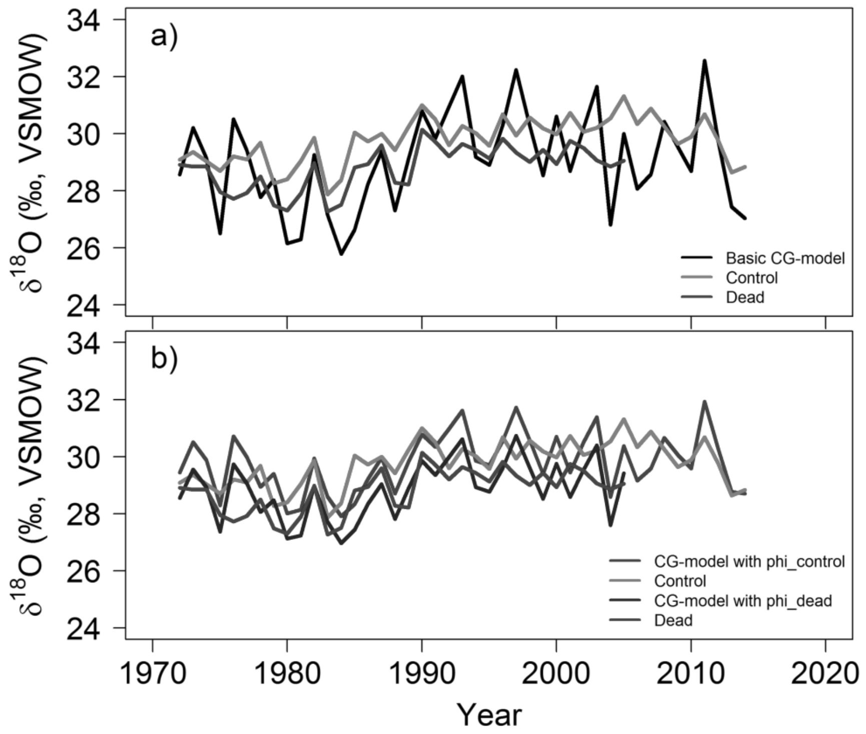

Figure 8. Time series of observed (control/now-dead) and predicted tree-ring cellulose δ^{18}O values using the basic CG-model (Eq. 7, a) and the modified CG-model (Eq. 9) with the two-pool correction (b).

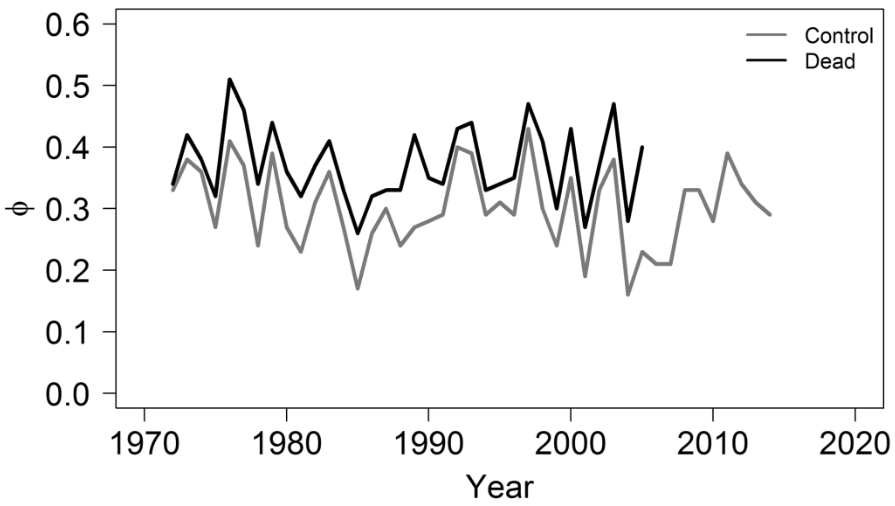

Figure 9. Modeled ϕ values representing the fraction of xylem water to total needle water using the modified tree-ring fractionation model (Eq. 9).

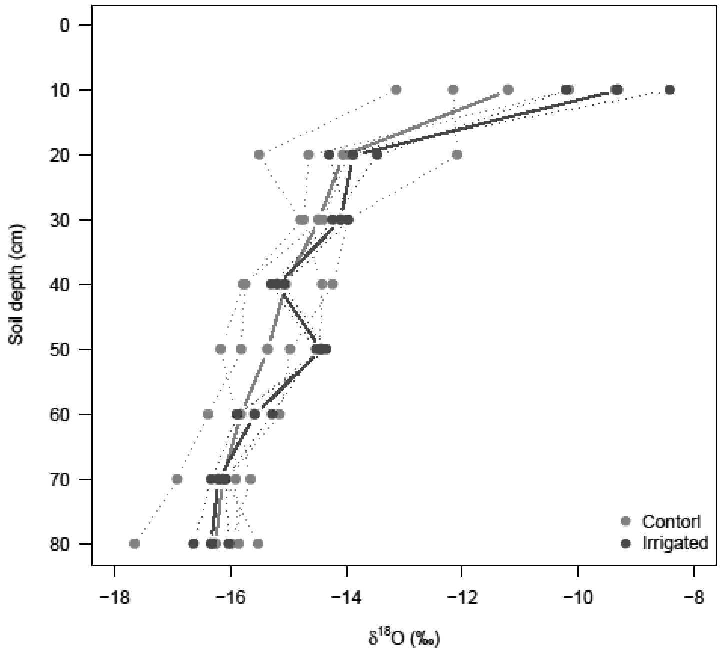

Figure S1. Individual (dotted lines, 4 sampling locations) and mean (solid lines, 2 sampling locations) δ^{18}O values of soil water with depth for control and irrigated plots sampled on 09.04.2014 (before irrigation started).

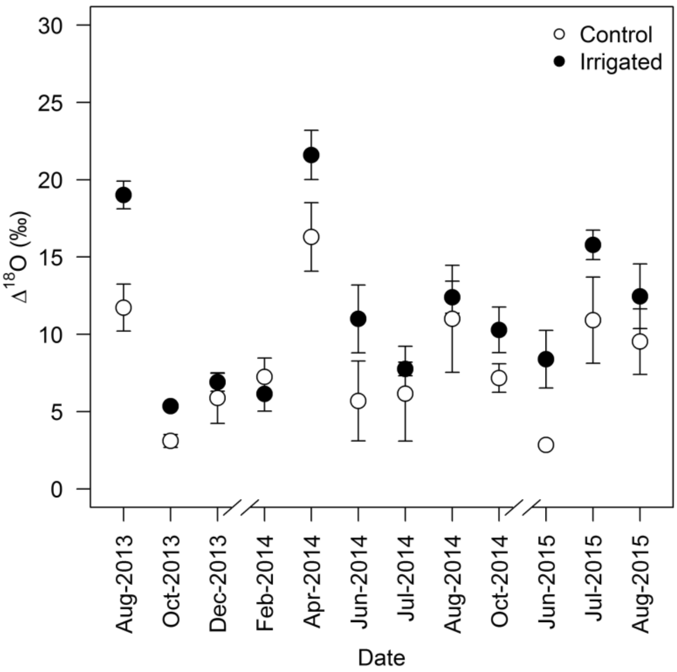

Figure S2. Seasonal changes in needle water enrichment in Pfynwald, calculated using as source water the soil water from depth 0-10 cm.

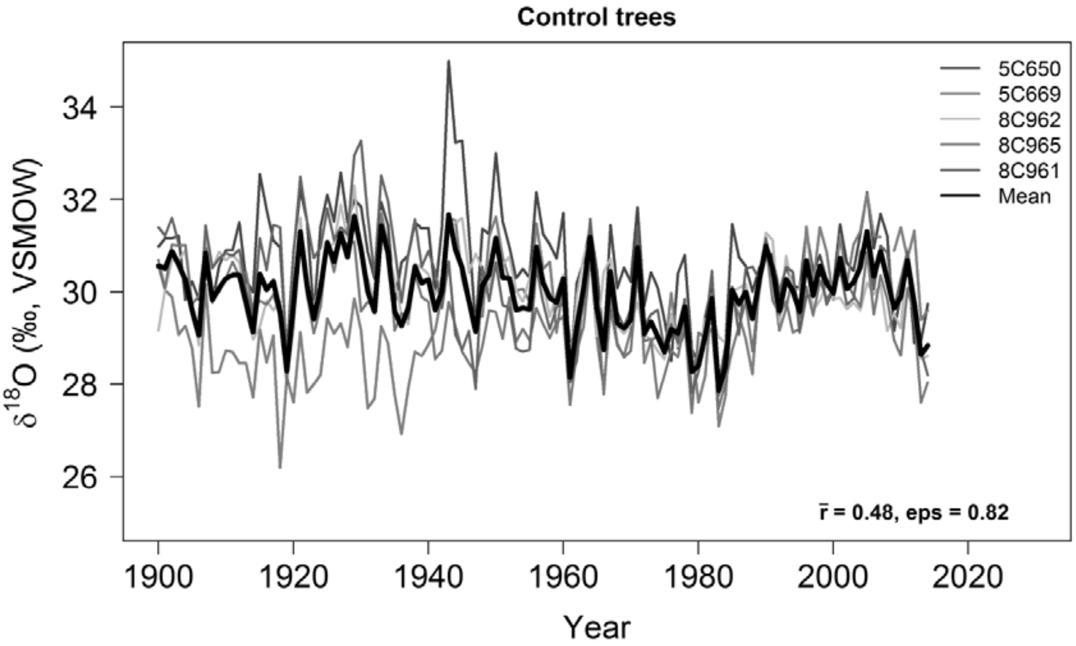

Figure S3. Individual series (coloured lines) and mean curves of tree-ring δ^{18}O of control trees.

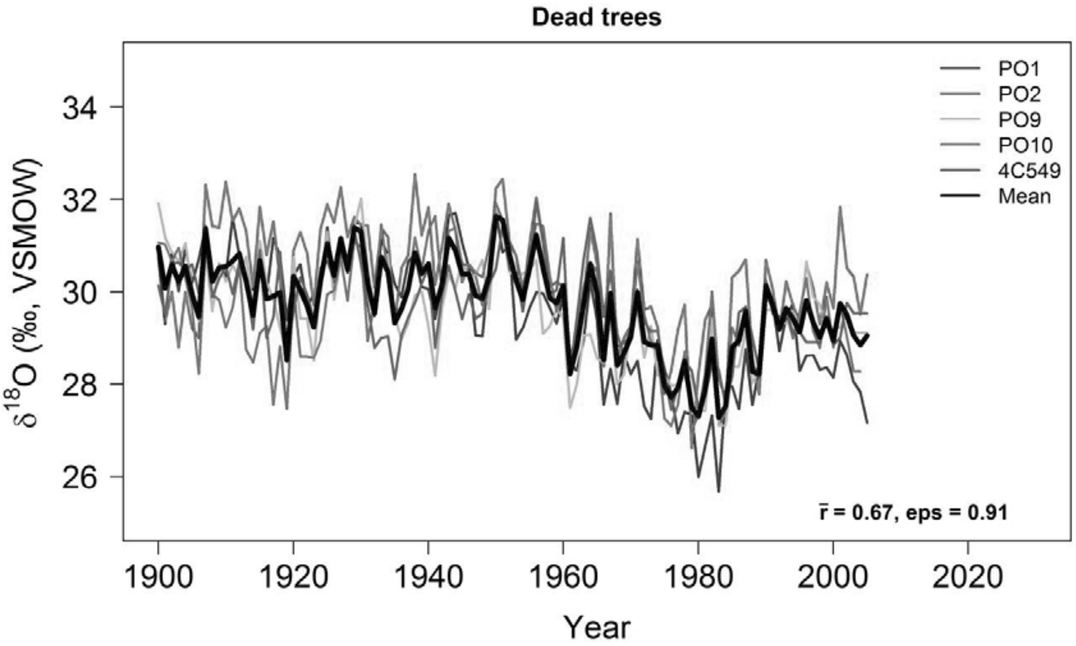

Figure S4. Individual series (coloured lines) and mean curves of tree-ring δ^{18}O of now-dead trees.

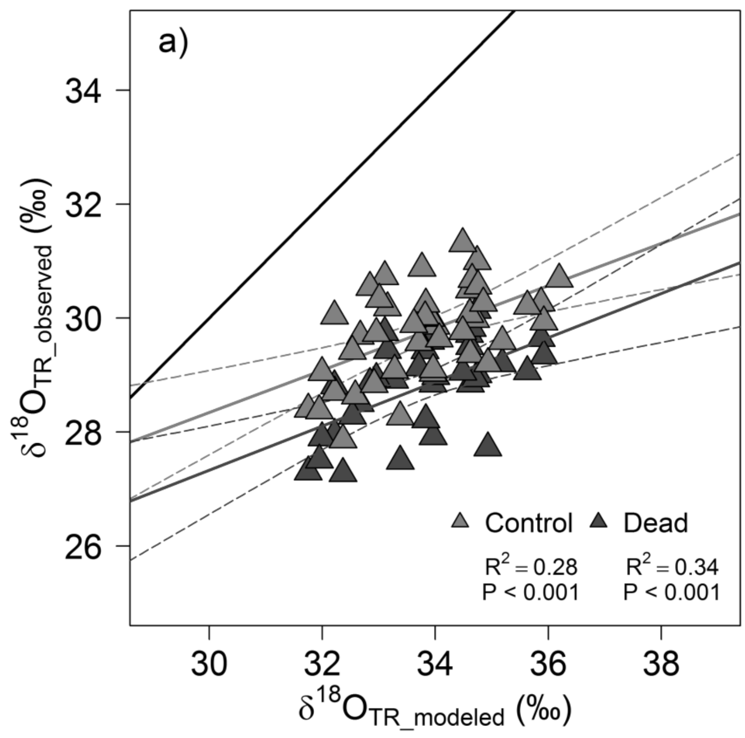

Figure S5. Relationship between observed and predicted tree-ring cellulose δ^{18}O using the model according to equation 8. The dashed lines indicate 95% confidence intervals.

Chapter 3

3. Influence of increasing air and soil temperatures on Scots pine from Central Yakutia

Galina Timofeeva[1,2,3], Harald Bugmann[2], Stefan Klesse[3,4], Rolf Siegwolf[1,3], Kerstin Treydte[3], Olga V. Churakova (Sidorova)[5,6], Yuri Skachkov[7] and Matthias Saurer[1,3]

[1]Laboratory of Atmospheric Chemistry, Paul Scherrer Institute (PSI), Villigen 5232, Switzerland

[2]Institute of Terrestrial Ecosystems, ETH Zurich, Zurich 8092, Switzerland

[3]Swiss Federal Institute for Forest, Snow and Landscape Research, (WSL), Birmensdorf 8903, Switzerland

[4]Laboratory of Tree-Ring Research, The University of Arizona, Bryant Bannister Tree-Ring Building, 1215 E. Lowell Street, Tucson, AZ 85721-0045, USA

[5]Institute for Environmental Sciences, University of Geneva, Climatic Change and Climate Impacts, 66 Boulevard Carl Vogt, CH-1205 Geneva, Switzerland

[6]Siberian Federal University, Institute Ecology and Geography, Laboratory of Ecosystem Biogeochemisty, Krasnoyarsk, Russia

[7]Melnikov Permafrost Institute, 36 Merzlotnaya Street, 677010 Yakutsk, Russia

Manuscript in preparation.

Abstract

Boreal forests are found at latitudes where the effects of climate change are expected to be particularly pronounced. Increasing temperatures and changing soil conditions could negatively impact these forests. Moreover, many of these forests grow on permafrost, making the hydrological cycle in such ecosystems quite complex. Therefore, it is important to investigate the response of boreal forests to current changes in climate as well as to estimate their adaptation potential to future climate change. Here, we investigated radial growth and physiology of Scots pine (*Pinus sylvestris* L.) in an extremely dry environment at the eastern edge of its continuous range in Central Yakutia (North-Eastern Siberia, Russia). We established a multi-proxy chronology using tree-ring width, carbon and oxygen isotope measurements over the last century for the first time for this area. We found a strong increase in air and soil temperatures in the last three decades that may have resulted in permafrost thawing and drying of the upper soil levels due to increased evaporation. We did not observe pronounced changes in stem radial growth, but the $\delta^{13}C$–climate relationship indicated a recently increased sensitivity to drought conditions and strong stomatal response. We further found an unusual negative relationship between soil temperature (at 20-120 cm depth) and $\delta^{18}O$ values in tree-rings, indicating an important influence of thawed permafrost water on the physiology of these trees in recent decades. Together with the changing relationship between $\delta^{13}C$ and $\delta^{18}O$, our results indicate increasing drought stress and a potential depletion of permafrost water availability in the upper soil in the study area.

Keywords: boreal forests, drought, permafrost, Svots pine, Siberia.

Introduction

Changes in global temperature, precipitation patterns and atmospheric moisture demand have been observed now for several decades and shown to be unusual in the context of natural variability (IPCC, 2013). It has also been observed that recent increases in temperature are faster and greater at high northern latitudes (Crespin *et al.*, 2013, Keisling *et al.*, 2017), a region comprising ca. 30% of the world's forest cover. Global climate models predicted that future redistributions of boreal forest could initiate important climate feedbacks (for instance, changes in land surface albedo) with a possible extension to lower latitudes, particularly under drastically increasing air and soil temperatures (Bonan *et al.*, 1992). It is therefore important to assess ongoing changes in growth and physiology of trees in this region to understand how much they are already affected by these climatic changes. An additional factor to be considered is that in many regions boreal forests are growing on continuous or discontinuous permafrost soils, enhancing complexity of the hydrological cycle compared to temperate forests or tropics (Shvidenko & Apps, 2006). It has already been observed that permafrost is melting under current climate change and more dramatic thawing is expected in the future (Demek, 1994, Romanovsky *et al.*, 2002, Koven *et al.*, 2013, Slater & Lawrence, 2013, Chadburn *et al.*, 2017). This could eventually lead to drying out of the upper soil as the melted water will evaporate, which reduces an important water source for tree growth. All above-mentioned issues will most likely affect entire ecosystems in these regions, including both plants and animals, and therefore have an impact on the global carbon cycle (Oechel & Vourlitis, 1997, Burke *et al.*, 2012, MacDougall *et al.*, 2012, Schneider von Deimling *et al.*, 2012, Kevin *et al.*, 2014, Desyatkin *et al.*, 2015, Schuur *et al.*, 2015, Guido *et al.*, 2016, Chasmer & Hopkinson, 2017).

Recently, tree decline has been reported for many regions and responses of various species to climatic changes, particularly to drought conditions, caught a lot of attention and have been highly investigated in different parts of the globe (Bigler *et al.*, 2006, Allen *et al.*, 2010, Galiano *et al.*, 2010, Rigling *et al.*, 2013, Vilà-Cabrera *et al.*, 2013, Voltas *et al.*, 2013, Hereş *et al.*, 2014, Allen *et al.*, 2015, Benavides *et al.*, 2015, Churakova (Sidorova) *et al.*, 2016a, Timofeeva *et al.*, 2017). Effects of climate change are expected to be particularly severe at treelines and edges of species distribution, where growing conditions are harsh (Gear & Huntley, 1991, MacDonald *et al.*, 1993, Grace *et al.*, 2002, Wilmking *et al.*, 2004, Gehrig-Fasel *et al.*, 2007, Harsch *et al.*, 2009, Sidorova *et al.*, 2010, Churakova (Sidorova) *et al.*, 2016b, Miller *et al.*, 2017). Therefore, strong effects of climate change and drought conditions on forests in regions like Central Yakutia (North-Eastern Siberia, Russia) are expected. This area is not only located on continuous permafrost, but also known for its extremely continental climate and low annual precipitation. In general, such regions are often poorly monitored and therefore the effects of climate change on tree-growth still not well understood (Nikolaev *et al.*, 2011a). Nevertheless, noticeable increases in air temperature, affecting permafrost have already been reported for this area (Demek, 1994, Balobayev *et al.*, 2009, Iijima *et al.*, 2010).

The combination of tree-ring growth measurements with stable isotopes of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) in tree rings is a powerful method for revealing tree physiological responses to climate change and droughts conditions. Variations of $\delta^{13}C$ values in plant organic matter provide valuable information on understanding how environmental conditions affect plant functions, such as photosynthetic activity, stomatal conductance and water-use efficiency (Farquhar *et al.*, 1989), whereas variations in $\delta^{18}O$ values are typically related to the isotopic composition of the source water, which is associated with precipitation, soil and groundwater (Dansgaard, 1964). Furthermore, variations in $\delta^{18}O$ are affected by transpiration and leaf water enrichment, particularly under drought conditions (Craig & Gordon, 1965, Farquhar & Lloyd, 1993).

