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# TREE RESPONSES TO NITROGEN DEPOSITION IN A CHANGING CLIMATE: USING CARBON AND NITROGEN STABLE ISOTOPES IN TREE-RINGS AND FOLIAGE

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GREGORY TOMLINSON

MSc Conservation & Forest Protection, Imperial College London

born on 02.08.1988
citizen of United Kingdom

accepted on the recommendation of

Prof. Dr. Nina Buchmann, examiner
Dr. Peter Waldner, co-examiner
Dr. Rolf Siegwolf, co-examiner

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# Summary

Anthropogenic emissions of greenhouse gases, particularly carbon dioxide, have induced fundamental changes in both global and regional climate conditions. At the same time, global nitrogen (N) availability has risen dramatically as a result of both increased fertilizer use and increased emissions of N oxides as a by-product of fossil fuel combustion. These past changes in N deposition rates and climate, particularly temperature, have had large impacts on forest ecosystem productivity, and will further impact forests throughout the 21st century. Tree isotopic composition of both carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) are often used in a multi-parameter approach with tree growth measurements to investigate tree response to past environmental changes. The application of tree $\delta^{15}N$ in environmental investigations is, however, rare in comparison to those using $\delta^{13}C$ or $\delta^{18}O$. This is partly due to technical problems associated with very low N concentrations in tree-rings (relative to C), while there are also uncertainties concerning our understanding of the movement of N within the tree. Furthermore, the lack of long-term N deposition datasets has made the response of tree growth to past changes in N availability, as well as interactions with changing climate conditions, difficult to determine.

This thesis consists of three studies aimed at determining the suitability of the application of tree $\delta^{15}N$ in environmental investigations, as well as assessing the past response of both tree growth and physiology to changes in N deposition and climate. This was achieved using tree-ring growth data and tree-ring and foliage isotopic data of $\delta^{15}N$ and $\delta^{13}C$ from both European beech (*Fagus sylvatica* (L.)) and Norway spruce (*Picea abies* (L.) Karst), together with selected environmental variables, from a number of sites across Switzerland.

*Chapter 1* describes a study in which trees under experimentally elevated N deposition since 1995, labelled with $^{15}N$ in 1995/6, and felled in 2010 were used to determine the extent of the movement of N across tree-rings of *P. abies*. Furthermore, the necessity of the extraction of mobile N compounds from wood material prior to N isotope analysis was also tested. Finally, it was possible to determine whether tree growth of these trees had increased as a result of the

experimentally increased N deposition. The $^{15}$N label was found throughout the tree sapwood, in tree-rings formed both before (back to 1951) and after (up to 2010) the label application, demonstrating the bidirectional mobility of N within the tree stem. Label recovery, however, was higher in tree-rings formed after labelling, indicating the trees actively metabolised N from previously formed tree-rings towards the newer tree-rings to fulfil N sink demands, as well as the continued uptake of the $^{15}$N label from the soil. We found no significant difference in the isotopic composition of either $\delta^{15}$N or $\delta^{13}$C values in tree-rings that had undergone extraction of mobile N compounds compared to control tree-rings. Finally, there was no increase in radial stem growth in response to the increased N deposition, suggesting the measured trees may not be N-limited. The high mobility of N within the tree stem indicates that the use of tree-ring $\delta^{15}$N in environmental investigations is difficult, while the removal of these mobile N compounds has no effect on the tree-ring N isotopic composition.

In *Chapter 2*, the spatial and temporal intra-tree variation of $\delta^{15}$N and $\delta^{13}$C was investigated using tree-ring chronologies and archived foliage samples of both *F. sylvatica* and *P. abies* from six long-term monitoring sites. This was complemented with selected environmental variables in order to determine the suitability of using tree-ring $\delta^{15}$N as a proxy for foliar $\delta^{15}$N in environmental investigations. Tree-ring $\delta^{15}$N was significantly less negative than foliar $\delta^{15}$N for both species, partly attributed to N recycling processes required to fulfil high N demands in foliage. While tree-ring $\delta^{13}$C was significantly related to foliar $\delta^{13}$C in *P. abies*, there was, however, no significant relationship between tree-ring and foliar $\delta^{15}$N in either species. The difficulties in applying tree $\delta^{15}$N values to study tree response to changing environmental conditions were emphasised by the fact that none of the environmental variables considered, including N deposition, were able to explain the variation in either tree-ring or foliar $\delta^{15}$N. Together, these results suggest that the use of tree-ring $\delta^{15}$N as a proxy for foliar $\delta^{15}$N in long-term environmental investigations would be unsuitable.

*Chapter 3* focused on the response of tree growth and physiology to changing environmental conditions during the 20th century. This was done using tree growth

measurements of both *F. sylvatica* and *P. abies* together with long-term datasets of selected environmental variables, including modelled N deposition, at eight sites across Switzerland. Furthermore, we used tree-ring $\delta^{13}$C values of *F. sylvatica* to investigate changes in tree intrinsic water-use efficiency ($WUE_i$) to changes in N deposition, temperature and precipitation at one mixed forest site (Lägeren) during the 20th century. Tree growth often increased significantly with increased precipitation and decreased significantly with increased temperature at the eight sites, highlighting the importance of water availability for tree growth in Switzerland. One high altitude site demonstrated increased tree growth with increasing temperatures, suggesting temperatures are below optimum for tree growth at this site. While tree growth increased significantly with N deposition at all eight sites, $WUE_i$ (the ratio of net photosynthesis to mean stomatal conductance) also increased significantly with both N deposition and summer temperature at Lägeren. High $WUE_i$ between 1960 and 1990 was attributed to increased drought-stress associated with N-induced increases in tree growth. High $WUE_i$ values in the more recent decades, however, were attributed to reduced stomatal conductance associated with high temperatures and subsequent increases in vapour pressure deficit (VPD). These results imply that the primary environmental driver of high $WUE_i$ at Lägeren has shifted from N deposition between 1960 and 1990 to temperature in the more recent decades, and that trees at Lägeren may become more susceptible to drought as temperatures increase during the 21st century, despite decreases in N deposition.

The results of this thesis demonstrate the limitations of using tree-ring $\delta^{15}$N in environmental investigations, primarily due to the high movement of N within the tree. In addition, with a long-term N deposition dataset, it was possible to show that the increase in N deposition in the second half of the 20th century significantly altered both tree growth and physiology.

# Zusammenfassung

Die anthropogenen Emissionen von Treibhausgasen, insbesondere von $CO_2$, haben sowohl regional als auch weltweit zu grundlegenden Veränderungen der Klimabedingungen geführt. Gleichzeitig ist die globale Verfügbarkeit von Stickstoff (N) durch die vermehrte Nutzung von Düngemitteln und dem erhöhten Ausstoss von Stickoxiden bei der Verbrennung fossiler Brennstoffe dramatisch angestiegen. Diese klimatischen Veränderungen, vor allem ansteigende Temperaturen und erhöhte Stickstoffeinträge, haben erheblichen Einfluss auf die Produktivität von Waldökosystemen, und werden diese auch im 21. Jahrhundert weiter beeinflussen. Die Isotopenverhältnisse von Kohlenstoff und Sauerstoff ($\delta^{13}C$ und $\delta^{18}O$) in Bäumen werden oftmals in Multi-Parameter-Ansätzen und in Kombination mit Messungen des Baumwachstums dazu verwendet, um die Reaktionen von Bäumen auf zurückliegende Umweltveränderungen zu untersuchen. Das Isotopenverhältnis von Stickstoff ($\delta^{15}N$) in Bäumen wird in Umweltstudien im Gegensatz dazu vergleichsweise selten herangezogen. Teilweise ist dies auf technische Probleme bei der Bestimmung des $\delta^{15}N$ bei den (im Vergleich zu C) sehr niedrigen Stickstoffkonzentrationen in den Jahrringen zurückzuführen. Zudem bestehen Unsicherheiten bezüglich der Verteilung und der Verlagerung von Stickstoff innerhalb der Bäume. In Ermangelung langfristiger Erhebungen zur N-Deposition sind die Reaktionen des Baumwachstums auf zurückliegende Veränderungen in der Stickstoffverfügbarkeit, sowie allfällige Wechselwirkungen mit veränderten Umweltbedingungen, schwierig zu ermitteln.

Die vorliegende Doktorarbeit setzt sich aus drei Studien zusammen, welche zum Ziel hatten, die Eignung von $\delta^{15}N$ in Bäumen bei Umweltstudien zu untersuchen. Weiterhin sollten die Reaktionen von Baumwachstum und -physiologie auf die Veränderung von N-Deposition und Klima in der Vergangenheit abgeschätzt werden. Dies wurde unter Verwendung von Wachstums- und Isotopendaten ($\delta^{15}N$ und $\delta^{13}C$) der Baumarten Buche (*Fagus sylvatica* (L.)) und Fichte (*Picea abies* (L.)) und unter Berücksichtigung ausgewählter Umweltvariablen an verschiedenen Standorten in der Schweiz analysiert.

Das *erste Kapitel* beschreibt eine Studie, in der Fichten seit 1995 unter experimentell erhöhtem Stickstoffeintrag wuchsen, und 1995/96 mit $^{15}$N markiert wurden. Die Bäume wurden 2010 gefällt, und das Ausmass der Mobilität von Stickstoff zwischen den Jahrringen der Bäume untersucht. Weiter wurde getestet, ob die Extraktion von mobilen Stickstoffkomponenten vor der Analyse von $\delta^{15}$N in Holz sinnvoll ist. Zuletzt konnte untersucht werden, ob die Bäume aufgrund des erhöhten Stickstoffeintrages eine Zunahme im Wachstum zeigten. Das $^{15}$N Label wurde im Splintholz des Baumes gefunden, sowohl in Jahrringen vor (bis 1951) als auch nach (2010) dem Markierungsereignis. Dies zeigt deutlich die bidirektionale Mobilität von Stickstoff im Stamm. Das höchste Isotopensignal konnte in jenen Jahrringen gefunden werden, welche nach dem Markierungsereignis gebildet wurden. Dies deutet darauf hin, dass Stickstoff in die neueren Jahrringe verlagert wurde, um damit dem erhöhten N-Bedarf in diesem Gewebe gerecht zu werden. Zudem lässt sich ableiten, dass die Bäume weiterhin $^{15}$N Label aus dem Boden aufnahmen. Es konnte weder für $\delta^{13}$C noch für $\delta^{15}$N ein signifikanter Unterschied zwischen Proben gefunden werden, bei denen mobile Stickstoffkomponenten belassen oder entfernt wurden. Ausserdem konnte keine Zunahme des Durchmesserwachstums der unter erhöhtem Stickstoffeintrag wachsenden Bäume detektiert werden, ein Hinweis darauf, dass die untersuchten Bäume am Standort nicht N-limitiert waren. Die hohe Mobilität des Stickstoffes innerhalb der Baumstämme macht deutlich, dass die Analyse von $\delta^{15}$N in Umweltstudien einer sorgfältigen Interpretation bedarf. Die Entfernung mobiler Stickstoffverbindungen aus den Stammproben hatte jedoch keinen Einfluss auf die Isotopenkomposition von $\delta^{15}$N.

Im *zweiten Kapitel* wurde die räumliche und zeitliche Variation von $\delta^{15}$N und $\delta^{13}$C in von Jahrring-Chronologien und archivierten Blattproben von *F. sylvatica* und *P. abies* an sechs Langzeituntersuchungsflächen erforscht. Diese Daten wurden mit ausgewählten Umweltvariablen ergänzt, um zu untersuchen, ob $\delta^{15}$N Werte in Jahrringen als indirekter Indikator für $\delta^{15}$N Werte in Blättern verwendet werden können. In beiden Arten wurden in den Jahrringen signifikant weniger negative $\delta^{15}$N Werte als in den Blättern nachgewiesen. Dies kann teilweise auf N-Recycling Prozesse zurückgeführt werden, die wichtig sind, um den hohen Stickstoffbedarf der

Blätter zu decken. Während in *P. abies* ein signifikanter Zusammenhang zwischen $\delta^{13}C$ in Blättern und Jahrringen gefunden wurde, konnte ein Zusammenhang zwischen $\delta^{15}N$ in Blättern und Jahrringen in keiner der beiden Arten bestätigt werden. Die Schwierigkeiten der Verwendung von $\delta^{15}N$ zur Untersuchung der Reaktion von Bäumen auf sich ändernde Umweltbedingungen werden dadurch unterstrichen, dass keine der untersuchten Umweltvariablen (eingeschlossen der N-Deposition) die Variation in $\delta^{15}N$ von Blättern oder Jahrringen erklären konnte. Zusammenfassend legen diese Ergebnisse nahe, dass die Verwendung von $\delta^{15}N$ in Jahrringen als Indikator für $\delta^{15}N$ in Blattmaterial in Langzeitstudien wenig geeignet ist.

Das *dritte Kapitel* der Dissertation untersuchte die Veränderung von Baumwachstum und -physiologie aufgrund von sich ändernden Umweltbedingungen im 20. Jahrhundert. Dafür wurden Wachstumsdaten der beiden Baumarten *F. sylvatica* und *P. abies* und Langzeiterhebungen ausgesuchter Klimavariablen, inklusive modellierter N-Depositionsdaten, an acht Standorten in der Schweiz analysiert. Zudem wurden anhand von $\delta^{13}C$ Werten in Jahrringen der Buche Veränderungen der intrinsischen Wassernutzungseffizienz (water-use efficiency, $WUE_i$) in Zusammenhang mit Veränderungen im Stickstoffeintrag, Temperatur und Niederschlag in einem Mischwald (Lägeren) während des 20. Jahrhunderts gebracht. An allen Standorten nahm das Baumwachstum dabei häufig mit erhöhtem Niederschlag signifikant zu und aufgrund erhöhter Temperaturen signifikant ab. Dies zeigt die herausragende Bedeutung einer ausreichenden Wasserversorgung für die Produktivität in Schweizer Wäldern auf. An einem hoch gelegenen Standort konnte ausserdem gezeigt werden, dass das Baumwachstum bei Temperaturerhöhungen signifikant zunimmt. Dies lässt darauf schliessen, dass die Temperatur dort unterhalb des Optimums für das Baumwachstum liegt. Während das Wachstum an allen acht Standaorten signifikant mit erhöhtem Stickstoffeintrag zunahm, stieg die $WUE_i$ (das Verhältnis von Photosynthese zu stomatärer Leitfähigkeit) mit steigendem Stickstoffeintrag als auch mit steigenden Temperaturen an. Hohe $WUE_i$ Werte zwischen 1960 und 1990 wurden in Zusammenhang mit erhöhtem Trockenstress durch das stickstoffinduziert erhöhte Baumwachstum gebracht. In den letzten Jahrzenten hingegen wurden erhöhte $WUE_i$

Werte auf die temperaturassoziierte Reduktion der stomatären Leitfähigkeit zurückgeführt. Diese Ergebnisse deuten darauf hin, dass an der Lägeren nicht mehr erhöhte N-Deposition zu hohen $WUE_i$ Werten führt (wie zwischen 1960-1990), sondern diese nun durch erhöhte Temperaturen zu erklären sind. Bäume an der Lägeren könnten daher aufgrund steigender Temperaturen im 21. Jahrhundert anfälliger für Trockenheit werden und dies unabhängig von einer Abnahme des Stickstoffeintrages.

Die Resultate dieser Dissertation demonstrieren die Einschränkungen bei der Verwendung von $\delta^{15}N$ in Jahrringen in Umweltstudien, welche vornehmlich von der hohen Mobilität von Stickstoff innerhalb des Baumes herrühren. Zusätzlich war es mit Hilfe von Langzeitdaten zum Stickstoffeintrag möglich zu zeigen, dass eine Erhöhung der N-Deposition während der zweiten Hälfte des 20. Jahrhunderts zu einer Veränderung in Baumwachstum und -physiologie geführt hat.

# General introduction

*Past and future environmental changes*

Since the industrial revolution, anthropogenic emissions of greenhouse gases have led to an increase in the mixing ratio of carbon dioxide ($CO_2$) in the atmosphere by approximately 40%, from 275-285 ppm in 1750 to 390.5 ppm in 2011 (Hartmann *et al.* 2013). This has contributed to fundamental changes in both global and regional climate systems, including an increase in global mean surface temperature of 0.85 ± 0.20°C between 1850 and 2012 (Stocker *et al.* 2013). Average temperatures in Switzerland have increased more than twice the global average during the 20th century, while associated changes to regional precipitation regimes have also resulted in an increased frequency of drought events (Rebetez 1999, Rebetez 2001). Temperatures in Europe are predicted to continue to increase throughout the 21st century, and extreme climatic events, such as droughts and floods, are also projected to increase in frequency (Kovats *et al.* 2014).