In this study, we analyzed recent trends in air and soil temperatures in Central Yakutia and investigated the impact of these changes on radial stem growth and physiology of Scots pines (*Pinus sylvestris* L.) from the extremely dry eastern edge of its distribution range in Central Yakutia. While there are already tree-ring width (TRW) chronologies for Scots pine available for the region (Nikolaev *et al.*, 2009, Nikolaev *et al.*, 2011a, Nikolaev *et al.*, 2011b), we established for the first time a multi-parameter chronology based on measurements of TRW, $\delta^{13}C$ and $\delta^{18}O$ values in tree-ring cellulose for the period of 1900-2013. In combination with the local climate station data, the chronologies were used to answer the following questions:

- How did air and soil temperatures change in the region during recent decades?
- Is there any growth response of Scots pine related to these changes?
- How did recent permafrost thawing affect radial stem growth and physiology of these trees?

Data and Methods

Study site

The study site is a Scots pine forest located in the area of the Spasskaya Pad (SP) scientific forest station about 20 km from Yakutsk (Yakutia, Northeast Siberia, 62°14' N, 129°37' E; ~ 220 m a.s.l). The station is located on continuous permafrost with drained sandy soils (Iijima *et al.*, 2010, Tei *et al.*, 2013b, Iijima *et al.*, 2014, Tei *et al.*, 2014). The boreal light taiga is the major ecosystem in this area dominated by larch (*Laricetum vacciniosum*), but also with some pine (*Pinetum arctostaphylosum*), birch (*Betuletum mixtoherbosum*) and mixed forests as well as meadows and thermokarst formations (so-called alaases).

Sampling of increment cores and tree-ring measurements

Twenty five Scots pine individuals were randomly sampled in the pine forest next to the SP station, irrespective of their ages and diameters at breast height (DBH), end of July 2014, taking two to four increment cores using a 5 mm increment borer (Haglöf, Långsele, Sweden). The visibility of the tree-ring boundaries was improved by cutting the surface with a core-microtome (Gärtner & Nievergelt, 2010). Tree-ring width (TRW) was measured with a precision of 0.01 mm at the Tree-Ring Laboratory of the Swiss Federal Institute for Forest, Landscape and Snow Research (WSL Birmensdorf, Switzerland), using a Lintab system connected to the TSAP-Win software V.3.5 (Rinntech, Heidelberg, Germany). All measured individual tree-ring series were first cross-dated visually with the existing master chronology from the study site (Nikolaev *et al.*, 2009, Nikolaev *et al.*, 2011a, Nikolaev *et al.*, 2011b) and validated, using the software COFECHA (Grissino-Mayer, 2001). The final chronology was established by averaging the cross-dated individual tree-ring series for the period 1900-2013.

Carbon and oxygen isotope measurements

Five trees were selected based on their synchronicity with the mean TRW chronology for further analyses of δ^{13}C and δ^{18}O values. First, tree rings were carefully separated with a surgical scalpel under a Wild M8 stereo microscope. Next, cellulose from the individual tree-rings was extracted and homogenized according to established protocols and homogenization procedures (for more detailed information see (Boettger *et al.*, 2007, Laumer *et al.*, 2009, Roden *et al.*, 2009). Subsequently, aliquots were packed into silver capsules to be combusted to CO by a pyrolysis method at 1420 °C in a thermal-conversion elemental analyzer (PYRO-cube, Elementar, Hanau, Germany) connected to an Isotope Ratio Mass Spectrometry (IRMS, Delta Plus XP) via a Conflo III interface (Thermo Fischer Scientific, Bremen, Germany). The main advantage of this system is that stable isotope ratios of both δ^{13}C and δ^{18}O can be measured simultaneously, which noticeably reduces the required sample amount and measurement time. Nevertheless, correction of obtained values is necessary, owing to a dilution of the sample gas by a small amount of carbon from the reactor that might lead to a dampening of the isotopic signal. To solve this issue, a small subset of the samples was additionally measured with a regular combustion method for the analysis of carbon isotopes in plant organic matter. With this method the whole range of δ^{13}C values was measured to establish a linear curve for the correction of the values measured by pyrolysis (Woodley *et al.*, 2012, Weigt *et al.*, 2015). Carbon isotope values were reported in the standard δ-notation as per mil (‰) difference from the Vienna Pee Dee Belemnite (VPDB) with an analytical precision < 0.2 ‰, whereas oxygen isotope values as per mil (‰) difference from the Vienna Standard Mean Ocean Water (VSMOW) with an analytical precision < 0.3 ‰. To account for the Suess effect, i.e. changing δ^{13}C of atmospheric CO_2, tree-ring δ^{13}C values of all five trees were corrected by subtracting the offset between actual and pre-industrial δ^{13}C values before establishing the final δ^{13}C chronology (McCarroll & Loader, 2004, Treydte *et al.*, 2009).

Climate data

We used data of mean monthly temperature averages and precipitation sums from the gridded time-series dataset CRU TS v. 3.24 of Climate Research Unit (CRU, 0.5° resolution, (Harris *et al.*, 2014) over the 1902-2013 period for the grid cell of 62°14' N, 129°37' E. We performed Pearson's correlation analysis starting from the year 1902 as we needed climate data of the previous December for winter. Measured soil temperature data at depths of 20 cm and below from 1985 to 2011 at the monitoring station in Pokrovsk (ca. 90 km away from Yakutsk) were additionally available for the correlation analysis. These data were obtained from the All-Russian Research Institute of Hydrometeorological Information - World Data Center (RIHMI – WDC, http://meteo.ru/).

Yakutsk is known as the coldest inhabited city on Earth with a mean annual temperature of -8.8 °C, and the area is also very dry with an annual precipitation amount of only 238 mm year^{-1} (Fig. 1, 1971-2000). According to the Köppen classification, climate of this area is defined as extreme subarctic with annual temperature amplitude reaching up to ca. 100 ºC. Mean monthly temperatures over the period of 1971-2000 range from -38.6 °C in January to 19.5 °C in July, whereas mean precipitation amounts range from 7 mm in March to 39 mm in July (Fig. 1). Precipitation maximum in summer, but its amount is still low. This is because of the prevailing southeasterly winds from the Pacific Ocean lose their moisture over the coastal mountains before they reach the area of the Lena river basin. In winter there is hardly any precipitation because of the formation of the so-called Siberian High, an intense accumulation of very cold dry air, resulting in light snow cover (Cohen *et al.*, 2001, Gong & Ho, 2002).

Data analysis

To quantify synchronicity and the strength of common variation within a chronology, the average inter-series correlation coefficients between individual series of each tree-ring parameter (rbar) and the expressed population signal (EPS) were calculated. An EPS of ≥ 0.85 usually indicates a sufficiently strong common growth signal among the trees to be representative for the site (Wigley *et al.*, 1984).

We determined the response of increment growth as well as tree-ring $\delta^{13}C$ and $\delta^{18}O$ values to climate, calculating Pearson's correlation coefficients for seasonal (winter: from previous December to February current year; spring: March to May; summer: June to August; fall: September to November) and annual averages of temperature and sums of precipitation. We tested the significance of the correlation coefficients by applying two-tailed Student's t-tests. To test the temporal stability of the climate–growth and isotope relationships, 30-year moving correlations between the tree-ring parameters and climate variables were also calculated for the 1902–2013 period.

Results

Climate data trends

Mean annual temperature increased significantly over the full study period of 1902-2013 (Fig. 2a, slope = 0.02 ºC year^{-1}, $R^2 = 0.24$, $P < 0.0001$, $n = 112$). However, most of the increase occurred over the period of 1984-2013 (slope = 0.07 °C year^{-1}, $R^2 = 0.39$, $P < 0.001$, $n = 30$), while there was no significant change for the period of 1902-1983 (slope = $- 1.61 \cdot 10^{-3}$ °C year^{-1}, $R^2 = 1.65 \cdot 10^{-3}$, $P > 0.05$, $n = 82$). Annual precipitation amount also increased significantly over the period of 1902-2013 (Fig. 2b, slope = 0.45 mm year^{-1}, R^2 = 0.07, $P > 0.05$, n = 112), mainly due to an increase at the beginning of the 20th century (significant over the period of 1902-1983: slope = 0.81 mm year^{-1}, R^2 = 0.11, $P < 0.01$, n = 82), while there was no significant increase over the recent period of 1984-2013 (slope = 0.80 mm year^{-1}, R^2 = 0.02, $P > 0.05$, n = 30).

The mean temperature trends were strongly seasonally dependent. Significant changes over the period of 1902-2013 occurred during winter (Fig. 3a, from previous December to February, slope = $3.29 \cdot 10^{-2}$ °C year^{-1}, R^2 = 0.19, $P < 0.0001$, n = 112), spring (Fig. 3b, from March to May, slope = 0.03 °C year^{-1}, R^2 = 0.30, $P < 0.0001$, n = 112) and summer (Fig. 3c, from June to August, slope = 0.01 °C year^{-1}, R^2 = 0.06, $P < 0.05$, n = 112). However, over the recent period of 1984-2013 significant changes occurred mainly during the spring (Fig. 3b, slope = 0.08 °C year^{-1}, R^2 = 0.21, $P < 0.05$, n = 30), summer (Fig. 3c, slope = 0.06 °C year^{-1}, R^2 = 0.27, $P < 0.01$, n = 30) and fall (Fig. 3d, from September to November, slope = 0.08 °C year^{-1}, R^2 = 0.22, $P < 0.01$, n = 30). Interestingly, there were no strong trends, although they were significant for spring and fall, over the period of 1902-1983 (winter: slope = $2.62 \cdot 10^{-3}$ °C year^{-1}, R^2 = $6.4 \cdot^{-4}$, $P > 0.05$, n = 82; spring: slope = 0.01 °C year^{-1}, R^2 = 0.05, $P < 0.05$, n = 82; summer: slope = - $2.65 \cdot 10^{-3}$ °C year^{-1}, R^2 = $4.48 \cdot 10^{-3}$, $P > 0.05$, n = 82; fall: slope = - 0.02 °C year^{-1}, R^2 = 0.08, $P < 0.05$, n = 82).

The mean annual soil temperatures at various depths, except at a depth of 120 cm, had significantly increased for the data available period of 1985-2011 with the strongest warming at a depth of 40 cm (Fig. 4, 20 cm: slope = 0.07 °C year^{-1}, R^2 = 0.22, $P < 0.05$, n = 27; 40 cm: slope = 0.09 °C year^{-1}, R^2 = 0.44, $P < 0.001$; 120 cm: slope = 0.03 °C year^{-1}, R^2 = 0.14, $P > 0.05$; 160 cm: slope = 0.04 °C year^{-1}, R^2 = 0.17, $P < 0.05$).

Tree-ring width, $\delta^{13}C$ and $\delta^{18}O$ chronologies

Stem growth expressed as tree-ring width (TRW) was generally very low for the period 1902-2013 (mean ± SD: 0.32 ± 0.12 mm). Raw individual TRW ranged from a minimum of 0.06 mm to a maximum of 0.87 mm for the same period. Individual trees shared a strong common variance (Fig. 5a, rbar = 0.54; EPS = 0.85, n = 25). The common variance between tree-ring widths of the five trees selected for the isotope analysis was comparable (rbar = 0.57; EPS = 0.87, n = 5). Carbon isotope values in tree rings showed an even stronger among-tree correlation (rbar = 0.75; EPS = 0.94, n = 5), with $\delta^{13}C$ values ranging from the minimum of -

24.31‰ to the maximum of -20.54‰ (Fig. 5b). Similarly, the oxygen isotope series were highly correlated among trees (rbar = 0.81; EPS = 0.96, n = 5) with δ^{18}O values ranging from the minimum of 19.93‰ to the maximum of 27.18‰ (Fig. 5c).

Moving correlations calculated for the 30-year windows between the individual tree-ring parameters had quite similar decadal fluctuations, but on different levels (Fig. 6). We observed a negative relationship between radial growth and tree-ring δ^{13}C values, and a positive relationship between growth and tree-ring δ^{18}O values, whereas δ^{13}C and δ^{18}O records showed a switch from positive to negative correlations after the mid-1960s, although rarely reaching a significant level (Fig. 6). The overall correlations between chronologies were neither strong nor significant (δ^{13}C *vs*. TRW: rbar = -0.25; δ^{13}C *vs*. δ^{18}O: rbar = 0.12, δ^{18}O *vs*. TRW: rbar = 0.30, n = 112).

Climate correlations

The strongest significant and negative correlation was found between radial stem growth and winter, spring, summer and annual mean temperatures (Fig. 7a), when considering the full period 1902-2013. The values of δ^{13}C in tree rings had a significantly positive correlation only with mean summer temperature (Fig. 7a), whereas oxygen isotope values were significantly and positively correlated with mean spring temperature in (Fig. 7a) and annual precipitation amount (Fig. 7b).

However, correlation coefficients remarkably changed for the analysis over the recent period 1984-2013. Radial growth positively correlated with spring precipitation (Fig. 8b). The values of δ^{13}C were highly correlated with mean summer temperature (Fig. 8a), as well as significantly negative with summer and annual precipitation sums (Fig. 8b). Significant and positive correlations for tree-ring δ^{18}O values were observed with both climate variables in spring (Fig. 8), although the absolute correlation coefficients were not very high.

Soil temperature and tree-ring widths were generally negatively correlated at soil depths of 20 and 40 cm (Fig. 9). Correlations were strongest for spring and summer at 20 cm soil depth (Fig. 9a), and for winter, spring and annual soil temperatures at 40 cm soil depth (Fig. 9b). Below a depth of 40 cm the relationships between soil temperature and tree-ring variables diminished. Tree-ring δ^{13}C values were found to be related mainly to winter and summer temperatures at 20 cm depth (Fig. 9a). We found a remarkable negative correlation between tree-ring $\delta^{18}O$ values and mean summer soil temperature at the two upper soil depths, 20 cm and 40 cm (Fig. 9).

Discussion

Changes in air and soil temperatures and their effects on permafrost in Central Yakutia

Our trend analysis of the climate data showed that a strong increase in air temperature started after the mid-1980s. This increase was strongly seasonal with most of the significant changes in spring and summer in the last three decades, whereas there was no significant trend in precipitation during this period. As precipitation is already extremely low in the region, the increase in temperature and concurrent higher atmospheric moisture demand are likely causing increased drought stress for the vegetation. Climate change induced increases in drought frequency, duration and intensity are in line with observations around the globe and are confirmed by regional and global climate models (Dai, 2013, IPCC, 2013, Keller *et al.*, 2017).

Furthermore, warming concurrent with no significant change in precipitation has influenced the soil conditions at our site, in particular the soil temperatures at various depths, resulting in warming of the ground. Unfortunately, there were no soil temperature data available prior to the mid-1980s at our study site. Observations of soil temperature are in general very limited in space and time in remote regions as our study site. Based on the observed warming, it is likely that permafrost thawing and deepening of the active layer has occurred at our site, as has been previously reported and is predicted to continue in eastern Siberia (Iijima *et al.*, 2010, Koven *et al.*, 2013, Slater & Lawrence, 2013, Hotaek *et al.*, 2014, Churakova (Sidorova) *et al.*, 2016b). Changing permafrost conditions interact with local landscapes and ecosystems, resulting in disturbances of topography, hydrology as well as the biogeochemical cycle (Guido *et al.*, 2016, Walvoord & Kurylyk, 2016). First indications of thawing permafrost effects on local landscapes and ecosystems have been detected in Central Yakutia. According to (Fyodorov-Davydov *et al.*, 2009, Fedorov *et al.*, 2014b, Desyatkin *et al.*, 2015), activated cryogenic processes (seasonal thaw deepening and shallower freezing), primarily caused by increasing air and soil temperatures, have led to shifts of many plants and animals ranges northward (Desyatkin *et al.*, 2015). The regular thawing of permafrost during summer is a crucial water source for plants in this arid region. Therefore, the depletion and future absence of this water pool due to increased evaporation could have serious consequences. Shifts in the distribution of local ecosystems, occurring quite rapidly in response to changing permafrost, are most likely expected to happen in the future and possible consequences have not been well investigated yet (Fyodorov-Davydov *et al.*, 2009, Fedorov *et al.*, 2014b, Desyatkin *et al.*, 2015). Moreover, degradation of the ice complex has intensified subsidence of ground surface in the recent decades, resulting in development of thermokarst lakes in the area of our study site (Fedorov *et al.*, 2014a, Fedorov *et al.*, 2014b). Evolution of such lakes is still poorly understood and requires more investigation as well (Tarasenko, 2013, Séjourné *et al.*, 2015). Therefore, studying plant responses to the above-mentioned changes in climate is extremely relevant, particularly at such dry sites as our study site.

Climate change effects on growth of Scots pines in Central Yakutia

Stem growth over the entire study period of more than one century did not show remarkable changes for Scots pine at our site. The growth was always quite low, but without any drastic decline in response to recent climate change as it has been observed for the same species in other xeric environments (Bigler *et al.*, 2006, Galiano *et al.*, 2010, Poyatos *et al.*, 2013, Rigling *et al.*, 2013, Vilà-Cabrera *et al.*, 2013, Voltas *et al.*, 2013, Hereş *et al.*, 2014, Timofeeva *et al.*, 2017). In the long-term, our statistical analysis showed that growth variations were mainly driven by air temperatures. Interestingly, not only summer, but also spring and winter mean air temperatures had a negative effect on radial growth of Scots pines. For summer temperature, this could be an indirect effect of atmospheric drought stress through increased vapor pressure deficit. Additionally, the negative relationship between growth and mean summer temperature has been associated to strong soil moisture deficit caused by increased soil evaporation during the warmest part of the growing season (Nikolaev *et al.*, 2009, Nikolaev *et al.*, 2011a).

However, trees growing at higher latitudes are expected to benefit from warming in winter and spring. Therefore, the negative influence of winter and spring warming on radial growth of Scots pines at our site is rather unexpected, but quite a similar response was also observed with Alaskan white spruce (*Picea glauca*). There was a decline in the positive relationship between temperature and stem radial growth in the late 20th-century, particularly at the forest-tundra margin across the Northern Hemisphere, most likely because of drought (Barber *et al.*, 2000). In fact, the temperature regime in the earlier part of the growing season (typically from April to May) is very important for cell production, and hence for growth, when an abrupt transition from negative to positive temperatures occurs (Nikolaev *et al.*, 2009, Nikolaev *et al.*, 2011a, Nikolaev *et al.*, 2011b). The climate correlation analysis above, however, was based on the full study period, but changes in the climate-growth relationship were observed when focusing on the last three decades. The negative relationship between radial growth and air temperature was not significant anymore, while spring precipitation became more important and positively affected growth of Scots pines. This indicates that snowmelt water in early spring is indeed important and became more and more important in recent decades as a water source to sustain growth.

As discussed above, rapid increases in air and soil temperatures at this site will most likely intensify soil evaporation processes, particularly in dry summers, leading to a reduction of soil moisture. However, as no clear growth decline was observed, it seems that the existence of permafrost at our site currently still helps trees to continue growing, providing them source water from thawing, although the upper soil depth may desiccate in the future if warming continues or even accelerates. On the contrary, larch trees (*Larix cajanderi* Mayr), growing on loamy soils few kilometers away from our site, showed indeed signs of decline (yellowing and browning of needles) during the 2007 growing season (Iwasaki *et al.*, 2010). The authors suggested that the decline could have been caused by root anoxia as an effect of waterlogging (Perata *et al.*, 2011, Kreuzwieser & Rennenberg, 2014), as the soil was extremely moist, due to stagnating water from permafrost thawing, particularly after two previous enormously wet years (Iwasaki *et al.*, 2010). This implies that excess soil water availability might also have a detrimental effect on tree growth depending on local site conditions, such as soil type, depth of active permafrost layer and probably tree species. Growth decline under drought can therefore be expected to be spatially very heterogeneous in this region.

Climate change effects on physiology of Scots pines in Central Yakutia

We used the analysis of stable isotope ratios to infer physiological changes of the studied Scots pine trees. In the long-term, tree-ring δ^{13}C values at our site were positively affected by summer temperatures only. This often indicates a typical stomatal response to drought conditions. Trees and plants, in general, tend to close their stomata to avoid water loss under drought stress, resulting in less discrimination of heavier carbon isotopes against lighter ones during photosynthesis (Farquhar *et al.*, 1989, McCarroll & Loader, 2004). Similar responses were observed for larch in Central and Northeastern Yakutia (Kagawa *et al.*, 2003, Sidorova *et al.*, 2008, Sidorova *et al.*, 2010, Tei *et al.*, 2013a, Tei *et al.*, 2014, Churakova (Sidorova) *et al.*, 2016b), but also in other drought-prone regions for *Pinus sylvestris* (Voltas *et al.*, 2013, Timofeeva *et al.*, 2017). The observed drought effects became even stronger in the last three decades, when correlations between tree-ring $\delta^{13}C$ values and mean summer air temperature enhanced, as well as a negative relationship with summer precipitation was found. Moreover, there was also a significant positive effect of soil temperature at the upper soil depth on tree-ring $\delta^{13}C$ values in the last three decades. These are clear indications of an enhancing drought effect on tree physiological processes related to photosynthesis and stomatal conductance, where we assume that photosynthesis was rather reduced under drought, which is supported by the negative correlation between summer temperature and growth.

Stable oxygen isotope values in tree-rings at our site were mostly positively affected by mean spring air temperature in the long-term. This relationship intensified in the last three decades, as well as the significantly positive influence of spring mean soil temperature, particularly at a depth of 40 cm. Furthermore, there was also a significantly negative effect of spring precipitation amount in the last three decades at our site. Such responses are consistent with theoretical expectations and in agreement with experimental results from other sites, as higher temperature generally results in both higher $\delta^{18}O$ of precipitation (taken up by the tree roots) and leaf water enrichment, which is later detected in the isotopic signature of tree-ring cellulose (Dansgaard, 1964, Gessler *et al.*, 2009, Roden *et al.*, 2009, Voltas *et al.*, 2013, Gessler *et al.*, 2014). In contrast, mean soil temperature in summer was surprisingly negatively correlated with tree-ring $\delta^{18}O$ values. This may indicate that source water with more depleted $\delta^{18}O$ was taken up from lower depths during dry periods in summer, probably coming from permafrost thawing at depths below 40 cm under drought conditions. Previous studies have also used oxygen isotopes to demonstrate the importance of melted permafrost water, which is often a water source for trees in this area, particularly under low precipitation amounts and high atmospheric moisture demand in summer (Sugimoto *et al.*, 2002). Our results are further in agreement with a study for larch from northeastern Siberia (Churakova (Sidorova) *et al.*, 2016b) and Central Siberia (Saurer *et al.*, 2016). Both studies showed a significant influence of melting of deeper soil layers under drought, considering the influence of drought on soil $\delta^{18}O$ as an anomalous or inverse isotope effect, because warm and dry conditions are normally associated with the $\delta^{18}O$ enrichment (Dansgaard, 1964, Saurer *et al.*, 2016). We further observed a change in the relationship between $\delta^{13}C$ and $\delta^{18}O$ variations from positive to negative in recent decades. A positive correlation is usually found due to concurrent effects of dry conditions on both isotope ratios (Saurer *et al.*, 1997). The unusual shift in the relationship could be related to the inverse isotope effect noted above for $\delta^{18}O$. Our results thus indicate that the importance of melting permafrost for water supply of the trees in such regions is increasing. Currently, water from melting permafrost still keeps these trees sufficiently supplied, but with continued and increased evaporation, this source of water may be depleted at some point in the future, thereby leading to much stronger drought stress conditions for these trees.

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Figures

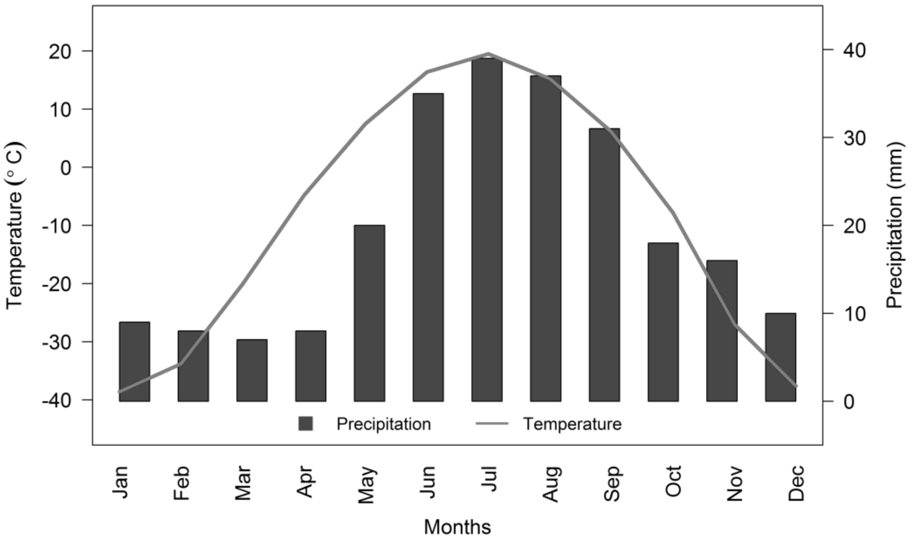

Figure 1. Climate diagram for Yakutsk over the period 1971-2000. Source: http://www.pogodaiklimat.ru/climate/24959.htm

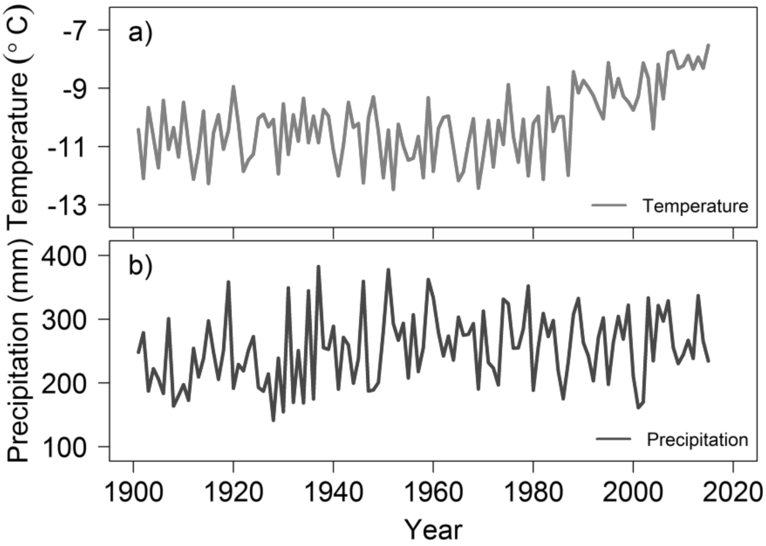

Figure 2. Annual meteorological variables for the CRU TS v. 3.24 grid cell of 62°14' N, 129°37' E: a) mean temperature and b) precipitation amount.

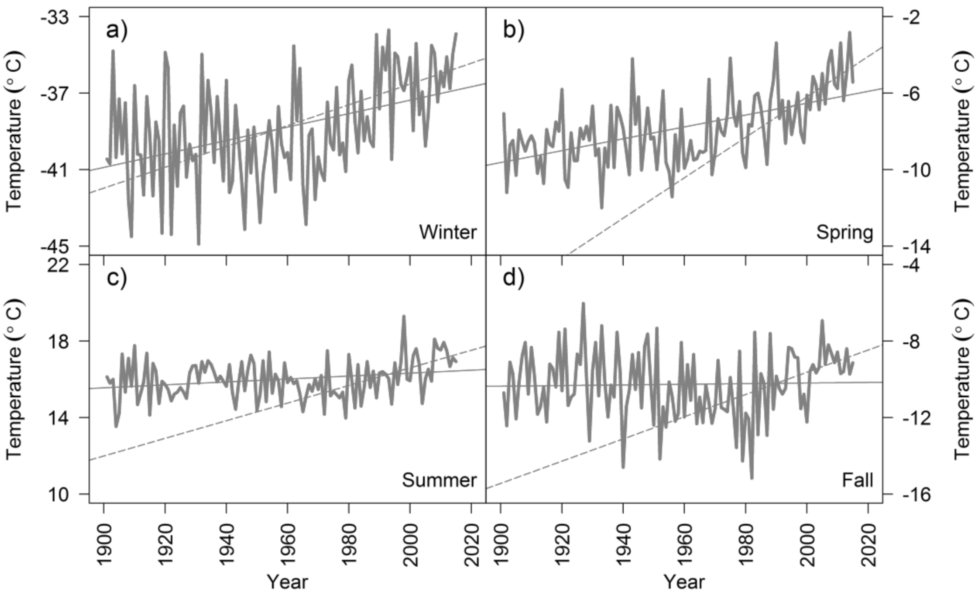

Figure 3. Seasonal mean temperatures for the CRU TS v. 3.24 grid cell of 62°14' N, 129°37' E: a) winter (previous December to February); b) spring (March to May); c) summer (June to August); d) fall (September to November). Temperature range on the y-axis is 12°C for all seasons. Solid lines indicate trends over the period of 1902-2013 and dashed lines indicate trends over the period of 1984-2013.

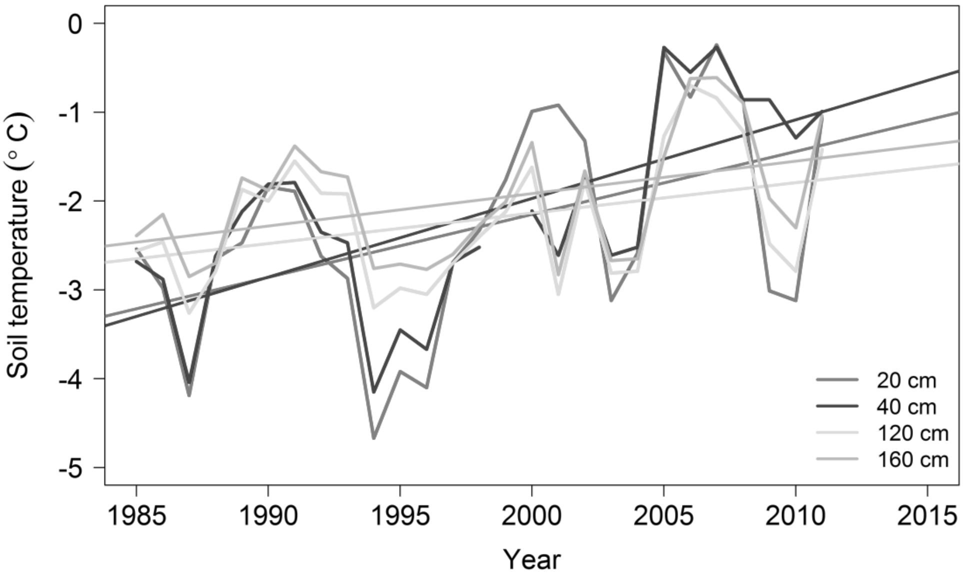

Figure 4. Time series of mean annual soil temperature at various depths observed at the monitoring station Pokrovsk (near Yakutsk) over the period of 1985-2011.

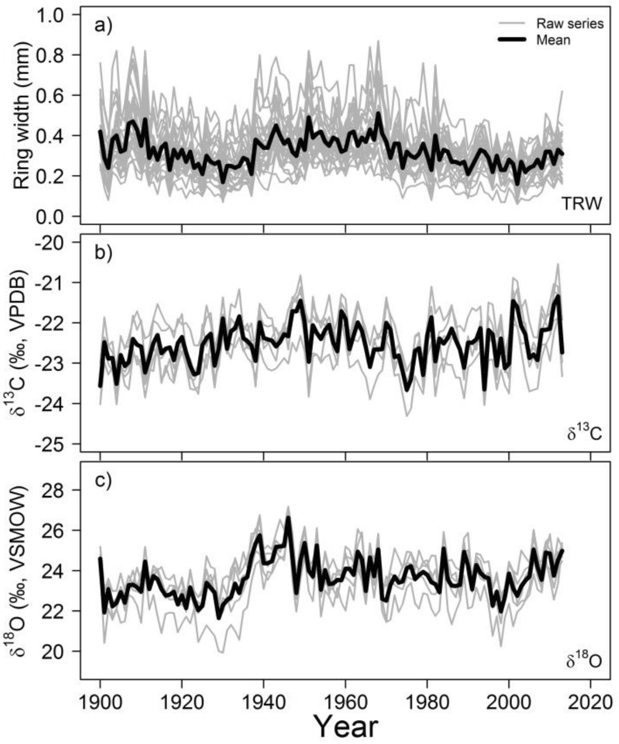

Figure 5. Individual series (gray lines) and mean curves: (a) tree-ring width, (b) $\delta^{13}C$ and (c) $\delta^{18}O$ values in tree rings.

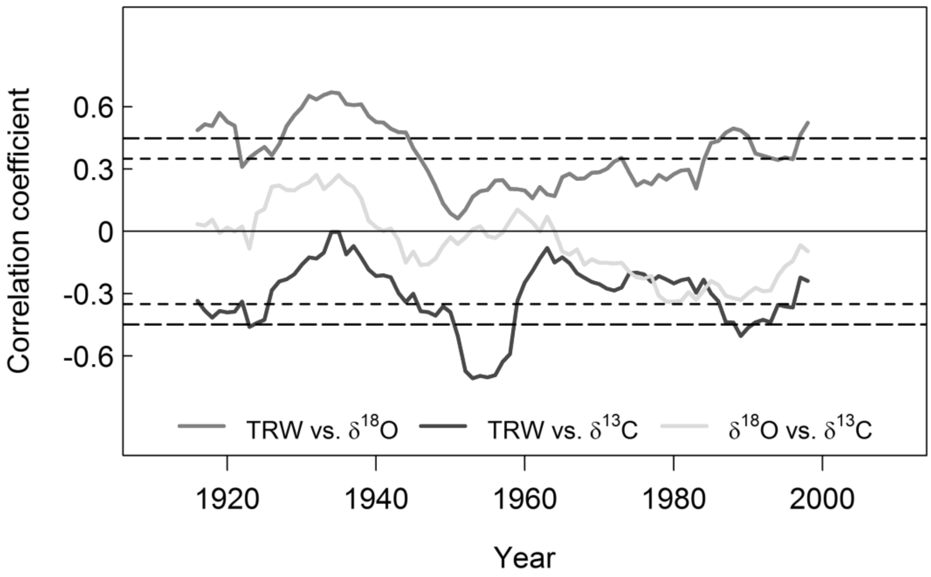

Figure 6. Thirty-year moving correlation coefficients between tree-ring parameters. The short-dashed line indicates the significance level at 95% and the long-dashed line at 99%.

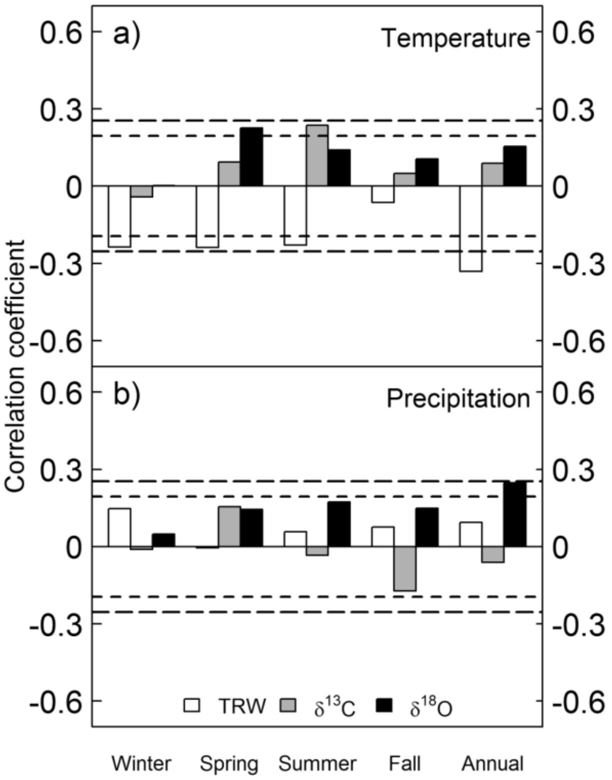

Figure 7. Pearson's correlation coefficients between tree-ring and climate variables for the period of 1902-2013 for various seasons and annual means for (a) temperature and (b) precipitation amount. The short-dashed line indicates the significance level at 95% and the long-dashed line at 99%.

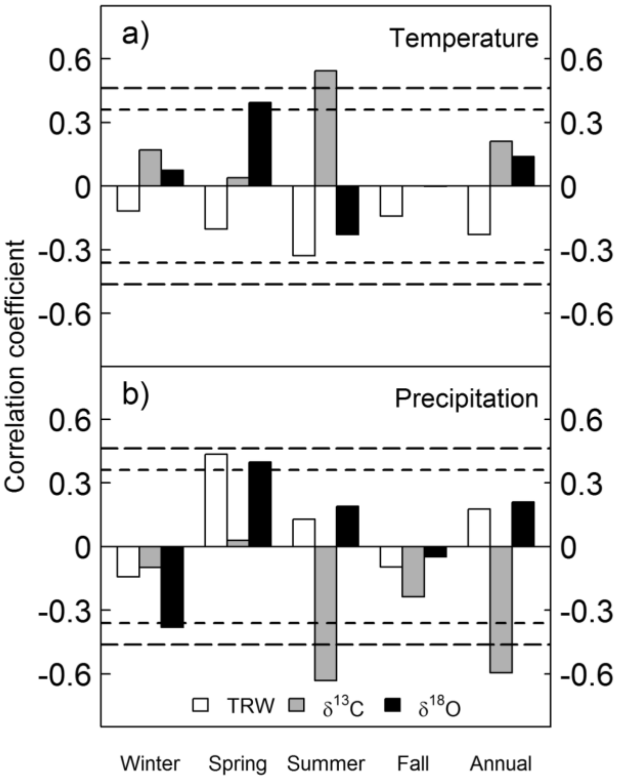

Figure 8. Pearson’s correlation coefficients between tree-ring and climate variables for the period of 1984-2013 for various seasons and annual means for (a) temperature and (b) precipitation amount. The short-dashed line indicates the significance level at 95% and the long-dashed line at 99%.

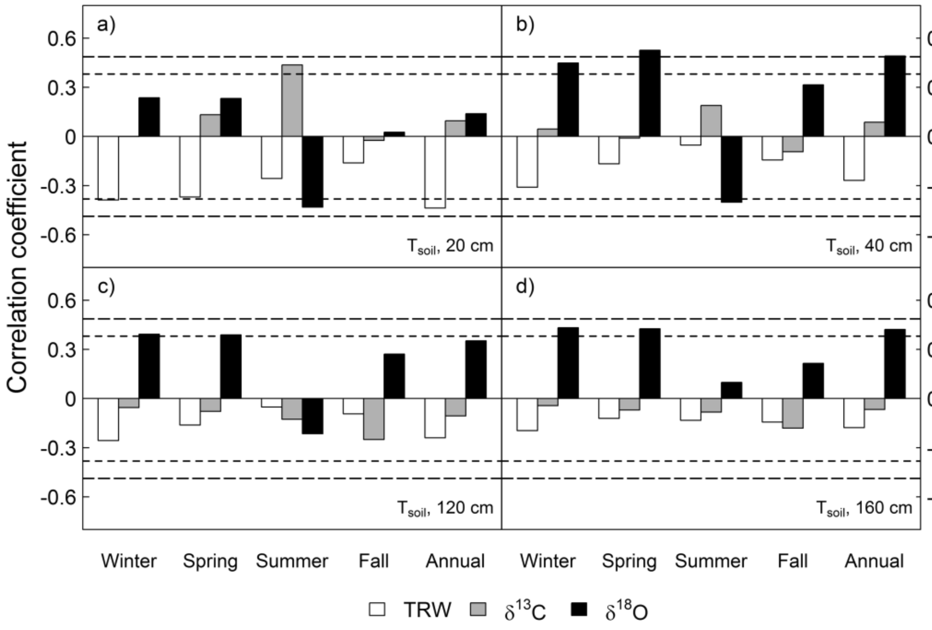

Figure 9. Pearson's correlation coefficients between tree-ring parameters and soil temperatures for the period of 1984-2013 for various seasons and annual means (a) at 20 cm depth and (b) 40 cm. The short-dashed line indicates the significance level at 95% and the long-dashed line at 99%.

Chapter 4

4. The fate of recently fixed carbon after drought release: towards unravelling C storage regulation in *Tilia platyphyllos* and *Pinus sylvestris*

Lucia Galiano[1,2], Galina Timofeeva[1,3,7], Matthias Saurer[1,3], Rolf Siegwolf[1,3], Jordi Martínez-Vilalta[4,5], Robert Hommel[6] and Arthur Gessler[1]

[1]Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstrasse 111, CH-8093 Birmensdorf, Switzerland;

[2]Institute of Hydrology, University of Freiburg, Freiburg D-79098, Germany;

[3]Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, CH-5232 Villigen PSI, Switzerland;

[4]CREAF, Cerdanyola del Vallès E-08193, Spain;

[5]Autonomous University of Barcelona UAB, Cerdanyola del Vallès E-08193, Spain;

[6]Eberswalde University of Sustainable Development, Schicklerstraße 516225 Eberswalde, Germany;

[7]Forest Ecology, Department of Environmental Systems Science, ETH Zurich, Universitätstrasse 16, CH-8092 Zurich, Switzerland

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Abstract

Carbon reserves are important for maintaining tree function during and after stress. Increasing tree mortality driven by drought globally has renewed the interest in how plants regulate allocation of recently fixed C to reserve formation. Three year-old seedlings of two species (*Tilia platyphyllos, Pinus sylvestris)* were exposed to two intensities of experimental drought during ~ 10 weeks and ^{13}C pulse labelling was subsequently applied with rewetting. Tracking the ^{13}C-label across different organs and C compounds (soluble sugars, starch, myo-inositol, lipids and cellulose), together with the monitoring of gas exchange and C mass balances over time, allowed for the identification of variations in C allocation priorities and tree C balances that are associated to drought effects and subsequent drought release. The results demonstrate that soluble sugars accumulated in *P. sylvestris* under drought conditions independently of growth trends; thus NSC formation cannot be simply considered a passive overflow process in this species. Once drought ceased, C allocation to storage was still prioritized at the expense of growth, which suggested the presence of 'drought memory effects', possibly to ensure future growth and survival. On the contrary, NSC and growth dynamics in *T. platyphyllos* were consistent with a passive (overflow) view of NSC formation.