Throughout the 20th century, nitrogen (N) fertilizers have been used on an industrial scale to enhance crop yield in agriculture in order to provide enough food for the rapidly expanding global population (Galloway *et al.* 2004). The creation of large amounts of N fertilizers was made possible by the Haber-Bosch process, through which ammonia ($NH_3$) is synthesised from atmospheric $N_2$ and hydrogen (Erisman *et al.* 2008). The application of increasing amounts of N fertilizers, together with a subsequent reduction in agricultural N-efficiency, has led to a substantial increase in the amount of reactive N ($N_r$) entering the atmosphere through various processes in the N cycle, such as denitrification and volatilization (Butterbach-Bahl *et al.* 2011, Erisman *et al.* 2011). At the same time, the combustion of fossil fuels in industry, transportation and heating has produced large amounts of nitrogen oxides ($NO_X$) as a by-product (Erisman *et al.* 2011). These air-borne oxides can travel long distances and are deposited in ecosystems worldwide (Galloway *et al.* 2004). Globally, the transport and deposition of $N_r$ to the Earth's surface has increased from ~34 Tg N $yr^{-1}$ in 1860 to ~100 Tg N $yr^{-1}$ in 1995, and is expected to increase to ~200 Tg N $yr^{-1}$ by

2050 (Galloway *et al.* 2008). In Europe, various measures have been implemented since the late 1980s in order to reduce $NO_X$ emissions. As a result, $NO_X$ emissions decreased remarkably in the 1990s and the deposition of $NO_X$ is predicted to decrease further, but contributions from agricultural N use will probably remain high (Winiwarter *et al.* 2011).

*Forest ecosystem response to changing environmental conditions*

Forests play an extremely important role in global carbon (C) sequestration, acting as a C sink for up to 2.4 ± 0.4 Pg C $yr^{-1}$ between 1990 and 2007 (Pan *et al.* 2011). Elevated atmospheric $CO_2$ concentrations are thought to have had a fertilization effect on forest productivity through increased tree photosynthetic activity, although the strength of this fertilization effect is under debate (Körner *et al.* 2007, Settele *et al.* 2014). In addition, higher temperatures have prolonged the length of the growing-season, further enhancing C sequestration rates (Linderholm 2006). However, a combination of increased drought stress and fire events are predicted to weaken global forest C sequestration rates, and may cause forest ecosystems to be a C sink by the end of the 21st century (Lindner *et al.* 2010, Settele *et al.* 2014). European forests are expected to experience increases in the frequency of drought events, increased fire and storm damage, and increases in pathogen and insect damage throughout the next century, significantly reducing forest productivity and altering species composition (Kovats *et al.* 2014). There is evidence to suggest that increased drought stress has already indirectly induced tree mortality in the inner alpine valleys of Switzerland (Rebetez & Dobbertin 2004).

In N-limited forest ecosystems, increased N deposition rates have initially been advantageous, causing increases in tree productivity and subsequent C sequestration through increased photosynthetic rates (Solberg *et al.* 2009). In an N manipulation experiment in Alptal, Switzerland, Krause *et al.* (2012) recorded increases in needle area, foliar N concentration and tree height in response to low-dose additions of N, resulting in a 22% increase in tree stem C sequestration. The beneficial effects of increased N availability, however, are negated once an ecosystem reaches a level of N saturation. N saturation can be defined as conditions in which the '*availability of* *mineral N may exceed the combined nutritional demands of plants and microbes'* (Aber *et al.* 1989). Once a forest ecosystem has reached N saturation, rates of N plant uptake via the roots will decrease, particularly if there is an increase in the canopy uptake of atmospheric N (Rennenberg & Gessler 1999), leading to increases in nitrate ($NO_3^-$) leaching rates from the soil. In order to maintain a charge balance within the soil, this will lead to an equivalent leaching of base cations from the soil, such as magnesium ($Mg^{2+}$), potassium ($K^+$) or calcium ($Ca^{2+}$) (Wallace *et al.* 2007). This will result in increased soil acidification rates through an increase in $H^+$ concentrations, as well as an increase in aluminum (Al) mobility in the soil (Aber *et al.* 1998). Increased Al mobility in the soil can decrease tree productivity through both Al poisoning and through associated nutrient imbalances in the soil, as tree uptake of calcium, an essential nutrient in both membrane and sapwood formation, is reduced (Wallace *et al.* 2007). In severe cases, this can lead to tree mortality (Aber *et al.* 1998). Other effects of N saturation on forest ecosystems include changes to understory species composition and decreases in biodiversity as a result of increased competition to N-efficient species, a decrease in symbiotic mycorrhizal relationships, and an increase in disease susceptibility (Gilliam 2006).

The response of tree productivity to increased N availability is, however, an on-going cause of debate due to the complex interactions between atmospheric N availability, soil N pools, and root and canopy N uptake (De Schrijver *et al.* 2008, de Vries *et al.* 2008, Magnani *et al.* 2007). In an investigation by de Vries *et al.* (2014), global N deposition was estimated to currently increase the forest C sink by 276-448 Tg C $yr^{-1}$, but this increase in tree productivity in response to increased N availability will diminish with time. In addition, the fertilizing effect of increasing N availability on tree growth may become weakened in time as other important nutrients required for tree growth, such as phosphorous, become limiting (Braun *et al.* 2010). The effects of both future climate change and changing N deposition rates on tree productivity are particularly difficult to predict and quantify due to the complex interactions between environmental variables on forest productivity (Hyvönen *et al.* 2007). For example, although N deposition may increase tree growth in N-limited forests, the predicted increase of drought events during the 21st century may further limit water availability for trees already experiencing increased water demands associated with increased tree stem and canopy growth (Betson *et al.* 2007). The understanding of how trees have responded to environmental changes throughout the 20th century is essential in determining the physiological response of trees to further changes in both climate and N deposition rates. However, the lack of long-term N deposition data for the 20th century has made these particular analyses difficult.

### *Dendrochronology and climatology*

Tree-ring width measurements are often used to investigate tree response to changing environmental conditions (Hughes 2002). In long-term investigations, the sensitivity of tree-ring width to changes in climate conditions, the precision of the analyses, and the vast network of well preserved tree-ring chronologies makes it is possible to reconstruct past climatic conditions dating back centuries (Fritts 1976). Due to the site and species-specific nature of tree-ring response to changing environmental conditions, the growing field of dendrochronology is providing valuable information on the best management practices and environmental policies to ensure continued forest productivity under predicted climate change (Babst *et al.* 2013). A standardization technique to remove stand- and age-related trends from tree-ring width chronologies is often applied in dendrochronological investigations in order to provide high frequency information that can be related to temporal variations in environmental conditions. However, this standardization leads to a dampening of the effect of long-term environmental changes on the tree-ring width chronology. Subsequently, tree basal area increment (BAI) can also be used as an additional tool in monitoring the long-term environmental effects on tree growth (Biondi & Qeadan 2008). While tree-ring width chronologies and BAI are extremely useful biological archives of tree response to different environmental conditions, they do not provide information on the underlying physiological mechanisms by which trees respond to these changing conditions. This information is often determined using a multi-parameter approach implementing tree growth measurements together with measurements of tree stable isotopic composition (Cernusak & English 2015).

### *Stable isotopes*

Stable isotopes are atoms of a particular element with the same number of protons and electrons but a different number of neutrons, resulting in very similar chemical properties but different physical properties due to differences in mass (Lajtha & Michener 1994). This difference in mass, together with differences in stable isotope natural abundance (*Table 1*), mean that stable isotopes can be used to study a wide-range of biological processes and pathways, as many active and passive processes will discriminate against the 'heavier' isotope (Peterson & Fry 1987). These processes that alter the ratio between 'light' and 'heavy' isotopes are termed fractionation events (Lajtha & Michener 1994).

*Table 1*. The International standards used during isotope analysis and the natural abundance percentage of each isotope for the isotopes of carbon, nitrogen and oxygen (Dawson *et al*. 2002)

| Element | Standard | Isotope | Natural abundance (%) |
|---|---|---|---|
| Carbon | Vienna - PeeDee belemnite (V-PDB) | $^{12}C$ | 98.982 |
| | | $^{13}C$ | 1.108 |
| Nitrogen | Atmospheric $N_2$ | $^{14}N$ | 99.63 |
| | | $^{15}N$ | 0.366 |
| Oxygen | Vienna – Standard mean ocean water (V-SMOW) | $^{16}O$ | 99.759 |
| | | $^{17}O$ | 0.037 |
| | | $^{18}O$ | 0.204 |

The stable isotopic composition, measured using a mass spectrometer and expressed in permil (‰), of a particular element is expressed in the δ-notation as deviations from an international standard (*Table 1*):

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \qquad (1)$$

where R is the ratio of 'heavy' to 'light' isotope, $R_{sample}$ is the ratio within the sample, $R_{standard}$ is the ratio within the standard (McCarroll & Loader 2004). Stable isotopes can provide information on both the origin (or source) of a sample and the fractionation events associated with various biological, chemical or physical processes. Investigations can focus either on stable isotopes in their natural abundance, or can manipulate the isotopic composition of a source substrate to act as a tracer in labelling experiments (Dawson *et al.* 2002).

*Stable isotopes of carbon and oxygen in plant tissues*

The source isotopic composition and fractionation events associated with both tree-ring and foliar isotopes of carbon ($\delta^{13}C$) are reasonably well understood, and therefore they are often used to study tree physiological processes (Farquhar *et al.* 1982), or used as proxies for certain climatic conditions (Helle & Schleser 2004). In C3 plants, $^{13}C$ isotopic composition is determined by the ratio of ambient to intercellular $CO_2$ concentration (regulated by stomatal conductance), and by the net fractionation associated with carboxylation with the RubisCo enzyme (regulated by photosynthetic activity) (Farquhar *et al.* 1989). Tree $^{13}C$ isotopic composition can also be used to determine tree intrinsic water-use efficiency ($WUE_i$), defined as the ratio of net photosynthesis to stomatal conductance (Farquhar *et al.* 1982, Ehleringer & Cerling 1995). $WUE_i$ is affected by a number of environmental variables that can alter both photosynthetic activity and stomatal conductance, such as atmospheric $CO_2$ concentrations, climate and N deposition (Guerrieri *et al.* 2010, Saurer & Siegwolf 2007).

*The potential and limitations of using tree N stable isotopic composition*

Tree-ring stable isotopes of nitrogen ($\delta^{15}N$) have also been used to study both environmental conditions (e.g. Savard *et al.* 2009) and tree physiology (e.g. Guerrieri *et al.* 2010). Studies using tree-ring $\delta^{15}N$ values are, however, few relative to those using tree-ring $\delta^{13}C$ or $\delta^{18}O$ values. This is in part due to: (1) the low concentrations of tree-ring N (approximately 500 times less than tree-ring C concentrations), (2) the mobility of N within the tree stem associated with N recycling processes, (3) a limited understanding of the relationship between tree-ring $\delta^{15}N$ and environmental conditions, and (4) the lack of long-term datasets on the $\delta^{15}N$ isotopic composition of source N, particularly N deposition.

The movement of N within the tree stem was first suggested in 1966 based on higher N concentrations found in the youngest tree-rings relative to the rest of the tree stem (Merrill & Cowling 1966). Subsequent labelling experiments using $^{15}$N tracers confirmed this high N mobility within the tree stem, with recovery of the $^{15}$N label in tree-rings formed both before and after the label application (Elhani *et al.* 2003, Nömmik 1966). As a consequence of this bidirectional movement of N within the tree sapwood, investigations using tree-ring $\delta^{15}$N have often employed an extraction technique to remove mobile N components prior to isotope analyses (Sheppard & Thompson 2000). This extraction procedure, however, further decreases the extremely low N concentrations in the wood samples, while there are questions as to whether or not the extraction procedure has a significant effect on the tree-ring $\delta^{15}$N isotope composition at all (Caceres *et al.* 2011, Doucet *et al.* 2011). In addition, while previous investigations have shown a drop in N concentration at the heartwood-sapwood boundary (Merrill & Cowling 1966), the extent of the movement of the mobile N compounds towards the heartwood is yet to be fully documented using a $^{15}$N-label. Thus, there is certainly some debate regarding the methodological approaches in the measurement of tree-ring $\delta^{15}$N values, particularly the necessity of an extraction treatment prior to isotope analyses.

Element concentration in tree foliage is often used as an indicator for the nutritional status of a tree (Mellert & Göttlein 2012). At the same time, while measurements of foliar $\delta^{15}$N are also used to study site-specific environmental conditions and regional N cycles (e.g. Garten 1993, Pardo *et al.* 2006), they lack the longevity of the isotope signals preserved in tree-ring chronologies. Therefore, an understanding of the relationship between tree-ring and foliar isotopic composition may allow future dendrochemical studies to use tree-ring $\delta^{15}$N values as proxies for foliar $\delta^{15}$N values in environmental investigations. However, current knowledge on the intra-tree isotopic variation of $\delta^{15}$N is poor, in particular how N recycling processes occurring between the tree stem and the foliage, as well as within the tree canopy (Evans 2001, Kolb & Evans 2002), affect the $\delta^{15}$N isotopic signature of tree-rings and foliage. Furthermore, it is also unclear as to how the $\delta^{15}$N composition of tree-rings and foliage respond to changing environmental conditions, whether the aforementioned movement of N within the tree stem masks potential environmental signals in the tree-ring $\delta^{15}N$ values, and whether these effects are species specific.

### *Thesis objectives*

Studies in the recent past have shown that both tree-ring and foliage N isotopic composition may be extremely useful in studying tree response to changing environmental conditions, particularly when used in a dual-isotope approach with tree $\delta^{13}C$. However, the current understanding of how physiological and environmental processes affect both tree-ring and foliar $\delta^{15}N$ is limited. There are open questions related to both the intra-tree variation of $\delta^{15}N$ and the effect of environmental variables in determining tree $\delta^{15}N$ composition, while there is still some debate concerning the methods applied during the analysis of tree-ring $\delta^{15}N$ values. At the same time, the effect of changing environmental conditions during the 20th century, particularly N deposition, on both tree growth and physiology in Switzerland is also unclear due to the scarcity of long-term N deposition datasets. This is of particular importance in helping to develop our understanding of how further environmental changes may alter forest productivity and species composition throughout the 21st century. Consequently, the main objectives of this thesis were to (1) assess the extent of the mobility of N within the tree stem and the necessity of extracting mobile N compounds prior to isotope analyses, (2) determine the relationship between tree-ring and foliage $\delta^{15}N$ and $\delta^{13}C$ values, and how they may be altered by environmental conditions, and (3) determine past physiological response of trees to changes in N deposition and climate, as well as the climate-growth response of trees across Switzerland, throughout the 20th century. With these objectives in mind, and through the application of tree-ring and foliage $\delta^{15}N$ and $\delta^{13}C$ isotope data together with measurements of tree-ring growth, the following research questions were formulated:

1. What is the extent of the mobility of N within the tree stem? Is the extraction of mobile N compounds prior to the isotopic analysis of tree-ring $\delta^{15}N$ necessary? How does this extraction procedure affect tree-ring $\delta^{13}C$? Are these effects the same in both trees labelled with $^{15}N$ and un-labelled trees?

2. How does tree-ring isotopic ratio of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ relate to the isotopic ratio of corresponding foliage? Is this effect species-specific? What is the effect of site-specific environmental conditions on both tree-ring and foliar isotopic composition? And is it possible to use tree-ring $\delta^{15}N$ values as proxies of foliar $\delta^{15}N$ values in long-term environmental investigations?

3. How did tree growth respond to changing climatic conditions and N deposition throughout the 20th century in Switzerland? Were these effects either species or site-specific? What was the physiological response of trees to changes in N deposition and climate during this time? And what does this mean for trees during future changes in climate and N deposition?

*Structure of the thesis*

The three thesis objectives are independent studies presented as individual chapters, for which a short summary is provided in the following:

**Chapter 1** – entitled *'The mobility of nitrogen across tree-rings of Norway spruce (Picea abies L.) and the effect of extraction method on tree-ring* $\delta^{15}N$ *and* $\delta^{13}C$*'* describes the use of a $^{15}N$-labelling experiment carried out in 1995 in the Alptal valley, Switzerland, to determine the mobility of N within the tree stem of *Picea abies*. In addition, the effect of the rapid-procedure extraction technique was also assessed on both tree-ring $\delta^{15}N$ and $\delta^{13}C$ values in both labelled and un-labelled trees. The low-dose application of N at Alptal since 1995 also made it possible to determine whether N deposition had a fertilizing effect on radial stem growth at this site. This chapter contributes to the debate concerning the removal of mobile N compounds prior to isotope analysis and also quantifies the radial mobility of the N within the tree stem via the movement of the $^{15}N$-label between tree-rings.