Keywords: allocation; carbon isotope; carbon storage; drought; growth; pulse labelling; recovery

Introduction

Tree and forest mortality driven by drought and heat have become a global concern in the last few decades (Allen et al. 2010; Choat et al. 2012). Climate models project global increases in drought frequency, intensity and duration (IPCC 2014; Allen et al. 2015). In addition, the interaction between drought stress and damage caused by pathogens and pests may intensify forest vulnerability (Oliva et al. 2014; Anderegg et al. 2015). Although the mechanistic basis underlying drought-induced tree death is still not well identified, there is agreement in that it involves the impairment of the vascular transport system and/or the carbon (C) economy (McDowell et al. 2008; Sala et al. 2010; McDowell et al. 2011, 2013). Not surprisingly, given the role of C storage for tree survival under stressful conditions (O'Brien et al. 2014), there is a growing interest in understanding how C storage is affected by drought and how plants regulate allocation of recent C assimilates to storage pools (Sala et al. 2012; Willey & Helliker 2012; Dietze et al. 2014).

Trees fix atmospheric CO_2 via photosynthesis (source) and distribute assimilates as substrate for growth, maintenance, storage, defence, export and reproduction (sinks; Chapin et al. 1990; Körner 2003). C storage is a major plant function that has been defined as the build-up of carbon in the plant that can be mobilized in the future to support biosynthesis when C supply and demand are not synchronous (Chapin et al. 1990). Mature trees may therefore store large amounts of mobile C pools, mostly non-structural carbohydrates (NSC) and neutral lipids (triacylglycerols) (see Hoch et al. 2003; Würth et al. 2005). Three storage processes have been recognized (Chapin et al. 1990): (1) accumulation when C supply simply exceeds demands (passive overflow); (2) reserve formation when the synthesis of storage compounds is up-regulated so that it competes for resources with growth and defence (active storage); and (3) recycling of compounds primarily involved in growth or defence to support future growth, which is normally considered unimportant for carbon. Although this distinction inherently involved passive overflow and actively regulated processes, the use of 'passive' and 'active' storage has become more common only recently (Dietze et al. 2014). The current discussion about the role of C storage under drought stress has renewed the interest in disentangling whether C storage is either a 'passive' or 'active' process or both (Sala et al. 2012; Wiley & Helliker 2012; Hartmann & Trumbore 2016; Martínez-Vilalta et al. 2016).

Stomatal closure during drought to prevent desiccation and hydraulic failure also reduces photosynthetic C uptake. It has been hypothesized that trees under drought stress are eventually forced into C storage dependency to meet continuous metabolic demands for maintenance, osmotic adjustments, tissue repair, and defence against pathogens (McDowell et al. 2008, 2011; Sala et al. 2012). However, numerous observational and experimental studies have analysed NSC dynamics in response to drought without any conclusive pattern emerging to date, as NSC concentrations may decrease (e.g. Galiano et al. 2011; Mitchell et al. 2013; Sevanto et al. 2014; Aguadé et al. 2015), increase or not change at all (Sala & Hoch 2009; Anderegg et al. 2012; Gruber et al. 2012; Hartmann et al. 2013a). Several non-exclusive explanations have been proposed to explain these mixed results: (1) cell division and expansion underlying growth are more sensitive to drought than photosynthesis (Muller et al. 2011; Dosio et al. 2011), and thus NSC pools may increase under drought, at least in early stages (McDowell 2011; Hagedorn et al. 2016); as a result, the degree of asynchrony between growth and photosynthesis decline determine the ‘carbon safety margin’ during drought, which seems to be species-specific (Mitchell et al. 2014); (2) NSC responses may vary across organs within a plant (Galvez et al. 2013; Hartmann et al. 2013b) reflecting the mobile nature of NSC and the need to consider the whole plant in this type of studies; (3) negative effects of drought on C mobilization and phloem transport may lead to C starvation at the cellular level irrespective of stored reserves (Sala et al. 2010); (4) NSC are likely never fully depleted because soluble sugars are required to remain above a certain threshold to sustain immediate plant functions (e.g., osmoregulation, transport, signalling; Sala et al. 2010; Hartmann & Trumbore 2016; Martínez-Vilalta et al. 2016); (5) NSC may be actively accumulated at the expense of short-term growth to optimize growth and survival in the long term (Sala et al. 2012; Wiley & Helliker 2012).

Observational and experimental studies thus far have mostly focused on plant physiological responses during the drought period (see Mencuccini 2014), whereas the tree’s ability to recover physiological functions once drought is released remains much less investigated (Hagedorn et al. 2016). Here, we applied ^{13}C pulse labelling to determine the fate of newly produced C assimilates directly after drought release. Seedlings of two species (*Tilia platyphyllos*, *Pinus sylvestris*) with contrasted leaf habit and wood anatomy were subjected to two intensities of experimental drought during ~ 10 weeks, and a subsequent ^{13}C pulse labelling was simultaneously applied with rewetting. Tracking the fate of ^{13}C-label across different plant organs (leaves, stem, coarse roots) and C compounds (soluble sugars, starch, myo-inositol, lipids and cellulose), together with the monitoring of light-saturated photosynthesis (A_{sat}), stomatal conductance (g_s), organ biomass and NSC concentrations, facilitated the identification of variations in C allocation priorities and tree C balances during recovery from drought. In addition, gas exchange, organ biomass, NSC concentrations and natural ^{13}C abundances in C compounds were also monitored during drought in order to relate the recovery effects to the drought-induced changes in the tree C balance. Specifically, our objectives were to determine: (1) whether NSC concentrations increase under drought conditions; (2) whether this increase is caused by active NSC formation (i.e. 'active' storage); and (3) to what extent C allocation strategies prioritize C storage at the expense of growth in seedlings recovering from drought, giving raise to 'drought memory effects'.

Data and Methods

Plant material and experimental design

Tilia platyphyllos (Scop.) is a deciduous broad-leaved tree with diffuse-porous wood anatomy, native to central and southern Europe. *T. platyphyllos* was observed to strongly reduce transpiration fluxes in response to the intense natural drought experienced in Central Europe in 2003 (see Scherrer et al. 2011), but recovered particularly well once drought ceased (Leuzinger et al. 2005). *Pinus sylvestris* (L.) is an evergreen coniferous tree native to Eurasia that grows under a wide range of environmental conditions and has great ability in adjusting its hydraulic architecture to water availability (Martínez-Vilalta et al. 2009; Poyatos et al. 2013; Salmon et al. 2015). Drought-driven mortality has been documented in *P. sylvestris* at the driest edge of its distribution in dry alpine valleys (Bigler et al. 2006; Rigling et al. 2013) and in the Mediterranean basin (Martínez-Vilalta & Piñol 2002; Vilà-Cabrera et al. 2013), and has been associated with depletion of NSC reserves (Galiano et al. 2011; Aguadé et al. 2015).

The study was carried out during summer 2014 in the greenhouse of the Swiss Federal Institute WSL (47º 21′ 37″ N, 08º 27′21″ E; 500 m a.s.l.) where temperature and relative air humidity varied along with the outside conditions and ranged from 9 to 37 ºC (mean 21 ºC) and from 22 to 74 % (mean 62 %), respectively. Additional artificial illumination was applied (Master Green Power CG T 400W Mogul 1SL/12, Philips Lighting Holding B.V., Eindhoven, The Netherlands) to simulate the average summer photoperiod (~ 15 h light) at Swiss latitudes. Photosynthetic active radiation (PAR) at canopy level was never < 200 µmol m^{-2} s^{-1}. A total of 108 three year-old seedlings of each of the two species were obtained from a commercial tree nursery (Kressibucher, Berg, Switzerland). At the beginning of May 2014, 216 pots were numbered and one seedling was planted into each 3.5-l pot filled with 1.8 kg soil substrate (mixture of 65 % clay mineral components and 35 % hummus; ökohum GmbH, Herrenhof, Switzerland) before leaves developed in *T. platyphyllos*. At planting time, seedling height and stem diameter at the base were 29.5±0.62 cm and 0.7±0.01 cm for *T. platyphyllos* and 27.5±0.30 cm and 0.50±0.006 cm for *P. sylvestris*. The bulk density of the mineral soil substrate was 0.51±0.0001 g cm^{-3}. During the acclimation period in the greenhouse, all seedlings were watered every second day to field capacity. All pots were fertilized with a standard controlled-release fertilizer every second month throughout the entire experiment (N:P:K – 18:6:12).

Equal numbers of pots from each of the two species (N = 36) were randomly allocated to one of three treatments: control/well-watered, moderate drought and severe drought. Soil volumetric water content (VWC) in control treatment was maintained at field capacity (see Figure 1). Soil VWC was monitored gravimetrically by weighting ten random pots per treatment every third day. Soil water potential (ψ_S) was derived from soil VWC according to Priesack & Durner (2006). Drought treatments were applied when seedlings showed vigorous condition and leaves were fully developed, starting 14 July 2014 in *T. platyphyllos* and 28 July 2014 in *P. sylvestris*. From this point onwards, no water was added until the moderate drought treatment reached the soil VWC threshold of 15 % (ψ_S ~ -0.12 MPa) and the severe drought treatment reached the soil VWC threshold of 8% (ψ_S ~ -1.3 MPa). In *T. platyphyllos*, moderate and severe soil VWC thresholds were reached after 5 and 24 days of irrigation cessation, respectively. In *P. sylvestris*, moderate and severe soil VWC thresholds were reached after 7 and 26 days, respectively. After approximately two months of water shortage, pots from both drought treatments were rewetted every second day to field capacity for a period of 20 days.

$^{13}CO_2$ pulse-labelling

^{13}C pulse labelling allows the investigation of C allocation dynamics, i.e. how fast and where the labelled C assimilates are allocated among different sink tissues and activities (Epron et al. 2012; Hartmann & Trumbore 2016) and has been successfully applied to assess the distribution of new assimilates under drought conditions (Ruehr et al. 2009; Blessing et al.2015, Hommel et al. 2016). $^{13}CO_2$ labelling was applied here two days after seedlings were rewetted, on 18 September 2014 in *T. platyphyllos* and 9 October 2014 in *P. sylvestris*. 18 pots per treatment were randomly selected and placed inside an airtight transparent plastic chamber (2.20 m x 1 m x 0.90 m; volume ca. 2000 l). The $^{12}CO_2$ concentrations within the chamber were continuously monitored with the GFS-3000 portable IRGA system (Heinz Walz GmbH, Effeltrich, Germany). For the labelling application, 5 g 99 % ^{13}C sodium bicarbonate (Cambridge Isotope Laboratories Inc, Tewksbury, MA, USA) was mixed with 5 g standard ^{12}C sodium bicarbonate and hydrochloric acid in an airtight sealed beaker outside the chamber to generate the 50 % labelled $^{13}CO_2$ gas. The ^{13}C labelled gas was pumped into the chamber as soon as the CO_2 concentration inside reached approx. 300 ppm due to photosynthetic CO_2 uptake. Labelling started for both species at 09:00 h. After 30 minutes the CO_2 concentration inside the chamber reached approx. 1700 ppm (i.e. ~ 700 ppm $^{13}CO_2$ and ~ 1000 ppm $^{12}CO_2$), which was maintained during the following 3.5 h. The use of fans inside the labelling chamber ensured a good mixing of the air. The mean air temperature inside the chamber during the labelling increased by no more than 4 ºC relative to the ambient air temperature in the greenhouse, and was 23.4 ºC for *T. platyphyllos* and 20.6 ºC for *P. sylvestris*. After the labelling, the transparent plastic chamber was removed and the greenhouse atmosphere was flushed with ambient air ensuring rapid removal of the remaining $^{13}CO_2$.

Sample collection and physiological measurements

Harvest time points were distributed unequally throughout the course of the experiment: 2-4 week intervals during the drought phase; and 1 hour, 6 h, 1 day/24 h, 2 d/48 h, 8 d/ 192 h and 20 d/480 h after the labelling and during the rewetting phase. Note that there was a time lag of 2 weeks between the harvest of the two species, starting 28 July 2014 in *T. platyphyllos* and 11 August 2014 in *P. sylvestris* (see Figure 1). At each harvest time, 3 individuals per species and treatment (control, moderate drought, severe drought) were entirely harvested and separated into leaves/needles, stem (10 cm from the root collar), coarse roots (> 2 mm) and fine roots (≤ 2 mm). Fresh weight (FW) was determined in all samples to compute tissue water content (WC; g g^{-1}) of each organ and at the whole-plant level (difference between FW and dry weight (DW) divided by DW). All samples were subsequently microwaved for 90 s at 600 W to stop enzymatic activity, oven-dried for 72 h at

65 ºC and ground to fine powder with a ball mill (MM400, Retsch GmbH, Haan, Germany). Leaf gas exchange measurements were conducted on mature, fully developed leaves immediately before destructive sample collection. Light-saturated photosynthesis (A_{sat}) and stomatal conductance (g_s) were measured between 12:00 and 16:00 h using a GFS-3000 portable IRGA system (Heinz Walz GmbH, Effeltrich, Germany) at a photosynthetic photon flux of 2000 µmol m^{-2} s^{-1}, a CO_2 concentration of 400 ppm, and a cuvette temperature and relative humidity of 24 ºC and 60 %, respectively. Pre-dawn (Ψ_{PD}) and mid-day (Ψ_{MD}) leaf water potentials were measured at three time points during the drought phase of the experiment using a Scholander-type pressure chamber (PMS instruments, Corvalis, OR, USA).

Carbon isotope composition of bulk material and specific carbon compounds and NSC concentrations were assessed at different harvest time points: $\delta^{13}C$ in bulk plant material was analysed after 2 months of drought prior to the ^{13}C pulse labelling (baseline) and at all harvest time points after labelling in three organs (leaves, stem, coarse roots) in all treatments and for both species. Compound-specific $\delta^{13}C$ signatures in cellulose, non-structural carbohydrates (NSC; including glucose, fructose and sucrose as soluble sugars, plus starch), myo-inositol (sugar alcohol) and lipids were measured after 2 months of drought prior to labelling (baseline) and at 6 h, 24 h and 192 h after labelling in three organs (leaves, stem, coarse roots) in control and severe drought treatments and for both species. $\delta^{13}C$ signatures in cellulose were only measured in lignified organs (stem, coarse roots) as we assumed that C allocation to leaf cellulose was quantitatively less important once leaves are fully developed. NSC concentrations were quantified at all harvest time points during the drought phase of the experiment and at 20 d after labelling in three organs (leaves, stem, coarse roots) in all treatments and for both species.

Extraction of carbon compounds for isotope analyses

Water-soluble carbon compounds (soluble sugars i.e. glucose, fructose and sucrose; and myo-inositol) were extracted from approx. 100 mg of homogenised dried material using 1.5 ml of Milli-Q water (18.2 MΩ cm at 25 ºC). The samples were heated at 85 ºC for 30 min, centrifuged at 10000 g for 2 min and 800 µl of the supernatant was transferred into 2-ml reaction vials. Neutral carbon compounds were then purified from ionic compounds using anion and cation exchange cartridges (OnGuard II H 1 cc, OnGuard II A 1 cc, Dionex Corporation, Sunnyvale, CA, USA; Rinne et al. 2012). Lipids and starch were extracted from the pellet that remained from the previous water-soluble fraction extraction, following the method described by Wanek et al. (2001) as modified by Göttlicher et al. (2006). The pellet was re-suspended by adding a monophasic mixture of methanol, chloroform and water (MCW; 12:3:5, v/v/v) at 70 ºC for 30 min. After three cycles of centrifugations at 10000 g for 2 min and further chloroform and water additions, the water and the lipophilic phases separated. Lipids were then extracted by transferring the lower lipophilic phase into glass vials. Before starch extraction, the remaining pellet was washed three more times with water to remove all soluble compounds. Starch was then gelatinized at 100 ºC for 15 min and broken down to glucose at 85 ºC for 120 min using α-Amylase from *Bacillus licheniformis* (Sigma Aldrich A4551 1 g). Hydrolysed starch was finally transferred into pre-washed centrifugal devices (Vivaspin 500, Sartorius, Göttingen, Germany) and centrifuged at 12000 g for 50 min to remove enzymes. After the centrifugation, 140 µl of the filtrated starch samples were transferred into pre-weighted tin capsules, which were stored in an oven at 60 ºC overnight. When the samples were dried, the tin capsules were re-weighted again to obtain a difference in weight before and after which is actually an approximate sample weight before isotopic measurements. In the case of lipids, 30 µl of sample was transferred into the tin capsules for drying before isotopic measurements, and the same procedure was applied to obtain the weight of each sample.

For cellulose extraction, approx. 15 mg of homogenised dried material was weighted and packed within heat-sealed Teflon bags (Fiber Filter Bags F57, Ankom Technology, Macedon, USA). In order to remove hemicelluloses from the samples, Teflon bags were soaked in a 5 % NaOH solution within Pyrex Erlenmeyer flasks at 60 °C for 2 h and washed three times with boiling distilled water. Afterwards, the samples were soaked in a 7 % $NaClO_2$ solution at 60 ºC for 30 h to remove resin and lignin and washed again three more times with boiling distilled water. All extracted solutions were stored at minus 20 ºC for further analyses.

Isotope analyses and calculations

$\delta^{13}C$ in the bulk plant material, cellulose, starch and lipids was determined by combustion method using an elemental analyser (EA: EA1110 CHN, Carlo Erba, Milan, Italy) linked in the HE downstream mode via a CONFLO II opensplit interface to an isotope ratio mass spectrometer (IRMS: Delta S, Finnigan MAT, Bremen, Germany) from 0.4-0.6 mg of sample material. Laboratory plant standards and international standards with known $\delta^{13}C$ values were used for calibration of the measurements resulting in a precision of 0.1 ‰. Compound-specific isotope analyses (CSIA) of soluble sugars and myo-inositol were conducted using a high-performance liquid chromatography (HPLC) coupled to the IRMS (delta V Advantage; Thermo, Bremen, Germany) with a LC Isolink interface (Thermo, Bremen, Germany). The mobile phase was 1 mM NaOH. For chromatographic separation a CarboPac PA20 anion-exchange column (3 × 150 mm, Dionex) at a temperature of 20°C was used (Rinne et al. 2012). Myo-inositol and pinitol co-elute due to their similar biochemistry as sugar alcohols and this combined peak is in the following referred to as myo-inositol. The δ^{13}C-standard deviation for repeated analysis of standard mixtures was on an average 0.3 ‰ for myo-inositol, 0.3 ‰ for glucose, 0.4 ‰ for fructose and 0.4 ‰ for sucrose.

The isotopic $^{13}C/^{12}C$ ratio in all samples was expressed in δ notation (‰) relative to the international Vienna Pee Dee Belemnite standard (VPDB). Thereafter δ^{13}C in bulk plant material was converted to atom% as follows (Ruehr et al. 2009):

$$\text{atom\%} = \frac{100 \cdot 0.0111802 \cdot \left(\frac{\delta}{1000}+1\right)}{1+0.0111802 \cdot \left(\frac{\delta}{1000}+1\right)} \qquad \text{Eqn 1}$$

where 0.0111802 is the standard value for the isotopic $^{13}C/^{12}C$ ratio of VPDB. To calculate excess ^{13}C (mg m^{-2}) for each organ (i.e. the amount of ^{13}C added to the respective organ due to ^{13}C labelling), atom% was normalized per organ DW and its natural isotope baseline prior labelling (Ruehr et al. 2009):

$$\text{excess } ^{13}\text{C} = \frac{\text{atom\%}_\text{s} - \text{atom\%}_\text{b}}{100} \cdot \text{B} \cdot \frac{\text{C\%}}{100} \qquad \text{Eqn 2}$$

where atom%$_s$ is the atom% of the organ sample; atom%$_b$ is the atom% of the natural unlabelled background averaged per species, treatment and organ (n = 3); B (mg m^{-2}) is the biomass DW averaged per species, treatment and organ (n = 3) and referred to the pot ground area (0.02 m^2); and C% is the percentage of C in the organic sample.