**Chapter 2** – entitled *'Can tree-ring* $\delta^{15}N$ *values be used as proxies for foliar* $\delta^{15}N$ *values in European beech (Fagus sylvatica L.) and Norway spruce (Picea abies L.)?* looks at the relationship over time between tree-ring and foliage $\delta^{15}N$ and $\delta^{13}C$

values in the two main tree species in Switzerland. Through the use of archived foliage samples collected biennially at long-term monitoring plots within Switzerland, we were able to compare the isotopic composition of tree-rings and foliage over time. This data, collected over six sites, was complemented with selected environmental data in order to try and explain intra-tree, inter-annual and inter-species isotopic variation. This chapter assesses how different site conditions, together with changing environmental conditions, influences both tree-ring and foliage $\delta^{15}N$ and $\delta^{13}C$ isotopic composition

**Chapter 3** – entitled *'Environmental drivers of tree growth and water-use efficiency of Fagus sylvatica (L.) and Picea abies (L.) Karst across Switzerland in the 20th century: Climate vs N deposition'* looks both at the environmental variables influencing tree growth at a number of sites in Switzerland, and the effect of changing N deposition rates on both tree growth and physiology throughout the 20th century. This chapter includes tree-ring and climate data from eight sites across Switzerland, including *Fagus sylvatica* and *Picea abies* sites, to determine both the site and species-specific effect of climate on tree growth. We used long-term modeled N deposition values spanning the 20th century to determine how N deposition has affected tree basal area increment (BAI) since prior to the rapid increase in N deposition rates in the second half of the 20th century. At one particular site, we combined long-term climate and N deposition data together with measurements of BAI and $WUE_i$ to determine tree physiological response to changing environmental conditions throughout the last century. This chapter contributes to our understanding of how trees have responded to previous changes in environmental conditions, and thus how they may react to future changes in climate and N deposition.

## References

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L. & Fernandez, I. (1998) Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited. *Bioscience,* **48,** 921-934.

Aber, J. D., Nadelhoffer, K. J., Steudler, P. & Melillo, J. M. (1989) Nitrogen Saturation in Northern Forest Ecosystems: Excess nitrogen from fossil fuel combustion may stress the biosphere. *BioScience,* **39,** 378-386.

Babst, F., Poulter, B., Trouet, V., Tan, K., Neuwirth, B., Wilson, R., Carrer, M., Grabner, M., Tegel, W., Levanic, T., Panayotov, M., Urbinati, C., Bouriaud, O., Ciais, P. & Frank, D. (2013) Site- and species-specific responses of forest growth to climate across the European continent. *Global Ecology and Biogeography,* **22,** 706-717.

Betson, N. R., Johannisson, C., Löfvenius, M. O., Grip, H., Granström, A. & Högberg, P. (2007) Variation in the $\delta^{13}C$ of foliage of *Pinus sylvestris* L. in relation to climate and additions of nitrogen: analysis of a 32-year chronology. *Global Change Biology,* **13,** 2317-2328.

Biondi, F. & Qeadan, F. (2008) A theory-driven approach to tree-ring standardization: Defining the biological trend from expected basal area increment. *Tree-Ring Research,* **64,** 81-96.

Braun, S., Thomas, V. F. D., Quiring, R. & Flückiger, W. (2010) Does nitrogen deposition increase forest production? The role of phosphorus. *Environmental Pollution,* **158,** 2043-2052.

Butterbach-Bahl, K., Gundersen, P., Ambus, P., Augustin, J., Beier, C., Boeckx, P., Dannenmann, M., Gimeno, B. S., Ibrom, A., Kiese, R., Kitzler, B., Rees, R. M., Smith, K. A., Stevens, C., Vesala, T. & Zechmeister-Boltenstern, S. (2011) Nitrogen processes in terrestrial ecosystems. In: Sutton, M. A., Howard, C. M., Erisman, J. W., Billen, G., Bleeker, A., Grennfelt, P., van Grinsven, H. & Grizzetti, B. (eds.) *The European nitrogen assessment:*

*sources, effects and policy perspectives*. Cambridge University Press, Cambridge, UK, 99-125.

Caceres, L. L. M., Mizota, C., Yamanaka, T. & Nobori, Y. (2011) Effects of pre-treatment on the nitrogen isotope composition of Japanese black pine (*Pinus thunbergii*) tree-rings as affected by high N input. *Rapid Communications in Mass Spectrometry,* **25,** 3298-3302.

Cernusak, L. A. & English, N. B. (2015) Beyond tree-ring widths: stable isotopes sharpen the focus on climate responses of temperate forest trees. *Tree Physiology,* **35,** 1-3.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H. & Tu, K. P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics,* **33,** 507-559.

De Schrijver, A., Verheyen, K., Mertens, J., Staelens, J., Wuyts, K. & Muys, B. (2008) Nitrogen saturation and net ecosystem production. *Nature,* **451,** E1-E1.

de Vries, W., Du, E. & Butterbach-Bahl, K. (2014) Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. *Current Opinion in Environmental Sustainability,* **9–10,** 90-104.

de Vries, W., Solberg, S., Dobbertin, M., Sterba, H., Laubhahn, D., Reinds, G. J., Nabuurs, G.-J., Gundersen, P. & Sutton, M. A. (2008) Ecologically implausible carbon response? *Nature,* **451,** E1-E3.

Doucet, A., Savard, M. M., Begin, C. & Smirnoff, A. (2011) Is wood pre-treatment essential for tree-ring nitrogen concentration and isotope analysis? *Rapid Communications in Mass Spectrometry,* **25,** 469-475.

Ehleringer, J. R. & Cerling, T. E. (1995) Atmospheric $CO_2$ and the ratio of intercellular to ambient $CO_2$ concentrations in plants. *Tree Physiology,* **15,** 105-111.

Elhani, S., Lema, B. F., Zeller, B., Brechet, C., Guehl, J. M. & Dupouey, J. L. (2003) Inter-annual mobility of nitrogen between beech rings: a labelling experiment. *Annals of Forest Science,* **60,** 503-508.

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z. & Winiwarter, W. (2008) How a century of ammonia synthesis changed the world. *Nature Geosci,* **1,** 636-639.

Erisman, J. W., van Grinsven, H., Grizzetti, B., Bouraoui, F., Powlson, D. & Reis, S. (2011) The European nitrogen problem in a global perspective. In: Sutton, M. A., Britton, C., Erisman, J. W., Billen, G., Bleeker, A., Greenfelt, P., van Grinsven, H. & Grizzetti, B. (eds.) *The European Nitrogen Assessment.* Cambridge University Press, Cambridge, 9-31.

Evans, R. D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science,* **6,** 121-126.

Farquhar, G. D., Ehleringer, J. R. & Hubick, K. T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology,* **40,** 503-537.

Farquhar, G. D., O'Leary, M. H. & Berry, J. A. (1982) On the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves. *Australian Journal of Plant Physiology,* **9,** 121-137.

Fritts, H. C. (1976) *Tree rings and climate,* London, UK, Academic Press, pp 567.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R. & Vöosmarty, C. J. (2004) Nitrogen cycles: past, present, and future. *Biogeochemistry,* **70,** 153-226.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S. P. & Sutton, M. A. (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science,* **320,** 889-892.

Garten, C. T. (1993) Variation in foliar $^{15}$N abundance and the availability of soil nitrogen on Walker Branch Watershed. *Ecology,* **74,** 2098-2113.

Gilliam, F. S. (2006) Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. *Journal of Ecology,* **94,** 1176-1191.

Guerrieri, R., Siegwolf, R., Saurer, M., Ripullone, F., Mencuccini, M. & Borghetti, M. (2010) Anthropogenic $NO_X$ emissions alter the intrinsic water-use efficiency ($WUE_i$) for *Quercus cerris* stands under Mediterranean climate conditions. *Environmental Pollution,* **158,** 2841-2847.

Hartmann, D. L., Klein Tank, A. M. G., Rusticucci, M., Alexander, L. V., Brönnimann, S., Charabi, Y., Dentener, F. J., Dlugokencky, E. J., Easterling, D. R., Kaplan, A., Soden, B. J., Thorne, P. W., Wild, M. & Zhai, P. M. (2013) Observations: Atmosphere and Surface. In: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P. M. (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., 159-254.

Helle, G. & Schleser, G. H. (2004) Interpreting climate proxies from tree-rings. In: Fischer, H., Kumke, T., Lohmann, G., Flöser, G., Miller, H., von Storch, H. & Negendank, J. W. (eds.) *The Climate in Historical Times.* Springer Berlin Heidelberg, 129-148.

Hughes, M. K. (2002) Dendrochronology in climatology – the state of the art. *Dendrochronologia,* **20,** 95-116.

Hyvönen, R., Ågren, G. I., Linder, S., Persson, T., Cotrufo, M. F., Ekblad, A., Freeman, M., Grelle, A., Janssens, I. A., Jarvis, P. G., Kellomäki, S., Lindroth, A., Loustau, D., Lundmark, T., Norby, R. J., Oren, R., Pilegaard, K., Ryan, M. G., Sigurdsson, B. D., Strömgren, M., Van Oijen, M. & Wallin, G. (2007) The likely impact of elevated [CO2], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *New Phytologist,* **173,** 463-480.

Kolb, K. J. & Evans, R. D. (2002) Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. *New Phytologist,* **156,** 57-64.

Körner, C., Morgan, J. & Norby, R. (2007) $CO_2$ fertilization: When, where, how much? In: Canadell, J., Pataki, D. & Pitelka, L. (eds.) *Terrestrial Ecosystems in a Changing World.* Springer Berlin Heidelberg, 9-21.

Kovats, R. S., Valentini, R., Bouwer, L. M., Georgopoulou, E., Jacob, D., Martin, E., Rounsevell, M. & Soussana, J.-F. (2014) Europe. In: Barros, V. R., Field, C. B., Dokken, D. J., Mastrandrea, M. D., Mach, K. J., Bilir, T. E., Chatterjee, M., Ebi, K. L., Estrada, Y. O., Genova, R. C., Girma, B., Kissel, E. S., Levy, A. N., MacCracken, S., Mastrandrea, P. R. & White, L. L. (eds.) *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1267-1326.

Lajtha, K. & Michener, R. H. (1994) *Stable isotopes in ecology and environmental science,* Oxford, Blackwell, pp 336.

Linderholm, H. W. (2006) Growing season changes in the last century. *Agricultural and Forest Meteorology,* **137,** 1-14.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M. J. & Marchetti, M. (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management,* **259,** 698-709.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P. G., Kolari, P., Kowalski, A. S., Lankreijer, H., Law, B. E., Lindroth, A., Loustau, D., Manca, G., Moncrieff, J. B., Rayment, M., Tedeschi, V., Valentini, R. & Grace, J. (2007) The human footprint in the carbon cycle of temperate and boreal forests. *Nature,* **447,** 849-851.

McCarroll, D. & Loader, N. J. (2004) Stable isotopes in tree rings. *Quaternary Science Reviews,* **23,** 771-801.

Mellert, K. H. & Göttlein, A. (2012) Comparison of new foliar nutrient thresholds derived from van den Burg's literature compilation with established central European references. *European Journal of Forest Research,* **131,** 1461-1472.

Merrill, W. & Cowling, E. B. (1966) Role of nitrogen in wood deterioration: amounts and distribution of nitrogen in tree stems. *Canadian Journal of Botany,* **44,** 1555-1580.

Nömmik, H. (1966) The uptake and translocation of fertilizer $^{15}$N in young trees of Scots pine and Norway spruce. *Studia Forestalia Suecica,* **35,** 1-18.

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., Phillips, O. L., Shvidenko, A., Lewis, S. L., Canadell, J. G., Ciais, P., Jackson, R. B., Pacala, S. W., McGuire, A. D., Piao, S., Rautiainen, A., Sitch, S. & Hayes, D. (2011) A large and persistent carbon sink in the world's forests. *Science,* **333,** 988-993.

Pardo, L., Templer, P., Goodale, C., Duke, S., Groffman, P., Adams, M., Boeckx, P., Boggs, J., Campbell, J., Colman, B., Compton, J., Emmett, B., Gundersen, P., Kjønaas, J., Lovett, G., Mack, M., Magill, A., Mbila, M., Mitchell, M., McGee, G., McNulty, S., Nadelhoffer, K., Ollinger, S., Ross, D., Rueth, H., Rustad, L., Schaberg, P., Schiff, S., Schleppi, P., Spoelstra, J. & Wessel, W. (2006) Regional assessment of N saturation using foliar and root $\delta^{15}$N. *Biogeochemistry,* **80,** 143-171.

Peterson, B. J. & Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics,* **18,** 293-320.

Rebetez, M. (1999) Twentieth century trends in droughts in southern Switzerland. *Geophysical Research Letters,* **26,** 755-758.

Rebetez, M. (2001) Changes in daily and nightly day-to-day temperature variability during the twentieth century for two stations in Switzerland. *Theoretical and Applied Climatology,* **69,** 13-21.

Rebetez, M. & Dobbertin, M. (2004) Climate change may already threaten Scots pine stands in the Swiss Alps. *Theoretical and Applied Climatology,* **79,** 1-9.

Rennenberg, H. & Gessler, A. (1999) Consequences of N deposition to forest ecosystems - recent results and future research needs. *Water, Air, and Soil Pollution,* **116,** 47-64.

Saurer, M. & Siegwolf, R. T. W. (2007) Human impacts on tree-ring growth reconstructed from stable isotopes. In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 49-62.

Savard, M. M., Begin, C., Smirnoff, A., Marion, J. & Rioux-Paquette, E. (2009) Tree-ring nitrogen isotopes reflect anthropogenic $NO_X$ emissions and climatic effects. *Environmental Science & Technology,* **43,** 604-609.

Settele, J., Scholes, R., Betts, R., Bunn, S. E., Leadley, P., Nepstad, D., Overpeck, J. T. & Taboada, M. A. (2014) Terrestrial and inland water systems. In: Field, C. B., Barros, V. R., Dokken, D. J., Mach, K. J., Mastrandrea, M. D., Bilir, T. E., Chatterjee, M., Ebi, K. L., Estrada, Y. O., Genova, R. C., Girma, B., Kissel, E. S., Levy, A. N., MacCracken, S., Mastrandrea, P. R. & White, L. L. (eds.) *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 271-359.

Sheppard, P. R. & Thompson, T. L. (2000) Effect of extraction pretreatment on radial variation of nitrogen concentration in tree rings. *J. Environ. Qual.,* **29,** 2037-2042.

Solberg, S., Dobbertin, M., Reinds, G. J., Lange, H., Andreassen, K., Fernandez, P. G., Hildingsson, A. & de Vries, W. (2009) Analyses of the impact of changes in atmospheric deposition and climate on forest growth in European monitoring plots: A stand growth approach. *Forest Ecology and Management,* **258,** 1735-1750.

Stocker, T. F., Qin, D., Plattner, G.-K., Alexander, L. V., Allen, S. K., Bindoff, N. L., Bréon, F.-M., Church, J. A., Cubasch, U., Emori, S., Forster, P., Friedlingstein, P., Gillett, N., Gregory, J. M., Hartmann, D. L., Jansen, E., Kirtman, B., Knutti, R., Krishna Kumar, K., Lemke, P., Marotzke, J., Masson-Delmotte, V., Meehl, G. A., Mokhov, I. I., Piao, S., Ramaswamy, V., Randall, D., Rhein, M., Rojas, M., Sabine, C., Shindell, D., Talley, L. D., Vaughan, D. G. & Xie, S.-P. (2013) Technical Summary. In: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P. M. (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* Cambridge University Press, Cambridge, United Kingdom & New York, NY, USA, 33-115.

Wallace, Z. P., Lovett, G. M., Hart, J. E. & Machona, B. (2007) Effects of nitrogen saturation on tree growth and death in a mixed-oak forest. *Forest Ecology and Management,* **243,** 210-218.

Winiwarter, W., Hettelingh, J.-P., Bouwman, A. F., de Vries, W., Erisman, J. W., Galloway, J., Klimont, Z., Leach, A., Leip, A., Pallière, C., Schneider, U. A., Spranger, T., Sutton, M. A., Svirejeva-Hopkins, A., van der Hoek, K. W. & Witzke, P. (2011) Future scenarios of nitrogen in Europe. In: Sutton, M. A., Britton, C., Erisman, J. W., Billen, G., Bleeker, A., Greenfelt, P., van Grinsven, H. & Grizzetti, B. (eds.) *The European Nitrogen Assessment.* Cambridge University Press, Cambridge, 551-569.