Isotopic concentrations in specific C compounds (cellulose, NSC, myo-inositol and lipids) from the samples collected after pulse-labelling were expressed as $\Delta\delta^{13}$C (‰) and calculated as follows:

$$\Delta\delta^{13}C_s = \delta^{13}C_s - \delta^{13}C_b \qquad \text{Eqn 3}$$

where $\delta^{13}C_s$ is the $\delta^{13}C$ of the organ sample and $\delta^{13}C_b$ is the $\delta^{13}C$ of the natural unlabelled baseline averaged per species, treatment and organ. Excess ^{13}C as described above for bulk plant material was not computed for specific C compounds because C concentrations would be needed and were only analysed in this study for NSC and lipids (see Blessing et al. 2015).

Non-structural carbohydrates concentrations

Non-structural carbohydrates (NSC) were defined as soluble sugars (glucose, fructose and sucrose) plus starch. A recent study on the comparability of NSC measurements across laboratories concluded that water extraction and quantification using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) are the methods with the least variable results (Quentin et al. 2015); and this was the methodology applied here. Soluble sugars were extracted from 6 mg of homogenised dried material using 1 ml of Milli-Q water (18.2 MΩ cm at 25 ºC). The samples were centrifuged at 14000 g for 10 min and the supernatant was diluted 1:50 (v/v) with distilled water within glass vials for sugar quantification. Glucose, fructose and sucrose were separated and quantified by HPAEC-PAD (DX-300, Dionex, USA) on an analytical Carbo-Pac PA20 Column from ThermoFisher using a NaOH elution gradient. Starch was extracted from another 6 mg fraction of homogenised dried material and gelatinized at 100 ºC for 60 min using 1 ml of Milli-Q water (18.2 MΩ cm). Starch was then incubated with an amyloglucosidase from *Aspergillus niger* (Sigma Aldrich 10115 5 g) at 50 ºC overnight, to break down starch and sucrose into glucose and glucose and fructose, respectively. Total soluble sugars resulting from the amyloglucosidase digestion were also separated and quantified by HPAEC-PAD. Starch was calculated as the total soluble sugars minus the soluble sugars resulting from the water extraction. All NSC values are expressed as mg g^{-1} of dry mass. A plant standard with known NSC concentrations was measured every 20 samples of the analysis sequence and used as an internal standard (mean NSC 64.03±1.18 mg g^{-1} across all NSC analyses).

Statistics

Statistical tests were computed independently for each combination of species, organ and harvest time point. Species responses cannot be directly compared because watering regimes and pulse-labelling set-ups were not simultaneous in *T. platyphyllos* and *P. sylvestris* and climatic conditions in the greenhouse changed over time. Response variables (soil VWC, plant WC, A_{sat}, g_s, leaf ψ_{PD} and ψ_{MD}, $\delta^{13}C$, $\Delta\delta^{13}C$, biomass and NSC) were thus tested for mean differences between treatments (control, moderate drought, severe drought) by using generalised least squares (GLS) models for each combination of species, organ and harvest time point. Function 'weights' was applied to the models in order to account for heterogeneity of variances across treatments. The residuals of all models were normally distributed.

The decrease of ^{13}C excess in leaves was described by the following exponential decay function:

$$\mathrm{N}(t) = \mathrm{N}_0\mathrm{e}^{-\lambda t} \qquad \text{Eqn 4}$$

where *t* is the time in hours after labelling; λ is the decay constant; N_0 is the initial quantity of ^{13}C excess at time = 0 (^{13}C peak); and N(*t*) is the quantity of ^{13}C excess after time *t*. Mean residence time (MRT = 1 /λ) and half life time (HLT = ln (2) / λ) were then computed for each species and drought treatment. All statistical analyses were performed with the R statistical software v3.2.5. (R Development Core Team, 2016) with the nlme package for GLS models. Significant differences were considered when $P < 0.05$.

Results

Water status and gas exchange

In general, whole-plant water content during drought followed the same pattern as soil volumetric water content for both species (Figure 1). After two weeks of treatment, whole-plant WC in seedlings under both drought intensities significantly differed from whole-plant WC in control seedlings for both species; and these differences were maintained or increased throughout the course of the drought. In *T. platyphyllos*, WC decreases in fine roots were clear under both drought intensities during the entire drought period and only after one month of severe drought in coarse roots (Figure S1). In *P. sylvestris*, WC decreases in fine roots also occurred under both drought intensities during the entire drought period, and only after one month of moderate and severe drought in stem and coarse roots (Figure S1). In both species, midday leaf water potentials (ψ_{MD}) were consistently lower under the moderate and, particularly, the severe drought treatments than for the control plants (Table 1).

Soil volumetric water content was completely recovered to field capacity after ten days of rewetting in *T. platyphyllos* (26 Sept 2014), but was still not recovered in *P. sylvestris* after 20 days (Figure 1). Whole-plant WC largely mirrored soil VWC and, consequently, the water status of *P. sylvestris* did not fully recover for any of the drought intensities by the end of the rewetting phase. In *T. platyphyllos*, coarse and fine roots equalled the water status of control individuals by the end of the experiment (Figure S1). In *P. sylvestris*, however, WC in fine roots did not reach the values of the controls for any of the drought treatments at the end of the experiment, and probably determined WC dynamics at the whole-plant level.

Stomatal conductance (g_s) responded differently to moderate drought in the two species (Figure 1). In *T. platyphyllos*, g_s under moderate drought only differed from control after two months of water shortage. By contrast, g_s under moderate drought in *P. sylvestris* and under severe drought for both species differed from the control after two weeks of water shortage. Thus, g_s patterns in *P. sylvestris* during the drought phase mainly mirrored WC in plants and soil. g_s recovered after 2-3 days of rewetting in those seedlings from both species that experienced moderate drought conditions during the drought phase of the experiment (Figure 1). Nonetheless, *T. platyphyllos* seedlings that experienced severe drought only recovered g_s after approximately ten days of rewetting (26 Sept 2014). In *P. sylvestris*, seedlings that experienced severe drought recovered g_s after three days of rewetting (10 Oct 2014), despite a small significant relapse occurring one day later. Light-saturated photosynthesis (A_{sat}) exhibited the same dynamics as g_s for both species and drought intensities (Figure 1).

NSC and biomass changes

Overall, NSC concentrations were 4-fold higher in *T. platyphyllos* (79.5±2.6, 180.4±5.6 and 308.4±11.4 mg g^{-1} for leaves, stem and roots, respectively; Figure 2A) than in *P. sylvestris* (55.5±3.6, 42.5±2.2 and 40.7±1.9 mg g^{-1} for needles, stem and roots, respectively; Figure 2B). Nonetheless, soluble sugars were proportionally more abundant in *P. sylvestris* (Figure 2).

In *T. platyphyllos*, soluble sugars in lignified organs tended to increase under severe drought over time but only significantly after one month of drought in stems (the increase was only marginally significant, $0.05 < P < 0.1$, for coarse roots after one month of drought, and for stem and coarse roots by the end of the drought; Figure 2A). Similarly, starch increased in coarse roots under both drought intensities by the end of the drought. By contrast, starch concentrations in stems were significantly lower under both drought intensities at the beginning of the drought, and equalled control values by the end of the drought period.

Overall decreases in biomass under both drought intensities (relative to the control plants) were observed in *T. platyphyllos* at the end of the drought period, despite being significant only for coarse and fine roots (and marginally for stems; Figure S2).

In *P. sylvestris*, an overall increase in soluble sugar concentrations under both drought intensities was observed in all organs during the entire drought period (Figure 2B). A similar pattern was observed for the absolute content of soluble sugars, which increased specially in needles and stems under both drought intensities over time (Figure S2; only results for NSC are shown). Starch concentrations were significantly higher under both drought intensities only at the end of the drought period in needles and coarse roots (Figure 2B). As for *T. platyphyllos*, overall decreases in biomass under both drought intensities were observed in *P. sylvestris* at the end of the drought period. These differences were significant for fine roots and marginally significant for stem and coarse roots; Figure S2).

In *T. platyphyllos* leaves, starch concentrations after 20 days of rewetting were significantly higher in the seedlings that experienced severe drought during the drought phase of the experiment (Figure 2A). In coarse roots, starch remained high in previously drought treated seedlings compared to the controls. After 20 days of rewetting, *T. platyphyllos* seedlings equalled biomass in coarse and fine roots across all watering regimes, despite a general (non-significant) tendency towards smaller organs in previously drought-stressed seedlings was still observed (Figure S2).

In *P. sylvestris*, soluble sugar concentrations in coarse roots were still significantly higher after 20 days of rewetting in those seedlings that had experienced severe drought compared to the control ones (Figure 2B). After 20 days of rewetting, *P. sylvestris* seedlings equalled biomass in fine roots across all watering regimes, but biomass reductions in coarse roots of drought-stressed seedlings became statistically significant compared to the controls (Figure S2).

Natural ^{13}C abundance

Overall, bulk δ^{13}C signatures in plant organs exposed to both drought intensities were significantly higher than in control organs for the two species (Table 2). *P. sylvestris* under severe drought exhibited significant ^{13}C enrichment in soluble sugars and starch in all organs. Similarly, *T. platyphyllos* under severe drought showed significant higher δ^{13}C signatures in all organs for soluble sugars, but only in leaves for starch. In both species, myo-inositol was significantly ^{13}C enriched under severe drought only in roots. Although increases in δ^{13}C in cellulose and lipids were common under severe drought in all organs and for both species, the differences were not significant relative to the control.

^{13}C pulse-labelling

Mean residence time (MRT) and half-life time (HLT) for bulk δ^{13}C in leaves of both species were similar between seedlings that experienced control and moderate drought conditions (Figure S3 and Table 3). Seedlings under severe drought, however, exhibited longer MRT and HLT. In *T. platyphyllos* stems, the ^{13}C label peaked simultaneously in bulk organic matter for control and moderate drought 1 h after labelling, while no obvious peak was observed for the severe drought treatment (Figure S3). In coarse roots, a first ^{13}C label peak occurred after 24 h in all watering regimes. In *P. sylvestris* stems, the ^{13}C label peaked earlier in the moderate drought treatment (1 h after labelling), and subsequently in the severe drought (6 h after labelling) and control (6-24 h after labelling) treatments (Figure S3). In coarse roots, the ^{13}C label showed the highest value earlier in the control and moderate drought treatments (6-24 h after labelling), and only 192 h after labelling in the severe drought treatment. In all cases, the amount of ^{13}C label allocated to the coarse roots was larger in those seedlings that previously experienced well-watered (control) conditions relative to moderate and severe drought ($P < 0.05$ for moderate and severe drought in *T. platyphyllos*; $P = 0.16$ for moderate drought and $P < 0.05$ for severe drought in *P. sylvestris*).

Compound specific carbon isotope analyses showed that ^{13}C-enrichment due to labelling was most strongly expressed in soluble sugars and starch compared to much lower values in myo-inositol, lipids, and cellulose for both species. Nonetheless, dissimilar patterns of C allocation were revealed across compounds and watering regimes (control vs. severe drought; Figure 3). In *T. platyphyllos* leaves, $\Delta\delta^{13}$C in soluble sugars, starch and lipids were highest 6 h after labelling and afterwards diminished over time (Figure 3A). The ^{13}C label in leaf soluble sugars and starch remained significantly longer in the severe drought than in the control treatment. $\Delta\delta^{13}$C in stem soluble sugars (24 h after labelling) and starch (192 h after labelling) was also higher in the severe drought treatment relative to the controls. Larger amounts of ^{13}C label contained in soluble sugars, myo-inositol and lipids were allocated to coarse roots in the control treatment compared to severe drought. By contrast, ^{13}C label in starch of coarse roots increased similarly over time for both watering regimes. Interestingly, 192 h after labelling, the ^{13}C label peaked in cellulose of coarse roots only in control seedlings.

In *P. sylvestris*, the ^{13}C label in soluble sugars of needles was also highest 6 h after labelling, and greater amounts of ^{13}C label appeared in stems and coarse roots of control seedlings in comparison to the severe drought-stressed ones (Figure 3B). In contrast, the ^{13}C label in myo-inositol was highest 192 h after labelling in needles and coarse roots of severe drought-stressed seedlings, whilst $\Delta\delta^{13}$C remained rather constant over time in the controls. ^{13}C label in starch was also highest 192 h after labelling in stem and coarse roots of severe drought-stressed seedlings. Similar patterns were observed for lipids in all organs and for both watering regimes, with larger amounts of ^{13}C label in the severe drought treatment 192 h after labelling. The ^{13}C label was also highest in cellulose of stems and coarse roots 192 h after labelling for both watering regimes, but in this case larger amounts were found in the control treatment.

Discussion

The dynamics, role and regulation of C storage remain a debated topic in the context of drought-induced tree death (Sala et al. 2012; Wiley & Helliker 2012; Hartmann & Trumbore 2016; Martínez-Vilalta et al. 2016). Tree's ability to recover the C reserves after drought has been much less investigated (but see Galiano et al. 2011; Zang et al. 2014; Jakob 2016). By combining the monitoring of NSC and biomass measurements at the organ and whole-plant level with ^{13}C pulse labelling and gas-exchange measurements, this experiment facilitated the identification of variations in C allocation priorities and tree C balances that are associated to drought effects and subsequent drought release.

C allocation to storage during drought

Our results indicate that NSC concentrations increased in *P. sylvestris* under drought conditions and this increase is caused by active NSC formation. For this species, we observed two different phases of NSC formation during drought (Figure 2 and Figure S2): (1) early in the drought period (after two weeks), A_{sat} was 50 % lower in seedlings exposed to both drought intensities relative to the control plants; even though overall growth was similar under all watering regimes and total NSC (both concentrations and absolute amounts) accumulated in all organs under both drought intensities; (2) late in the drought period (after two months), total NSC (both concentrations and absolute amounts) still accumulated, especially under severe drought conditions, although A_{sat} decreased to ~ zero under severe drought and growth also declined under both drought intensities relative to the controls. Interestingly, soluble sugars were always the NSC fraction that increased the most with increasing drought. These NSC patterns suggest that, at least in the short-term, soluble sugars may be actively accumulated as an osmotic response to maintain vascular integrity as water availability decreases (Woodruff & Meinzer 2011; Sala et al. 2012). These results contradict the prediction that isohydric species such as *P. sylvestris* (Irvine et al. 1998) rapidly deplete their C reserves under drought conditions (McDowell et al. 2008) and suggest that NSC formation for this species cannot be simply considered a passive overflow process (Hartmann et al. 2015; see also Salmon et al. 2015). Nonetheless, under prolonged drought, the relatively isohydric strategy of *P. sylvestris*, together with reduced canopy leaf area, may eventually force trees to draw from stored carbohydrates to meet the continued metabolic demands (McDowell et al. 2008, 2011; Galiano et al. 2011; Poyatos et al. 2013).

Increases in absolute contents of NSC were not commonly observed in *T. platyphyllos* under drought conditions, and NSC concentration dynamics were thus likely more related to mere changes in biomass and A_{sat} over time (Figure 2 and Figure S2): (1) early in the drought period (after two weeks), A_{sat} was 50 % lower in seedlings exposed to severe drought relative to the controls, and total NSC concentrations tended to diminish under drought whilst growth remained comparable to that of control seedlings; (2) late in the drought period (after two months), A_{sat} decreased to ~ zero under severe drought and growth also tended to decline under both drought intensities. Consequently, total NSC concentrations under drought remained similar (stem) or even increased (coarse roots) relative to those in control seedlings. This pattern could be explained by the fact that, contrary to *P. sylvestris*, *T. platyphyllos* contained large NSC amounts (mostly starch; Hoch et al. 2003) in all organs and additional NSC formation may not be important in this species to maintain physiological functioning under drought conditions (at least up to the drought intensities studied here; see Morris et al. 2016; Martínez-Vilalta et al. 2016).

Natural ^{13}C abundances indicated that overall NSC became isotopically heavier in drought-stressed seedlings after two months of drought (Table 1). ^{13}C enrichment in soluble sugars under drought conditions can be explained by two main processes: (1) decreases in the intercellular CO_2 partial pressure induced by stomatal closure that leads to decreasing $^{13}CO_2$ discrimination by Rubisco (Farquhar et al. 1982); and/or (2) increases in the amount of ^{13}C-enriched soluble sugars originating from degradation of the generally ^{13}C-enriched starch (see Tcherkez et al. 2003). Considering the starch dynamics observed in this study, the ^{13}C enrichment in soluble sugars of *P. sylvestris* was more likely due to the former process, which would indicate once again that new ^{13}C enriched assimilates were still allocated to C storage and thus further enriching starch in ^{13}C under drought conditions. By contrast, NSC dynamics in *T. platyphyllos* indicated some degree of starch breakdown under drought conditions, which means that in this species ^{13}C enrichment in soluble sugars could occur by either of the two processes described above.

Myo-inositol is an organic osmolyte that serves as a precursor to a number of metabolites related to membrane biogenesis, cell signalling and biosynthesis of other organic osmolytes directly involved in osmotic adjustment (e.g. pinitol; Loewus & Murthy, 1999). It should be noted, however, that myo-inositol here was not distinguished from pinitol due to their similar biochemistry (see Material and Methods). Myo-inositol was also ^{13}C-enriched in the coarse roots of seedlings from both species exposed to severe drought (Table 2). Considering the low growth rates of those coarse roots by the end of the drought period, its ^{13}C enrichment might indicate yet again that osmoprotectant synthesis pathways may be activated under harsh conditions, in this case to protect root cells against drought stress (Nguyen & Lamant 1988; Rathinasabapathi 2000).

C allocation to storage during drought release

Stomatal conductance and A_{sat} recovered rapidly to control values after rewetting, despite *T. platyphyllos* lagged by a few days relative to *P. sylvestris*. Nevertheless, MRT and HLT of δ^{13}C in leaves after pulse labelling indicated prolonged retention of recent assimilates aboveground in seedlings previously exposed to drought conditions for both species, suggestive of phloem transport impairment. Other studies have also documented reduced phloem transport velocities under drought conditions (Ruehr et al. 2009; Barthel et al. 2011) and subsequent rewetting (Zang et al. 2014). Similarly, and especially in *T. platyphyllos*, less C was translocated belowground to the coarse roots of previously drought-stressed seedlings, which could have led to NSC accumulation in the leaves of *T. platyphyllos* by the end of the experiment.

Interestingly, ^{13}C-label tracking in specific C compounds revealed opposite C allocation patterns between control and previously drought-stressed seedlings for both species. In *T. platyphyllos*, previously drought-stressed seedlings incorporated most ^{13}C-label into storage (starch) in stem and coarse roots eight days after labelling. Simultaneously, control seedlings incorporated C into both storage (starch) and growth (cellulose) in coarse roots. Six and 24 hours after labelling, simultaneous incorporation of ^{13}C-label into myo-inositol and cellulose of control coarse roots might indicate that biosynthetic pathways are being activated for growth production (cf. see above). In *P. sylvestris*, seedlings recovering from drought incorporated ^{13}C-label into storage (starch and lipids), osmoprotection (myo-inositol) and, to a lower extent, growth (cellulose) in the stem and coarse roots eight days after labelling. Simultaneously, control seedlings mainly incorporated C into growth (cellulose) and, to a lower extent, storage (lipids) in the stem and coarse roots.

Storage formation in seedlings recovering from drought may be the result of two processes: (1) plant 'memory effects' after drought may still prioritize C storage at the expense of growth to optimize growth and survival in the long term (i.e. 'active' storage; Sala et al. 2012; Wiley & Helliker 2012); (2) growth may be constrained by other limitations than insufficient C availability and surplus C may overflow to storage (McDowell 2011; Palacio et al. 2014). The dynamics of C mass balance during drought did not support active storage for *T. platyphyllos*, and thus the latter process appears more plausible in this species. On the contrary, the fact that growth was not constrained in *P. sylvestris* and NSC still accumulated suggest that 'drought memory effects' may still favour C storage over growth during recovery to ensure future growth and survival (Sala et al. 2012). These results support that C storage may be an important sink under drought conditions and subsequent recovery in *P. sylvestris* and highlight that other C pools (lipids, sugar alcohols) should be considered as potential competing C sinks (Fischer & Höll 1992; Hoch et al. 2003).

^{13}C pulse labelling, together with the monitoring of gas exchange and C mass balances over time, is a powerful tool to understand how plants regulate C partition to storage and growth among other sink activities (Hartmann & Trumbore 2016). At least in the short-term, soluble sugars may be actively accumulated in *P. sylvestris* as an osmotic response to drought effects, and NSC formation cannot be simply considered a passive overflow process for this species (Sala et al. 2012). Once drought ceased, C allocation to storage was still prioritized at the expense of growth in *P. sylvestris*, which suggested that ‘drought memory effects’ may increase future growth and survival. Our study, however, did not allow the distinction whether C storage was directly upregulated or indirectly stimulated through downregulation of growth (‘active’ versus ‘quasi-active’ storage, respectively; Wiley & Helliker 2012). Research using molecular and genetic tools may shed more light on these questions to completely understand C storage regulation in woody plants and its role in stress responses at different time scales (Dietze et al. 2014).