# Chapter 1

## The mobility of nitrogen across tree-rings of Norway spruce (*Picea abies* L.) and the effect of extraction method on tree-ring $\delta^{15}N$ and $\delta^{13}C$

Tomlinson, G.[1], Siegwolf, RTW.[2], Buchmann, N.[3], Schleppi, P.[1], Waldner, P.[1], Weber, P.[1]

[1]WSL Swiss Federal Institute for Forest, Snow and Landscape Research, CH-8903 Birmensdorf, Switzerland

[2]Ecosystem Fluxes Group, Paul Scherrer Institute (PSI), CH-5232 Villigen, Switzerland

[3]Department of Environmental Systems Science, ETH Zurich, CH-8092 Zurich, Switzerland

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## Abstract

The use of stable nitrogen (N) isotope ratios ($\delta^{15}N$) in dendroecological studies is often preceded by an extraction procedure using organic solvents to remove mobile N compounds from tree-rings. Although these mobile N compounds may be capable of distorting potential environmental signals in the tree-ring $\delta^{15}N$, recent investigations question the necessity of such an extraction. We used an on-going experiment with simulated elevated N deposition previously labelled with $^{15}N$, in conjunction with control trees, to investigate the necessity of extracting mobile N compounds (using a rapid extraction procedure) for tree-ring $\delta^{15}N$ and $\delta^{13}C$ studies, as well as N and C concentration analyses. In addition, we examined the magnitude of radial redistribution of N across tree-rings of Norway spruce (*Picea abies*). The $^{15}N$-label, applied in 1995/96, was found in tree-rings as far back as 1951, although the increased N availability did not cause any significant relative increase in tree growth. The rapid extraction procedure had no significant effect on tree-ring $\delta^{15}N$ or $\delta^{13}C$ values in either labelled or control trees, nor on N concentration. C concentrations, however, were significantly higher after extraction in control samples, with the opposite effect observed in labelled samples. Our results indicate that the extraction of mobile N compounds through the rapid extraction procedure is not necessary prior to the analysis of Norway spruce $\delta^{15}N$ or $\delta^{13}C$ values in dendrochemical studies. $\delta^{15}N$ values, however, must be interpreted with great care, particularly when used as proxy for the N status of trees, due to the very high mobility of N within the tree stem sapwood of Norway spruce over several decades.

## Introduction

Tree-ring stable isotopes of carbon (C) and oxygen (O) have often been used to investigate tree physiological processes (Saurer & Siegwolf 2007), reconstruct past environmental conditions (Loader *et al.* 2007), and investigate tree response to increased nitrogen (N) deposition (Krause *et al.* 2012a). Recent studies have also demonstrated the potential use of tree-ring stable isotopes of N as a proxy for N deposition (Choi *et al.* 2005, Doucet *et al.* 2012), for soil N content (Weber *et al.*

2008), as well as an additional tool in investigating tree physiological responses to changes in the regional N cycle (Bukata & Kyser 2007, Guerrieri *et al.* 2010). Studies utilising tree-ring $\delta^{15}N$ are, however, rare in comparison to those of C and O; two of the reasons for this lack of studies are the low concentrations of N in tree-rings and the movement of mobile nitrogen compounds across tree-rings (Hart & Classen 2003).

The translocation of mobile N compounds is an important physiological process that allows the tree to reallocate N to meet the various N sink demands of growing tissues on both vertical and radial scales within the tree stem (Lévy *et al.* 1996, Lim & Cousens 1986, Salifu & Timmer 2003). These mobile N compounds are comprised of non-structural tree-ring components, including tannins, polyphenols, lipids, waxes, resins and other extractives (Elhani *et al.* 2003), and have been hypothesised to move through the horizontal xylem ray cells of the tree stem (Mead & Preston 1994). The tree will primarily remobilise N compounds towards the tree N sinks, such as towards the annual production of new tree-rings and foliage (Millard 1996, Sheppard & Thompson 2000, Turner 1977). The species-specific translocation process usually involves the movement of N from older tree rings to younger, more active parts of the tree, (e.g. bark, but also to young developing foliage), evidence for which is the sharp decrease in N concentrations during the transition from sapwood to heartwood (Lévy *et al.* 1996).

The signal disturbance caused by the translocation of mobile N compounds is usually dealt with by the removal of these mobile N compounds prior to isotope analysis. There are many examples of $\delta^{15}N$-related dendrochemical studies that employ such an extraction technique (Doucet *et al.* 2012, Elhani *et al.* 2005, Guerrieri *et al.* 2011, Guerrieri *et al.* 2010). Studies in the recent past, however, have begun to question the necessity of this extraction (Caceres *et al.* 2011, Doucet *et al.* 2011), and whether or not the tree-ring N isotope ratio is significantly altered through the extraction (Hart & Classen 2003). For instance, its effect on the N isotope composition is often dependent on the species as well as the wood type (heartwood or sapwood) (Doucet *et al.* 2011). The extraction procedure itself, including the use of organic solvents, has also been investigated (Bukata & Kyser

2005). The extraction of mobile N compounds from wood material prior to isotope analysis is still under debate, and the effects of extraction on the N isotope composition and total N concentration are far from definite.

Thus, our study aims to (1) examine the effect of the extraction of mobile N compounds on Norway spruce (*Picea abies* L.) tree-ring N and C isotope ratios and concentrations, and (2) assess the mobility of N across coniferous sapwood tree-rings. To do this, we used trees from a plot previously labelled with $^{15}$N in 1995/96 that have also been used since to study simulated elevated N deposition. Thus, it was also possible to determine the effect of N fertilization on tree growth.

## Methods

### *Site description*

The studied site is situated approximately 1200m above sea level in the Alptal valley of central Switzerland (47°02'N, 8°43'E), with a slope of approximately 20% with a west aspect. Nitrogen deposition at the site is moderate, with bulk and throughfall deposition totalling 12kg and 17kg N/ha/year, respectively (Schleppi *et al.* 1999), equally divided between $NO_3^-$ and $NH_4^+$. Parent rock material is Flysch, while the major soil types are clay-rich Gleysols (46% clay, 46% silt, 6% sand). The forest stand consists of Norway spruce (*Picea abies* L.) and silver fir (*Abies alba*), with trees up to 260 years old (Krause *et al.* 2012b). The site has no record of significant disturbances prior to 2010.

### *$^{15}$N labelling and N addition experiments*

An on-going low-dose N addition experiment has been conducted on the study site in Alptal since April 1995, referred to as the N addition site. A small catchment of approximately 1500m$^2$ has been subject to an increased nitrogen deposition of approximately 25kg N/ha/year in the form of $NH_4NO_3$ through a sprinkler system positioned approximately 1.5m above the ground. The additional nitrogen is mixed with rainwater collected from the site on a large plastic sheet and applied during rain intervals throughout the year.

Furthermore, the N addition site was also subject to $^{15}N$ labelling during the first year of N addition (April 1995 to March 1996) by applying 1400‰ $^{15}NH_4{}^{15}NO_3$ (219mmol/m$^2$; 0.88 atom %) through the sprinkler system. Previous investigations have found that the soil was the largest sink/pool for the $^{15}N$ label in both the short- (< 1 week) and long-term (up to 14 years) following its application (Krause *et al.* 2012b). Tree biomass, in contrast, was found to contain only approximately 10% of the $^{15}N$-label in 2010 (14 years after its application), suggesting the trees were still taking up some of the label from the soil more than 10 years after the original application (Krause *et al.* 2012b).

Although not for this specific purpose, a trench of 80cm depth dug around the perimeter of the site (assumed to be of sufficient depth given the impermeable gleyic subsoil of the site) ensured that both the additional N and $^{15}N$ applied to the N addition site could not be transported outside the designated experimental area, contaminating adjacent control areas.

### *Samples*

Stem wood material back to 1939 was taken from five individual Norway spruce trees within the N addition site, each felled in 2010, and a further five trees found outside the N addition area were used as control trees. The area from which the control tree samples were taken lies adjacent to the N addition site, with very similar site conditions in regards to soil, climate, slope, species composition, deposition, and disturbance history. To represent average growth and isotope composition of these trees, material was taken from two different radial positions in each stem.

The tree-ring widths of the collected material were measured between the years 1980-2010 using the TSAP-Win tree-ring software (Version 3.5) connected to a LINTAB3 measuring system (Rinn 1996). The material was subsequently cross-dated both visually and using standard methods (Cook & Kairiukstis 1990, Fritts

1976) before being verified statistically using the TSAP and COFECHA routines (Holmes 1983). Where possible, the heartwood/sapwood boundary was identified visually at four different points on the tree discs; the four different points allowed for the determination of the average year at which the tree heartwood began.

*Nitrogen extraction protocol*

The bulk wood cores from each tree were divided in two halves; half underwent N extraction, and the other half had no N extraction, to be used as the control. Tree-rings were separated with a razor blade under a microscope between the years 1939-2008, and were then pooled every two years (e.g. 2005/06, 2007/08 etc.).