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Figures and Tables

Table 1. Means and standard errors (n=3) of Ψ_{PD} and Ψ_{MD} (MPa) values for both species and all watering regimes during the drought phase of the experiment. Different letters indicate significant differences between watering regimes for Ψ_{PD} and Ψ_{MD} at each time point ($P < 0.05$).

	Control		Moderate D		Severe D	
	ψ_{PD}	ψ_{MD}	ψ_{PD}	ψ_{MD}	ψ_{PD}	ψ_{MD}
Tilia platyphyllos						
28 July 2014	-0.20±0.00 a	-1.53±0.06 a	-0.23±0.03 b	-1.63±0.09 a	-0.33±0.03 c	-1.70±0.06 a
11 August 2014	-0.26±0.03 a	-1.16±0.09 a	-0.36±0.03 a	-1.40±0.15 a	-0.66±0.09 b	-1.80±0.06 b
14 September 2014	-0.33±0.03 a	-1.10±0.06 a	-0.43±0.09 a	-1.23±0.09 a	-1.06±0.16 b	-2.53±0.09 b
Pinus sylvestris						
11 August 2014	-0.60±0.06 a	-1.10±0.06 a	-0.76±0.06 a	-1.53±0.12 b	-0.70±0.11 a	-1.60±0.10 b
28 August 2014	-0.36±0.06 a	-0.86±0.03 a	-0.56±0.06 a	-1.26±0.03 b	-0.96±0.09 b	-1.56±0.09 c
6 October 2014	-0.36±0.03 a	-1.10±0.06 a	-0.83±0.09 b	-1.43±0.12 b	-0.76±0.12 b	-1.63±0.06 b

Table 2. Means and standard errors (n=3) of $\delta^{13}C$ values for bulk material and carbon compounds (cellulose, soluble sugars, myo-inositol, starch and lipids) extracted from leaves/needles, stem and coarse roots of both species at the end of the drought phase (10 weeks) and before labelling (14 September 2014 for *Tilia platyphyllos*, and 6 October 2014 for *Pinus sylvestris*). Carbon compounds were only extracted in control and severe drought treatments. Cellulose was only extracted from lignified organs (stem and coarse roots). Different letters indicate significant differences among watering regimes for each organ and carbon compound ($P < 0.05$).

	Watering	$\delta^{13}C$ (‰)					
Organ	regime	Bulk	Cellulose	Soluble sugars	Myo-inositol	Starch	Lipids
Tilia platyphyllos							
Leaves	Control	-26.85±0.09 a	—	-25.81±0.88 a	-28.50±0.79 a	-25.99±0.33 a	-27.99±0.46 a
	Moderate D	-26.28±0.14 b	—	—	—	—	—
	Severe D	-25.43±0.46 b	—	-19.73±0.41 b	-27.33±0.70 a	-23.22±0.31 b	-25.81±1.32 a
Stem	Control	-27.26±0.20 a	-26.00±0.20 a	-25.48±0.54 a	-30.94±0.34 a	-24.57±0.37 a	-27.63±0.17 a
	Moderate D	-26.00±0.21 b	—	—	—	—	—
	Severe D	-25.92±0.78 ab	-24.67±1.23 a	-19.80±0.47 b	-29.61±0.84 a	-23.62±0.86 a	-27.38±0.15 a
Coarse roots	Control	-26.51±0.14 a	-25.88±0.14 a	-23.71±0.78 a	-29.55±0.58 a	-24.54±0.11 a	-26.46±0.41 a
	Moderate D	-25.27±0.40 b	—	—	—	—	—
	Severe D	-25.09±0.85 ab	-23.93±1.88 a	-18.40±0.19 b	-26.08±0.78 b	-22.68±0.71 a	-24.81±3.00 a
Pinus sylvestris							
Needles	Control	-30.30±0.46 a	—	-31.66±0.06 a	-30.16±0.76 a	-29.33±0.55 a	-27.50±0.48 a
	Moderate D	-29.12±0.27 b	—	—	—	—	—
	Severe D	-29.16±0.20 b	—	-22.60±1.08 b	-29.46±0.12 a	-24.48±0.19 b	-26.87±0.03 a
Stem	Control	-30.16±0.50 a	-28.38±0.49 a	-30.19±0.16 a	-30.93±0.49 a	-29.24±0.57 a	-25.71±1.58 a
	Moderate D	-28.91±0.31 b	—	—	—	—	—
	Severe D	-29.21±0.19 ab	-28.07±0.11 a	-23.12±0.55 b	-31.20±0.24 a	-25.70±0.46 b	-24.44±1.55 a
Coarse roots	Control	-29.30±0.46 a	-27.78±0.57 a	-29.86±0.34 a	-31.68±0.12 a	-27.44±0.36 a	-26.00±0.16 a
	Moderate D	-27.88±0.30 b	—	—	—	—	—
	Severe D	-28.04±0.44 ab	-27.25±0.19 a	-22.45±0.47 b	-30.83±0.15 b	-26.00±0.35 b	-25.64±0.65 a

Table 3. Mean residence time (MRT) and half life time (HLT) in hours derived from the decay constant (λ) for recent assimilates (^{13}C excess) in leaves/needles of both species. The coefficient of determination (R^2) expresses the goodness of the fit for each watering regime, and P-values indicate that fitted coefficients (N_0, λ) were always significantly different from zero.

Organ	Watering regime	λ	Std error λ	MRT	HLT	R^2	P-value N_0	P-value λ
Tilia platyphyllos								
^{13}C excess bulk leaves	Control	0.0471	0.0047	21.20	14.69	0.95	<0.001	<0.001
	Moderate D	0.0454	0.0063	22.01	15.26	0.91	<0.001	<0.001
	Severe D	0.0389	0.0052	25.68	17.80	0.91	<0.001	<0.001
Pinus sylvestris								
^{13}C excess bulk needles	Control	0.0439	0.0048	22.76	15.77	0.94	<0.001	<0.001
	Moderate D	0.0407	0.0052	24.52	16.99	0.92	<0.001	<0.001
	Severe D	0.0259	0.0040	38.58	26.74	0.89	<0.001	<0.001

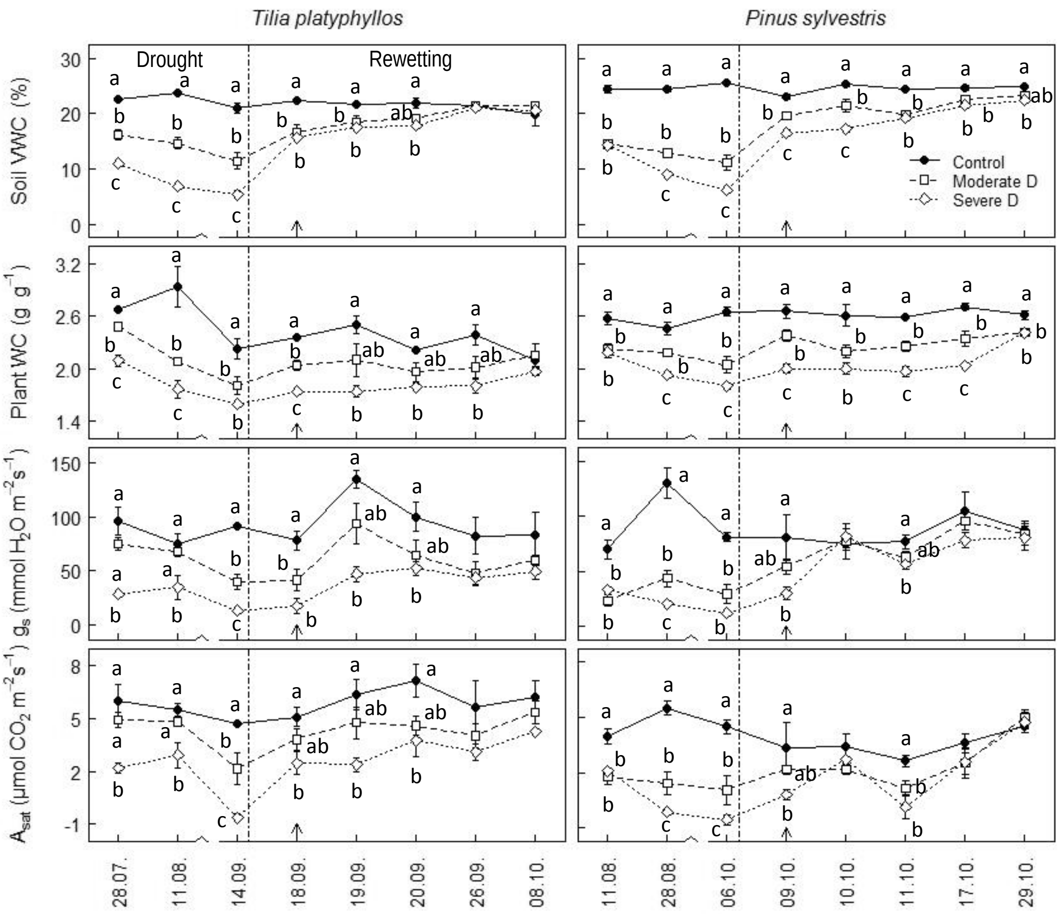

Figure 1. Temporal dynamics of soil volumetric water content (Soil VWC), whole-plant water content (Plant WC), stomatal conductance for H_2O (g_s) and light-saturated leaf photosynthesis (A_{sat}) for control (closed circles), moderate drought (open squares) and severe drought (open diamonds) treatments in both species. The dotted vertical lines denote the starting point of rewetting to field capacity after 10 weeks of moderate or severe drought (16 September 2014 in *T. platyphyllos*, and 7 October 2014 in *P. sylvestris*). Upward arrows on X-axes indicate the labelling date. Dates after labelling on X-axes correspond to the harvest time points: 1 day/24 h, 2 d/48 h, 8 d/ 192 h and 20 d/480 h. Error bars represent standard errors (n=3). Different letters indicate significant differences among watering regimes at each harvest time point ($P < 0.05$).

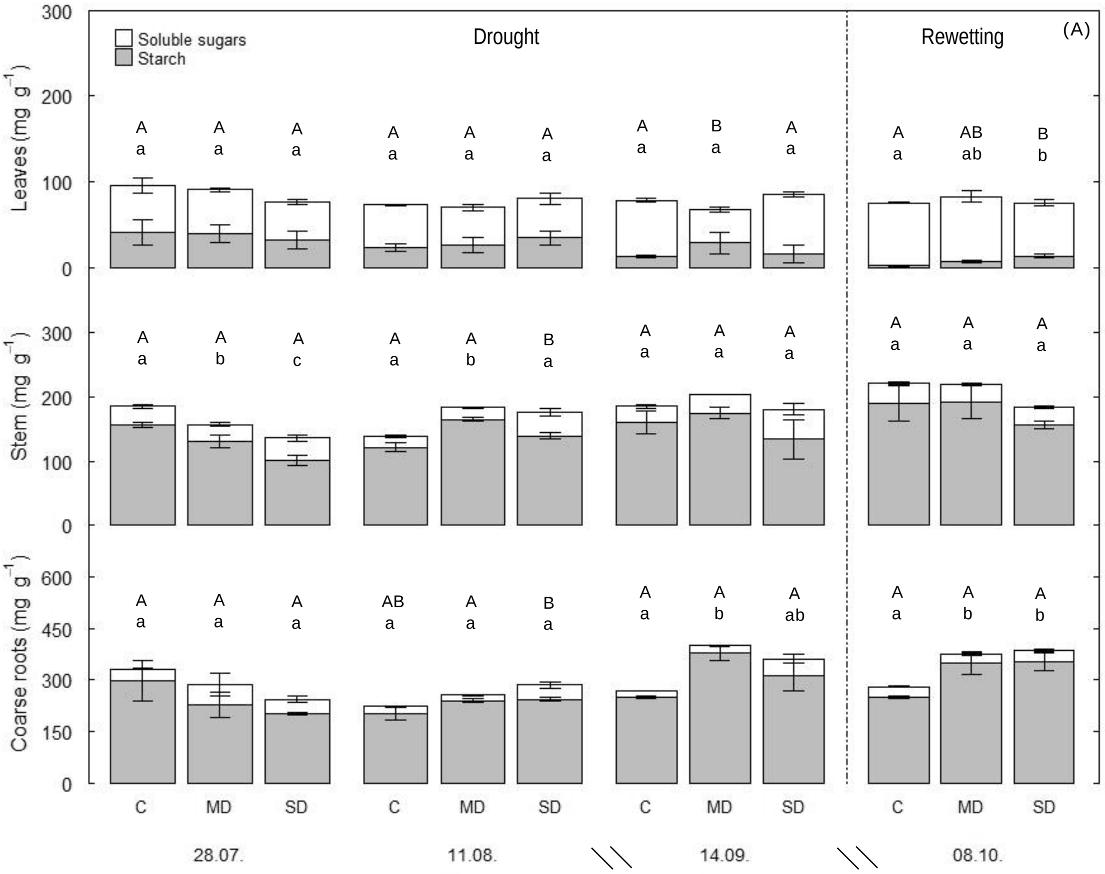

Figure 2A. Concentrations of soluble sugars and starch in leaves/needles, stem and coarse roots of *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings for control, moderate drought and severe drought treatments (C, MD and SD in X-axes, respectively). The dotted vertical line separates the drought (10 weeks) and the rewetting (20 days) phases of the experiment. Error bars represent standard errors (n=3). Different capital and lower case letters indicate significant differences among watering regimes at each harvest time point for soluble sugars and starch, respectively ($P < 0.05$).

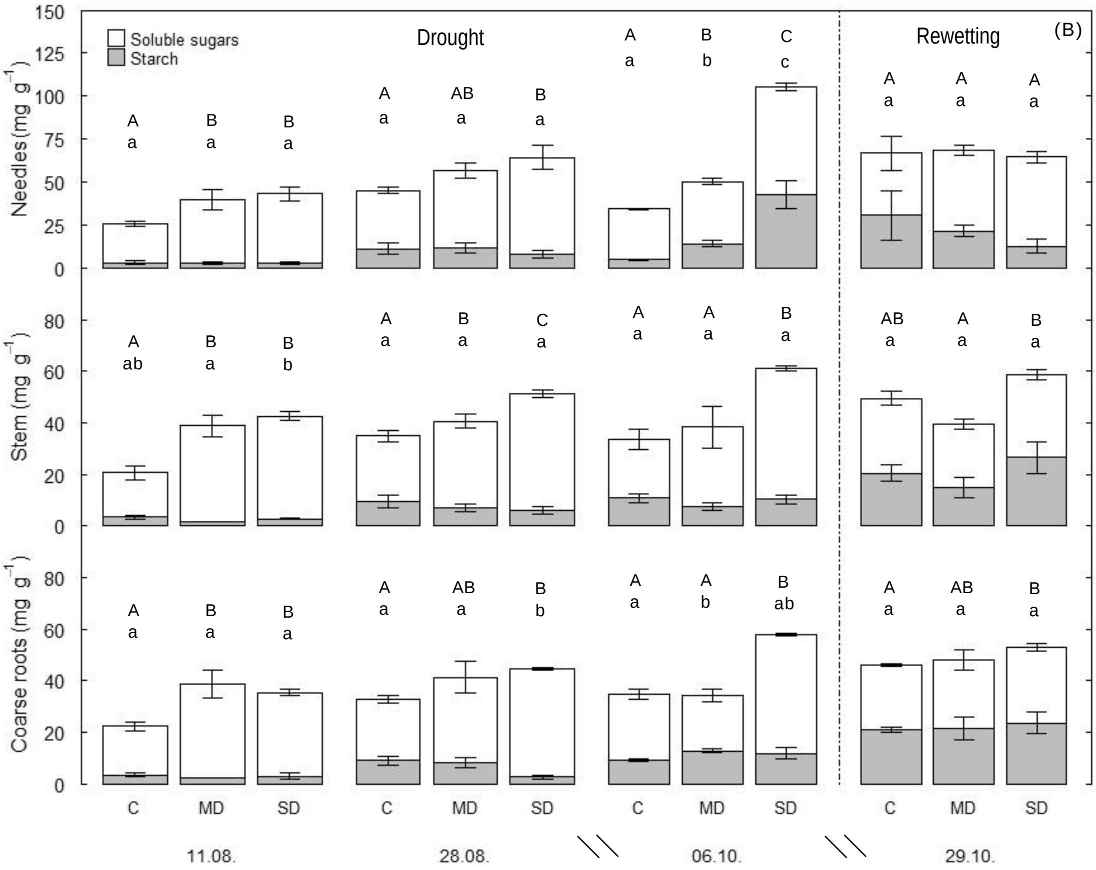

Figure 2B. Concentrations of soluble sugars and starch in leaves/needles, stem and coarse roots of *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings for control, moderate drought and severe drought treatments (C, MD and SD in X-axes, respectively). The dotted vertical line separates the drought (10 weeks) and the rewetting (20 days) phases of the experiment. Error bars represent standard errors (n=3). Different capital and lower case letters indicate significant differences among watering regimes at each harvest time point for soluble sugars and starch, respectively ($P < 0.05$).

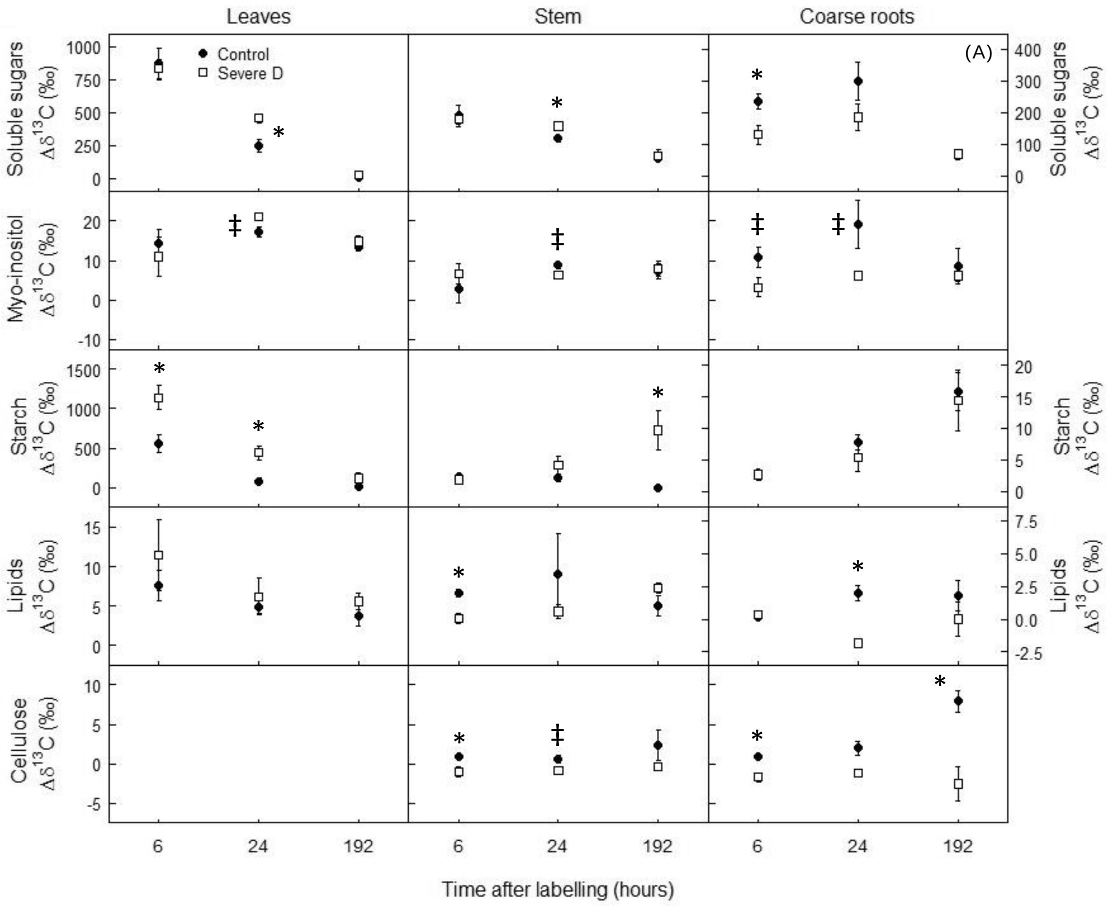

Figure 3A. Temporal dynamics of $\Delta\delta^{13}C$ ($\delta^{13}C_{sample}$ - $\delta^{13}C_{background}$) for soluble sugars, myo-inositol, starch, lipids and cellulose extracted from leaves/needles, stem and coarse roots of *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings 6 hours, 24 h/1 day and 192 h/8 d after the labelling (i.e. rewetting phase) in control (closed circles) and severe drought (open diamonds) treatments. $\delta^{13}C$ of cellulose was only measured in lignified organs (stem and coarse roots). Error bars represent standard errors (n=3). Left/right position of tick marks on y-axis inside each panel indicates relevant left/right y-axis scale. Significant differences between watering regimes at each harvest time point are given (*$P < 0.05$; ‡$0.05 < P < 0.1$).

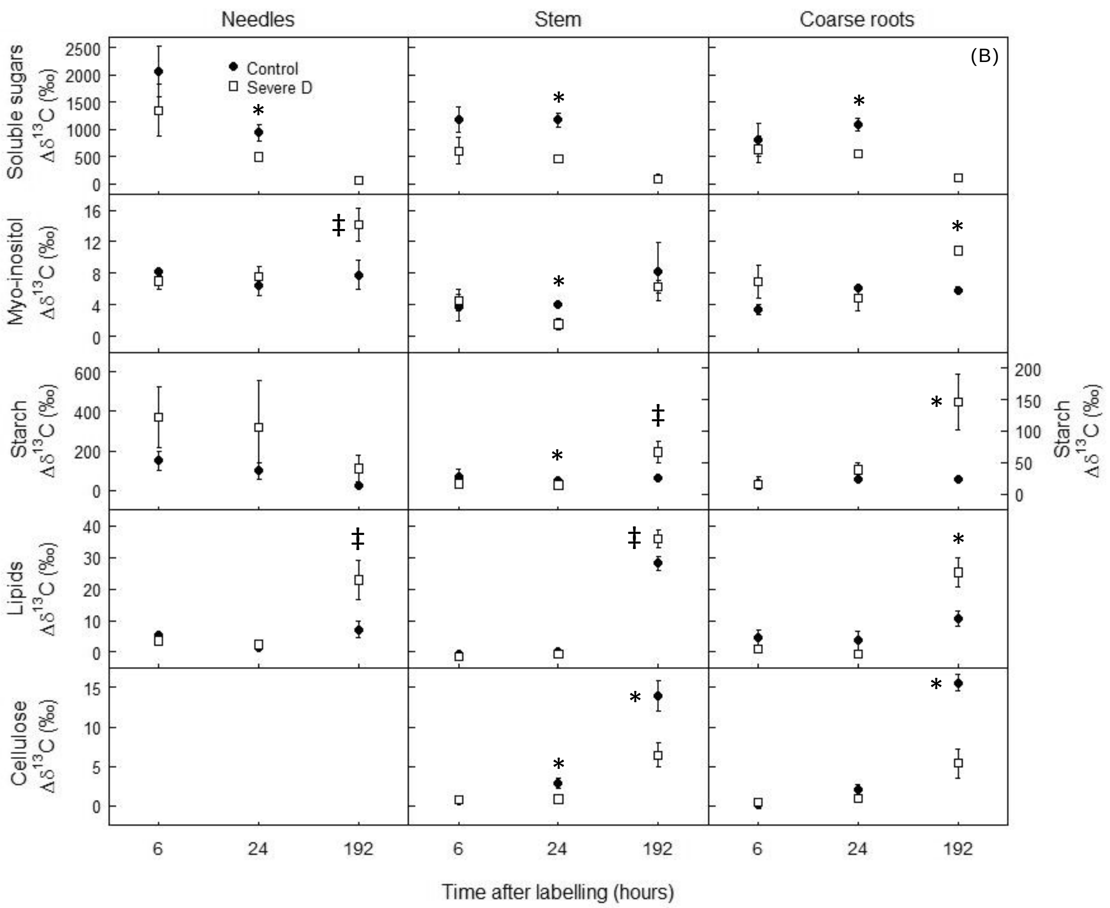

Figure 3B. Temporal dynamics of $\Delta\delta^{13}C$ ($\delta^{13}C_{sample}$ - $\delta^{13}C_{background}$) for soluble sugars, myo-inositol, starch, lipids and cellulose extracted from leaves/needles, stem and coarse roots of *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings 6 hours, 24 h/1 day and 192 h/8 d after the labelling (i.e. rewetting phase) in control (closed circles) and severe drought (open diamonds) treatments. $\delta^{13}C$ of cellulose was only measured in lignified organs (stem and coarse roots). Error bars represent standard errors (n=3). Left/right position of tick marks on y-axis inside each panel indicates relevant left/right y-axis scale. Significant differences between watering regimes at each harvest time point are given (*$P < 0.05$; ‡$0.05 < P < 0.1$).

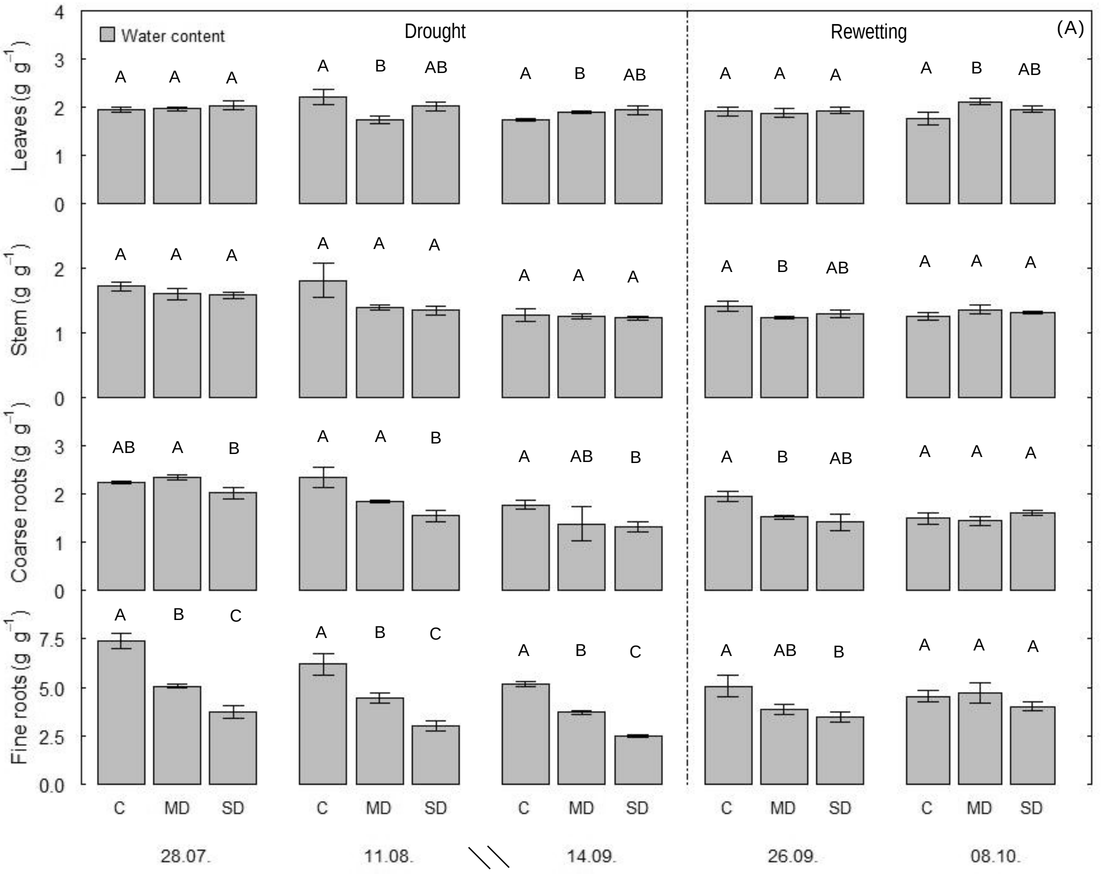

Figure S1A. Water content of leaves/needles, stem, and coarse and fine roots in *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings for control, moderate drought and severe drought treatments (C, MD and SD in X-axes, respectively). The dotted vertical line separates the drought (10 weeks) and the rewetting (20 days) phases of the experiment. Error bars represent standard errors (n=3). Different letters indicate significant differences among watering regimes at each harvest time point ($P < 0.05$).

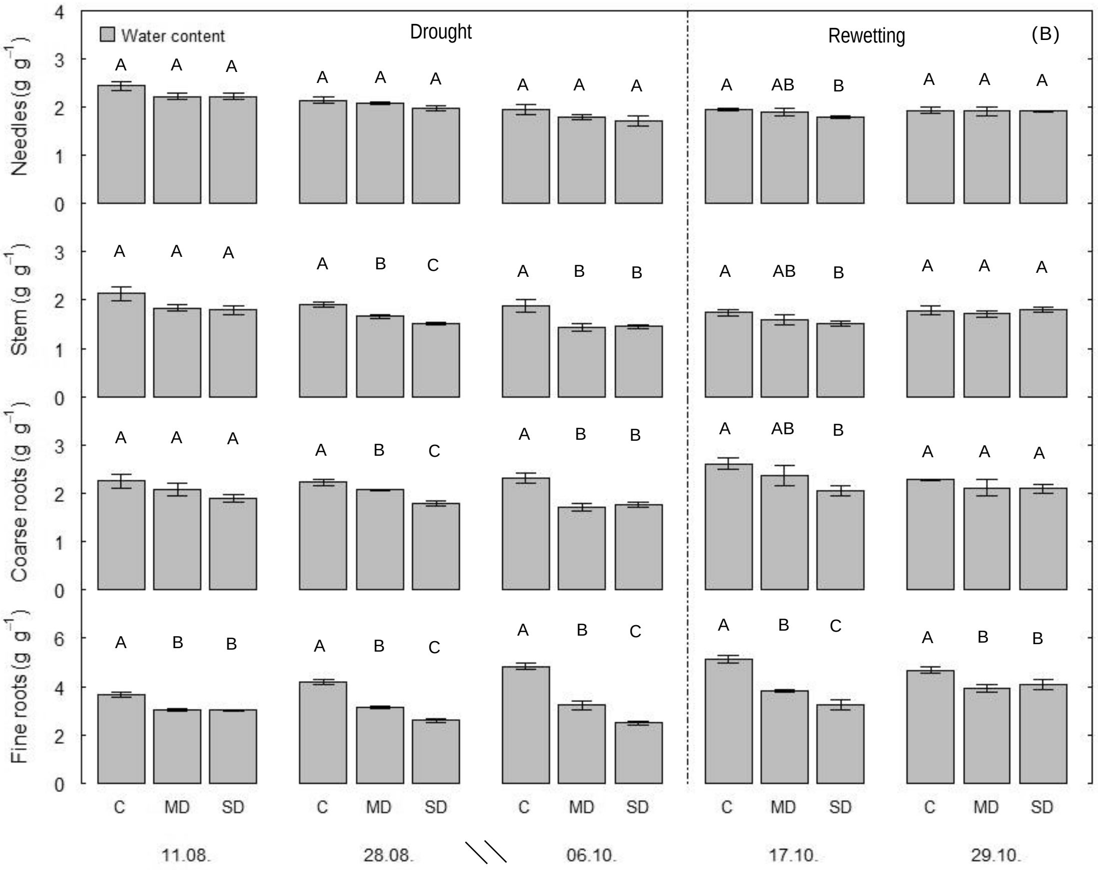

Figure S1B. Water content of leaves/needles, stem, and coarse and fine roots in *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings for control, moderate drought and severe drought treatments (C, MD and SD in X-axes, respectively). The dotted vertical line separates the drought (10 weeks) and the rewetting (20 days) phases of the experiment. Error bars represent standard errors (n=3). Different letters indicate significant differences among watering regimes at each harvest time point (P < 0.05).

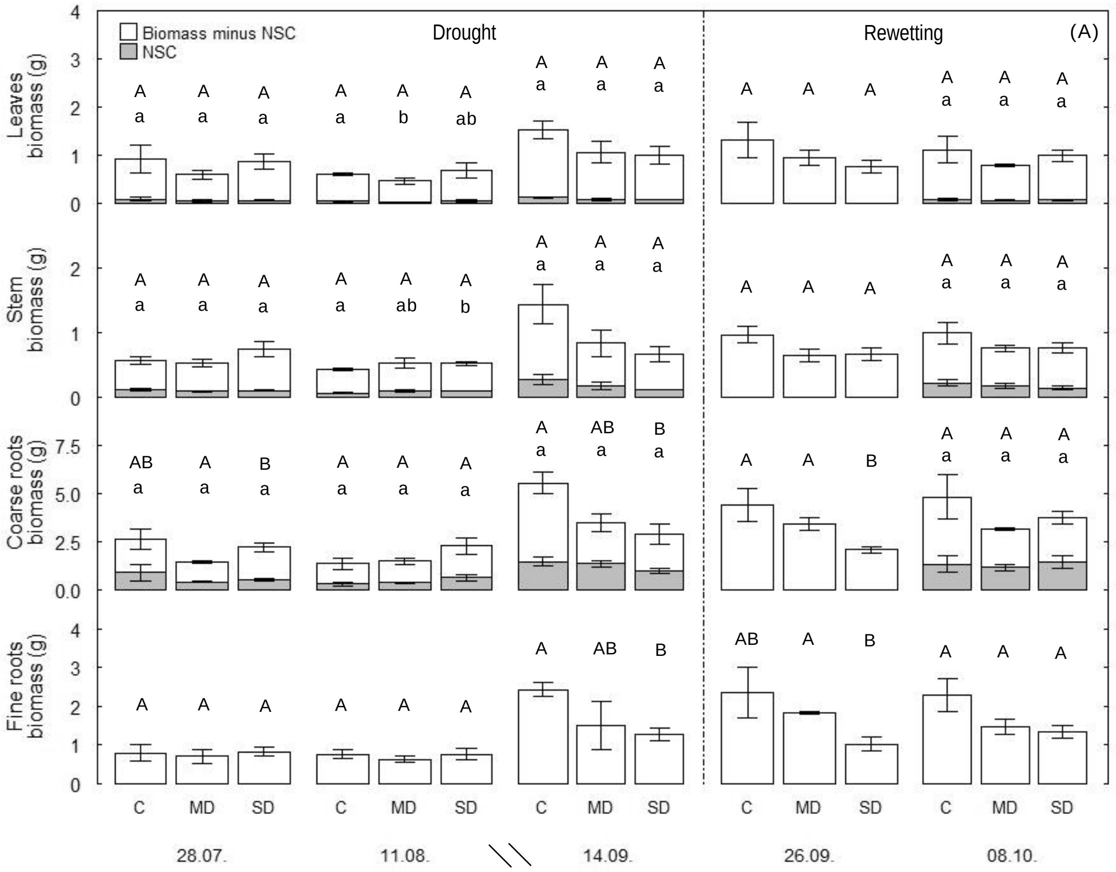

Figure S2A. Biomass of leaves/needles, stem and coarse roots in *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings for control, moderate drought and severe drought treatments (C, MD and SD in X-axes, respectively). The lower grey and upper white part of the stacked bars represent the absolute content of non-structural carbohydrates (NSC) and biomass excluding NSC, respectively. The dotted vertical line separates the drought (10 weeks) and the rewetting (20 days) phases of the experiment. NSC were not measured in fine roots and at harvest time point 8 d/192 h after labelling (26 September 2014 in *Tilia platyphyllos*, and 17 October 2014 in *Pinus sylvestris*). Error bars represent standard errors (n=3). Different capital and lower case letters indicate significant differences among watering regimes at each harvest time point for biomass and NSC, respectively ($P < 0.05$).

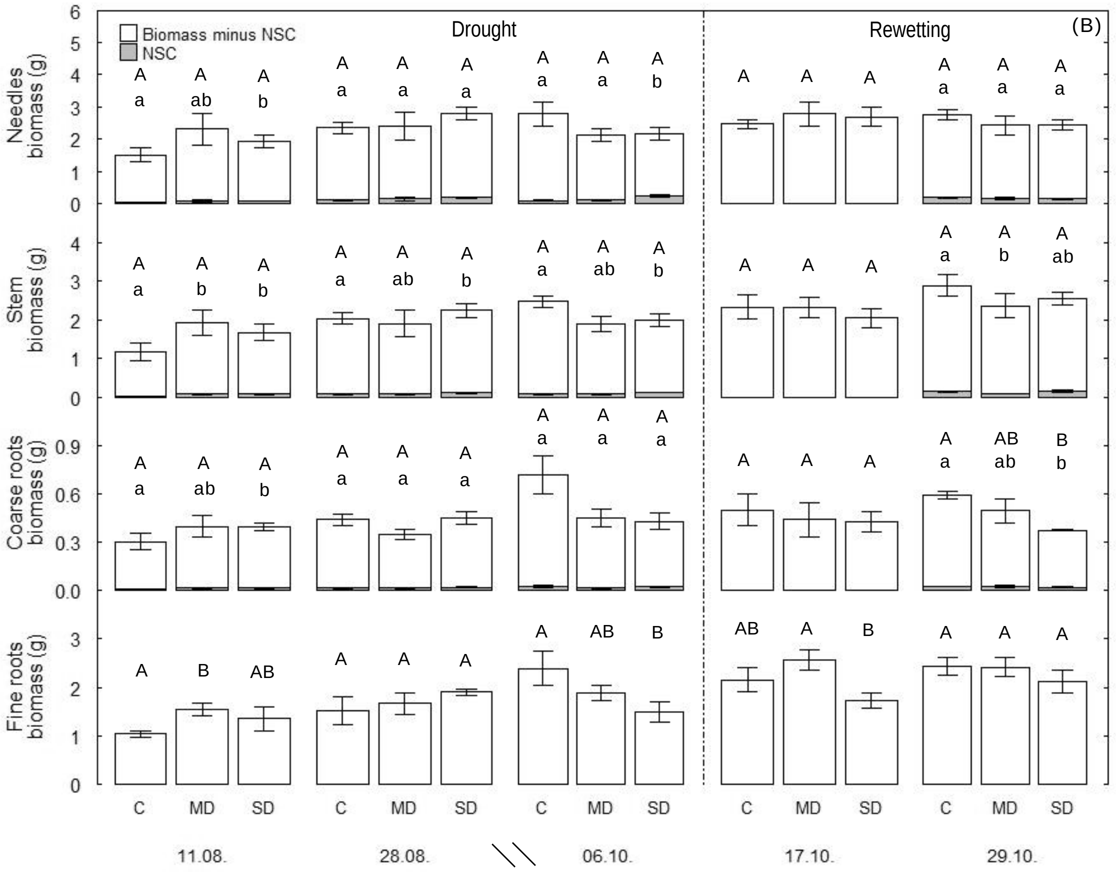

Figure S2A. Biomass of leaves/needles, stem and coarse roots in *Tilia platyphyllos* (A) and *Pinus sylvestris* (B) seedlings for control, moderate drought and severe drought treatments (C, MD and SD in X-axes, respectively). The lower grey and upper white part of the stacked bars represent the absolute content of non-structural carbohydrates (NSC) and biomass excluding NSC, respectively. The dotted vertical line separates the drought (10 weeks) and the rewetting (20 days) phases of the experiment. NSC were not measured in fine roots and at harvest time point 8 d/192 h after labelling (26 September 2014 in *Tilia platyphyllos*, and 17 October 2014 in *Pinus sylvestris*). Error bars represent standard errors (n=3). Different capital and lower case letters indicate significant differences among watering regimes at each harvest time point for biomass and NSC, respectively ($P < 0.05$).

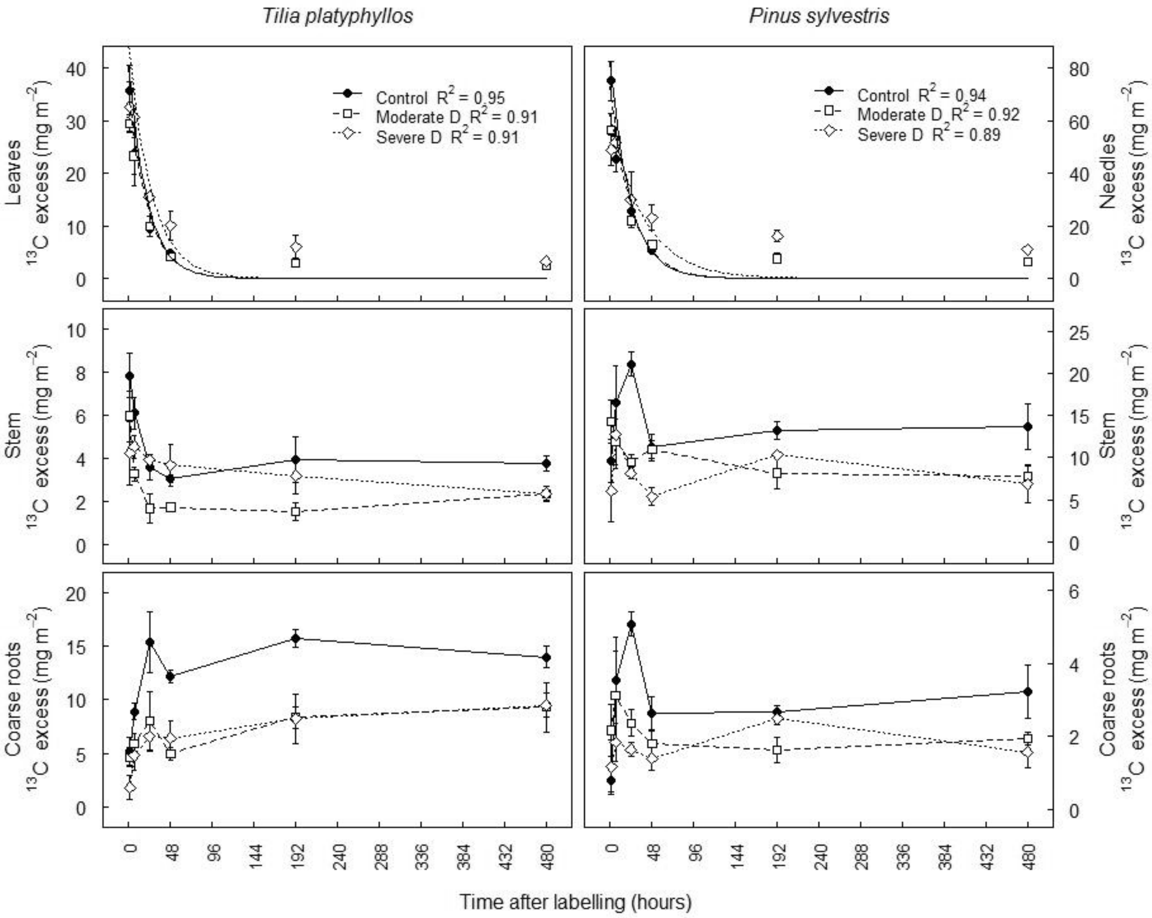

Figure S3. Dynamics of ^{13}C excess in the bulk material of leaves/needles, stem and coarse roots of both species for control (closed circles), moderate drought (open squares) and severe drought (open diamonds) treatments. Error bars represent standard errors (n=3). Exponential decay functions were fitted separately for control (solid line), moderate drought (coarse dashed line) and severe drought (fine dashed line) treatments in leaves/needles of both species. Lines in lower panels (for stem and coarse roots) simply join data points for a given treatment and are provided to aid interpretation.

Chapter 5

5. Synthesis and Outlook

The main objective of this dissertation was the investigation of the long-term responses of Scots pine to past and current climate variability, particularly to drought, at two edges of the Scots pine distribution in Europe (Valais, Switzerland) and Asia (North-Eastern Siberia, Russia). Both study sites are among the driest where Scots pine grows, with annual precipitation amounts of 605 and 238 mm, respectively, but with strongly different climate regimes, i.e. temperate vs. extremely continental. Additionally, the Siberian site has the source water influenced by permafrost thawing in summer, which may be changing due to ongoing warming and therefore needs careful consideration.

Specific emphases of this dissertation were the elucidation of Scots pine vulnerability to drought as well as a better understanding of the mechanisms responsible for its decline, applying a combination of methods with the main focus on stable isotopes. At the site in Switzerland, the study was complemented by results of an irrigation experiment to investigate the short-term responses of Scots pine not only to drought, but also to drought release. In addition, a greenhouse experiment with Scots pine seedlings helped to identify allocation strategies of freshly produced carbohydrates during drought and drought release.

In the Synthesis chapter, the main findings of the dissertation are discussed combining the results and conclusions from all experiments. The chapter additionally discusses methodological aspects and ends with an outlook for future research.

Key findings of this dissertation

Combination of radial growth and stable carbon isotope variability to identify responses of Scots pine to drought

Simultaneous measurements of radial stem growth and stable carbon isotopes in tree-ring cellulose were used at both study sites (Chapters I & III). The two variables are influenced by different environmental factors, and their combined analysis therefore potentially helps to identify physiological mechanisms operating under drought conditions. Stem growth is reflecting a complex mixture of processes, including the integrated availability of carbohydrates, sink activity and respiration losses, while the abundance of carbon isotopes is strongly related to stomatal responses, thus giving insights into the isohydric *vs.* anyisohydric behavior of the trees. This in return helps to identify plants' water-use efficiency.

At the study site in Switzerland (Pfynwald, Valais) two groups of trees, living (surviving) *vs.* recently died, were investigated. Here, I found that trees that died later initially featured lower growth and higher water-use efficiency, indicating a more conservative water-use strategy compared to the surviving individuals. This could have caused a lack of carbohydrates for further growth investment for these trees, as the freshly fixed carbon (C) was most likely used for maintenance. The most distinct decline of trees that recently died with respect to their radial stem growth and water-use efficiency started in the mid-1980s (Chapter I). These trees did not behave according to expectations in the final phase of decline: their water-use efficiency suddenly decreased, while growth was becoming lower. This may indicate that trees experienced disturbance of their stomatal conductance and photosynthetic activity. Furthermore, the drought-stressed trees had shorter needles and showed needle discoloration, which could be an additional reason of reduced metabolic activity. Such unexpected short-term behavior may be caused by a highly conservative strategy of tree physiology during a longer period prior to the distinct decline, which entailed a chronic weakening. This indicates that it is useful to consider two periods, i.e. prior and during the decline, for such studies (Chapter I).

At the study site in Russia (Central Yakutia, North-Eastern Siberia, Russia) I investigated living trees only over the period of 1900-2013, owing to the absence of a sufficient number of standing dead trees to produce a robust tree-ring chronology (Chapter III). Although precipitation amount at this site was even lower than at the Swiss site, I did not observe distinct changes in both radial stem growth and isotope variability, despite growth being generally low. However, I observed first indications of drought response in $\delta^{13}C$ values in tree-ring cellulose. Over the last three decades, when there was a strongly positive temperature trend, $\delta^{13}C$ values were strongly and positively correlated with temperature and negatively with precipitation amount, which was not the case before (Chapter III).

Therefore, my results show that differences in climate regimes and soil conditions play an important role in terms of the drought response, although both sites are commonly very dry

(Chapters I & III). The presence of permafrost, owing to the extremely continental climate, in fact may help Scots pine in Siberia to continue growing and possibly surviving at least in the next few decades, whereas Scots pines at the western edge of distribution have already been strongly affected by drought and there will likely be enhanced mortality if drought conditions will increase and intensify in the future (Chapters I & III).

The combined analysis of radial growth and carbon isotope variability in tree rings proved highly useful to study different tree response strategies to drought as highlighted in a conceptual diagram (Fig. 1). The diagram shows differences between surviving and dead trees in the phase before the distinct decline and eventual mortality, where the surviving trees are considered as the reference. This phase can comprise several decades and was termed here the “early life phase” (before the distinct decline). We do not consider here the final phase of decline, because there the behavior may change (Chapter I). Trees that have isohydric behavior during the early life phase tend to form smaller-sized cells, which results in lower radial stem growth and higher water-use efficiency owing to their conservative strategy. Therefore, trees following this strategy are falling in the upper, left-hand sector of the diagram, as was observed for Scots pine at the Swiss study site (Fig. 1). These trees are expected to be prone to carbon starvation rather than to hydraulic failure. This response may be species-specific, as a contrasting behavior was observed for Norway spruce (*Picea abies* L. Karst.) at two sites in Norway (Hentschel *et al.*, 2014). Trees that died later in their study had higher growth and were not following a strict water-use strategy, i.e. had lower water-use efficiency. Therefore, these trees were prone to hydraulic failure rather than carbon starvation, as expected for trees that form larger-sized cells. They also featured higher growth and did not use a conservative water-use strategy, and therefore such behavior would be more typical for species with anisohydric behavior.

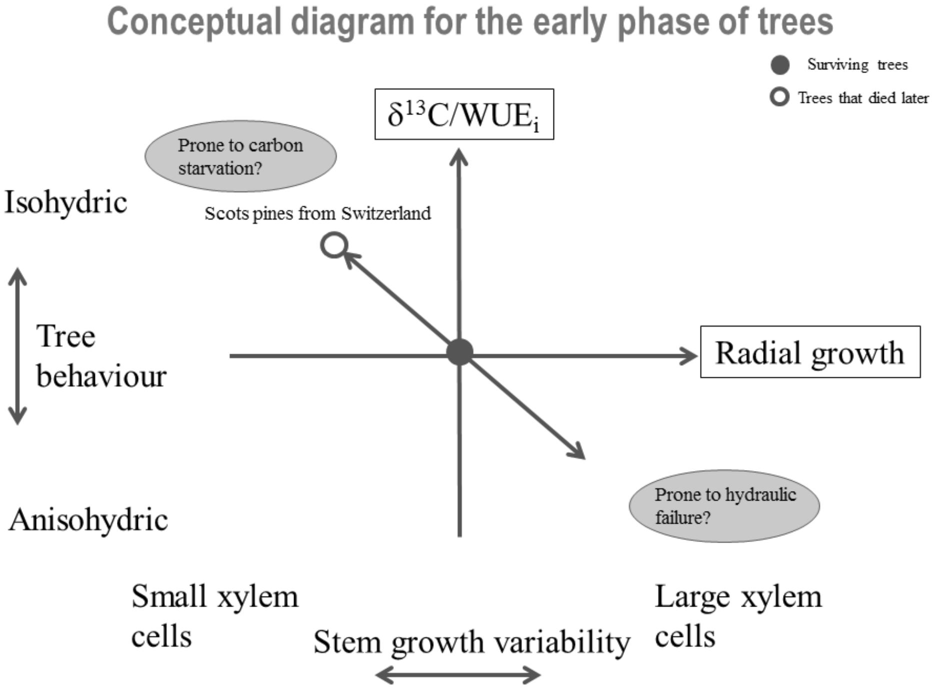

Figure 1. Conceptual diagram for surviving and dead trees before the distinct decline (“early life phase”). The x-axis reflects radial growth, whereas the y-axis $\delta^{13}C$ values in tree rings or qualitative intrinsic water-use efficiency (WUE_i). The blue circle indicates surviving trees and the white circle trees that died. Surviving trees are considered as the reference in this case. The location of the white circle on the diagram indicates the position of dead trees from my study site in Pfynwald (Valais, Switzerland).

Such a conceptual diagram, based on measurements of radial stem growth and carbon isotopes, could be helpful for an evaluation of isohydric *vs.* anisohydric tree strategies, and be possibly useful for estimatingthe tree’s fate under future enhanced drought, based on their behavior even before a distinct decline occurred.

Modeling of oxygen isotope variability in needles and tree rings

Interpretation of oxygen isotope variability in plant organic matter is still a greater challenge than of carbon isotope variability, particularly in tree-rings (Gessler *et al.*, 2014). This is because of the many fractionation processes at the soil and the leaf/needle level and during sugar transport and the formation of cellulose in the stem. It is often unclear how much of the leaf/needle enrichment isotopic signature is incorporated into the cellulose in comparison with the signature of the source water. To improve understanding and interpretation of oxygen isotope variability in leaves/needles, modelling of the leaf/needle water enrichment is a powerful tool. However, it is currently still a challenge to apply such models for improving our mechanistic understanding of the oxygen isotope signals in tree-ring cellulose as various involved processes and isotope fractionations are quantitatively not well known yet.

One of the goals of this dissertation was therefore to investigate the oxygen isotope variability in needles of the irrigated and non-irrigated control trees as well as in tree-ring cellulose of now-living and now-dead trees, applying a basic and a novel modelling approach (Chapter II). I observed that the novel modelling approach, based on the two-pool correction of the basic Craig-Gordon model (Craig & Gordon, 1965), was suitable for this purpose. This improved model (Roden *et al.*, 2015, Song *et al.*, 2015) much better explained the observed greater needle water enrichment in the irrigated trees, compared to the drought-stressed non-irrigated trees. This pattern can be explained by the higher degree of needle compartmentalization, where the water volume of the xylem relative to the total needle water (ϕ) is relatively large, owing to suberization and tissue shrinkage around the xylem (Chapter II). This was often observed under drought stress (e.g., discoloration, decrease in length and thickness of existing needles) as well as for old needles (Roden *et al.*, 2015). Applying the same model on tree-ring cellulose, I was also able to explain the greater enrichment of $\delta^{18}O$ values in the now-living trees compared to the now-dead ones, particularly during the period of distinct decline of the now-dead trees. Moreover, I was able to identify the ϕ values for the period of modelling, which may thus serve as an indicator of decline and eventual mortality for prediction purposes in similar studies (Chapter II).

Thus, I have demonstrated that needle morphological changes are reflected in the $\delta^{18}O$ of stem cellulose, which considerably adds to our knowledge on the factors influencing tree-ring isotope variations in general. Although a detailed modelling of $\delta^{18}O$ is not always possible because of a lack of information for remote sites, my result is important information for the interpretation in other studies on drought impacts. Declining trends in $\delta^{18}O$ could be an indication of tree decline. Although at our study site in Siberia we did not have trees that were declining, a modelling approach would also be useful for the interpretation of stable oxygen variability in those trees (Chapter III). Trees at this site are strongly influenced by the source water. The influence of the soil water is even greater under drought conditions, when melted permafrost water is available and taken up by the trees. As this water is isotopically depleted, an “inverse” isotope effect is observed, as lower $\delta^{18}O$ is found under warmer conditions. Under such conditions, soil water modelling may be pivotal for understanding tree-ring isotope variations (Saurer *et al.*, 2016). My findings indicate that there is no unique model for all species and all site conditions and the existing models need to be adapted to the species- and site-specific conditions.

Irrigation and drought release

The effect of drought on tree physiology can principally be investigated in two ways under controlled conditions: trees can either be subjected to drought or just the opposite can be done by supplying them with additional water (irrigation). The latter approach may have some advantages particularly for trees that are already under severe drought stress. Irrigation studies further can provide insights whether drought effects are irreversible or not. In this dissertation, the irrigated trees showed rapid increases in radial growth in the growing season following the beginning of the irrigation experiment in the Pfynwald (Chapter I). Depleted values of $\delta^{13}C$ in tree-ring cellulose were also observed in the same growing season compared to the non-irrigated control trees, indicating an increase in either stomatal conductance or photosynthetic activity. These results indicate that water availability was indeed a limiting factor for these trees and additional water input could improve their growth and health status. However, it is still not clear how trees allocate carbon during drought and drought release. Answering this question for mature trees in a forest is a big challenge, owing to methodological difficulties of pulse-labelling experiments in the field and high expenses to track freshly produced carbohydrates in various tree compartments for mature individuals.

However, an experiment with seedlings under controlled conditions is more practical and could provide insights on this issue. In this dissertation, a drought experiment with 3-year-old seedlings of *Tilia platyphyllos* and *Pinus sylvestris* with subsequent drought release (i.e., re-watering) after ca. 2 months of drought was carried out in a greenhouse. The results showed that growth and accumulation of soluble sugars in drought-stressed *P. sylvestris* seedlings were independent of each other. Therefore, I conclude that the formation of non-structural carbohydrates in this species cannot simply be considered as a passive overflow process under severe drought conditions (chapter IV). The control seedlings invested their carbon more into growth (cellulose) as well as to a smaller degree into storage (lipids) in the stem and coarse roots. During drought release, the drought-stressed seedlings of both species rapidly increased stomatal conductance and photosynthetic activity, albeit with a few days of delay for *T. platyphyllos*. Even during drought release, the drought-stressed *P. sylvestris* seedlings continued allocating carbon to storage at the cost of growth. Such behavior may be due to the so-called “drought memory effect” (Galiano *et al.*, 2017), when seedlings prioritize the investment of carbon into storage to gain carbon for future growth and survival, if drought would continue, as observed in the case of *T. platyphyllos* (Chapter IV). This experiment helped to identify carbon allocation strategies during drought and drought release, particularly differences between a broadleaf and a coniferous species. However, it must be kept in mind that mature trees may behave differently from seedlings and, thus, further investigation of mature tree responses during and after drought remains necessary.

Methodological aspects

Combination of different methods – multi-proxy approach

To answer the questions of this dissertation, I used combined measurements of radial growth, stable isotopes of carbon and oxygen (Chapters I, II & III), and compound-specific isotope analysis (CSIA) of soluble sugars, starch, lipids and cellulose (Chapter IV). In addition, I also used gas exchange measurements (transpiration, stomatal conductance, photosynthetic rate) and needle morphological data (Chapters II & IV). An approach based on a wide range of different techniques is important for tackling a complex question like the drought response of trees, but it has rarely been applied in the literature so far, at least not to this extent.

To analyse the data, I also applied diverse methods, including isotope fractionation modelling and statistical anaylsis. To investigate the relationships between climate and tree-ring variables, I performed correlation analyses with various climate variables and tree-ring parameters, both with raw data and after removing the age trends as well as trends unrelated to climate. In addition, 30-year averaged moving correlations were used to investigate the temporal relationship between tree-ring parameters and climate variables. Therefore, the results of this dissertation are unique in providing insights not only on the long-term, but also the short-term (irrigation experiment) tree responses to climate change, particularly to drought conditions, using a combination of multiple tree-ring parameters. Furthermore, the results of this work identified the utility of recent modelling approaches of needle water enrichment as well as stable oxygen isotope variability in tree-ring cellulose. The modelling conducted in my thesis can therefore be considered as an important and powerful tool compared to purely statistical approaches. Moreover, the results of CSIA helped to improve our understanding on carbon allocation mechanisms during drought and drought release.

Study sites

The study sites for this dissertation are located at the western (Pfynwald, Valais, Switzerland) and eastern (Central Yakutia, North-eastern Siberia, Russia) edges of the continuous range of Scots in Eurasia. These sites obviously have significantly different climate regimes, but both are very dry and feature sandy soils. Although it may be more strategically consistent to analyse two forests with comparable site conditions, our goal was to investigate responses of Scots pines to drought conditions at sites where climate change is quite pronounced already now and more severe drought events are expected in the future. I propose that studying rather contrasting sites is useful for better understanding Scots pine physiology, which is a species surprisingly wide-spread across Eurasia, and that this is more likely to be more relevant, if different site conditions are compared.

Greenhouse experiment

Indoor experiments with seedlings have proven to be of great value for improving our understanding of the mechanisms underlying tree responses to drought and re-watering under controlled conditions (Ruehr *et al.*, 2009, Blessing *et al.*, 2015, Pflug *et al.*, 2015, Hagedorn *et al.*, 2016). Such experiments are relatively easy to design, set up and perform, but it is still a challenge, if not impossible, to carry out such studies in the long-term. Normally, they last from a few months up to a few years at best. However, the size of seedlings allows for analyzing all tree compartments (leaves/needles, stem, coarse and fine roots) simultaneously, and to apply CSIA individually, which is more challenging with large trees. Although it is difficult to extrapolate results found for saplings in the greenhouse to adult trees in the field, such experiments are still very useful for the identification of carbon allocation mechanisms during drought and drought release.

Outlook

Although significant progress has been made in this dissertation about understanding the drought responses of Scots pine, there are still many unsolved questions in the realm of its physiology and climate change. As a recommendation for future studies, I definitely endorse the application of similar multi-proxy approaches at other study sites with other species. Different species have very different drought response strategies, as observed, for example, in my pot experiment with *Tilia platyphyllus* and *Pinus sylvestris*, implying that my findings cannot be directly applied to other species.

I also recommend taking into account the importance of the source water for modelling stable oxygen isotope variability in needles and especially in tree-ring cellulose. Therefore, a frequent sampling of the soil and additionally precipitation water at the study site is recommended to investigate the seasonal changes of $\delta^{18}O$ values in any kind of plant organic matter. For study sites such as mine in Siberia, I also recommend to measure $\delta^{18}O$ values of the soil down to a well-accessible depth, and possibly in the permafrost itself. My novel finding that needle-morphological changes are reflected in the $\delta^{18}O$ signature of tree-rings may also be tested in other drought-mortality studies where a group of declining and surviving trees is compared. It would also be relevant to know whether my finding for Scots pine from Switzerland is observed in other species and at other sites as well.

Lastly, the application of the CSIA of individual soluble sugars with mature trees, rather than "just" with seedlings, may be of great value for investigating tree decline and mortality mechanisms under drought conditions, although the analysis is time-consuming and costly. Such procedures could benefit from complementary measurements of tree hydraulic properties, which were unfortunately not applied in my dissertation.

In fact, a pulse-labelling experiment with mature trees in a forest in combination with radial growth measurements would be of great value. As climate change will almost certainly be progressing in the next decades, it remains important to investigate and ascertain the adaptation potential of tree species to changes of climatic conditions. Considering the local, regional and global importance of forests, this is clearly a challenge not only for science, but for society in general.

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