For the N extraction, we used a modified version of the rapid procedure from Sheppard & Thompson (Sheppard & Thompson 2000), which has often been used to treat tree cores prior to tree-ring N isotope analysis (Doucet *et al.* 2012, Elhani *et al.* 2005, Elhani *et al.* 2003, Guerrieri *et al.* 2011, Guerrieri *et al.* 2010). The cores were placed in a soxhlet apparatus for 4hrs in toluene and ethanol (50:50), 4hrs in ethanol and 4hrs in distilled water. Both the extracted cores and the control cores were then oven-dried at 60°C for 48hrs. The extraction procedure removed approximately 7.8% of the total weight of the wood compared to the control samples, after taking into account the weight loss caused by oven drying (2.4%).

*Analysis*

All tree-ring samples between the years 1991/92 - 2007/08 were measured for $\delta^{15}$N and $\delta^{13}$C values, as well as N and C concentrations, in both extracted and un-extracted samples (9 extracted and 9 un-extracted samples per tree, n = 180). Samples from 2009/10 were excluded due to the incomplete formation of tree-rings in 2010. Further tree-rings were analysed for $\delta^{15}$N values in un-extracted samples back to 1939/40: alternate samples were measured between 1963/64 and 1987/88, whilst samples were also measured from 1939/40 and 1951/52. This resulted in a further 9 samples per tree, with the exception of two control trees from which it was not possible to analyse prior to 1963/64 due to insufficient wood material (additional n = 86).

Following extraction and oven-drying, the samples were milled and homogenised using a MM200 centrifugal ball mill (Retsch, Haan, Germany). The resulting wood powder was then weighed into tin capsules (0.50-0.55mg for $\delta^{13}C$ and 15.0-15.1mg for $\delta^{15}N$). The N and C samples were measured separately due to the high C/N ratios in wood material; during measurements of $\delta^{15}N$ values, a blank (empty tin capsule) was also measured after each wood sample for the same reason (Saurer *et al.* 2004). Samples were analysed at the Paul Scherrer Institute (Switzerland) by combustion under excess oxygen in an elemental analyser (EA-1100, Carlo Erba, Milano, Italy) connected in continuous-flow mode to a mass spectrometer (Delta-S, Finnegan, Bremen, Germany) with a precision of 0.1 and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Isotope values are expressed in the δ-notation as relative deviations from the international standards; Vienna Pee Dee Belemnite for carbon ($\delta^{13}C = ((^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1)$; [‰]), and atmospheric $N_2$ for nitrogen ($\delta^{15}N = ((^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1)$; [‰]).

The tree-ring $\delta^{13}C$ values were corrected for the decline in atmospheric $\delta^{13}C$ since the beginning of the industrial period (approximately 1850AD) caused by the combustion of fossil fuels (McCarroll *et al.* 2009). This was achieved by using records of past atmospheric $\delta^{13}C$ obtained from ice cores and from historical records from Mauna Loa (Leuenberger 2007).

The results of this study were statistically analyzed using a number of Analysis of Variance (ANOVA) tests in the statistics program *R* (version 2.15.1). A series of individual ANOVA models were used to determine significant drivers ($p \leq 0.05$) on C and N isotope ratios and concentrations between 1995/6 and 2007/8 (i.e. after the start of the labelling and N addition experiments). In these models, site conditions (N addition or control), treatment (extracted or un-extracted) and time (years) were used as explanatory variables. The models were simplified by removing the least significant term (which included interactions between the explanatory variables), until only significant factors remained in the model. Similar models were used to determine the significance of site conditions (N addition or control) and time (years) on the tree-ring $\delta^{15}N$ values between 1939/40 and 2007/08, and on tree growth between 1980 and 2010.

## Results

### *Tree growth*

Mean tree-ring chronologies were produced for both the N addition and control trees between 1980-2010. The tree-ring widths of control trees were significantly greater ($p \leq 0.001$) than the tree-ring widths of N addition trees between the years 1980-2010 (*Fig. 1*). The change in tree-ring widths from 1980-1994 to 1995-2010, i.e. before and after the start of the N addition experiment, was not significantly different between the N addition and control trees ($p = 0.86$). On average, the heartwood/sapwood boundary was found approximately at the year 1946 (±5.9), ranging from 1927 to 1970 for individual trees.

### *Labelling and N addition*

The labelling/N addition experiment caused a significant increase in tree-ring $\delta^{15}N$ values (un-extracted samples) ($p \leq 0.001$) and N concentration ($p = 0.013$) relative to the control between 1995/6 and 2007/8 (*Fig 2a, b*). Further analysis revealed that the $^{15}N$ label applied to the N addition plot in 1995/96 was found to cause a significant increase in $\delta^{15}N$ values in all measured tree-rings dating back to 1939 relative to the control trees ($p \leq 0.001$) (*Fig. 3*). The average difference in $\delta^{15}N$ values between the labelled and un-labelled trees was 9.4‰ (average values of 0.3 and -8.9‰ for labelled and un-labelled trees, respectively).

Tree-ring $\delta^{13}C$ values did not differ significantly between the N addition and control trees between 1995-2008 ($p = 0.90$) (*Fig. 2c*). Trees from the labelling/N addition experiment did, however, show a significant decrease in carbon concentration relative to tree-ring samples of control trees ($p \leq 0.001$) during the same time period (*Fig. 2d*).

### *Extraction procedure*

The extraction procedure had no significant effect on tree-ring $\delta^{15}$N values between the years 1991-2008 ($p = 0.16$) (*Fig. 2a)*. The correlation between the $\delta^{15}$N values of extracted and un-extracted samples was highly significant for the trees with N addition ($p \leq 0.001$), but insignificant for the control trees ($p = 0.49$) *(Fig. 4a)*. The extraction procedure also had no significant effect on tree-ring N concentrations ($p = 0.13$) (*Fig. 2b*).

Similar to $\delta^{15}$N, tree-ring $\delta^{13}$C values were not significantly affected by the extraction procedure between 1991-2008 ($p = 0.93$) (*Fig. 2c*), and the correlation of the $\delta^{13}$C values between the two sample types was highly significant for both N addition and control trees ($p \leq 0.001$) (*Fig. 4b*). The extraction did, however, have a significant effect on the tree-ring C concentration between 1991-2008 ($p = 0.003$), although the effect was not uniform between the N addition (decrease in C concentration) and control trees (increase in C concentration) (*Fig 2d*).

## Discussion

### *Tree growth*

Tree-ring widths of control trees were greater than those of N addition trees both before and after the start of the N addition, in accordance with previous studies at Alptal (Krause *et al*. 2012a). The large standard error bars of the control tree growth are indicative that there was greater variability in growth in the control trees than in the N addition trees. Noteworthy is the fact that the N addition experiment had no significant impact on the change in tree-ring width between the two N treatments. A number of studies have demonstrated elevated tree growth in response to increased N availability (Hättenschwiler *et al*. 1996, Solberg *et al*. 2004, Solberg *et al*. 2009), while fewer studies have found no effect (Seftigen *et al*. 2012) or decreased tree growth (Emmett 1999). The impact of increased N availability on tree growth is site-specific, and depends almost wholly on the N status of each site. Based on the fact that we found no evidence of either increased or decreased growth in the N addition trees, we suggest that the Alptal site is neither primarily limited by N availability, nor near levels of N saturation. Evidence of increased N leaching from the N addition plot, however, implies the site may be approaching levels of N saturation (Schleppi *et al.* 2004).

### *Labelling & N addition experiment*

#### *Nitrogen*

The tree-ring $\delta^{15}N$ values of N addition trees increased by up to 17‰ in response to the $^{15}N$ label addition, while the $\delta^{15}N$ values of control trees remained consistently between approximately -8 and -10‰. The label in the N addition trees peaked 2-3 years after its application (i.e. 1997-1998). This lag effect is hypothesised to be the result of additional factors hindering the immediate uptake of N, such as soil microbial immobilization, anaerobic soil conditions and a reduced period of active nutrient uptake as a result of the relatively high altitude of the site (Schleppi *et al.* 1999). Furthermore, the continued presence of elevated levels of $^{15}N$ in the N addition trees following the initial label application can be interpreted as (1) the translocation of N compounds to newer tree-rings, and (2) the continued uptake of $^{15}N$ label from the soil. Monitoring label recovery at the Alptal site over time revealed that the majority of the label was retained in the soil (up to 61% in 2009) and was available for root uptake many years after the initial label was applied (Krause *et al.* 2012b).

Rather unexpected were the difference in $\delta^{15}N$ values between the N addition and control tree-ring samples far preceding the start of the labelling experiment. Due to the close proximity of the N addition and control trees, we can deduce that this is at least in part due to the movement of the $^{15}N$ label into older tree-rings. The $^{15}N$ label was present in tree-rings as far back as 44 years prior to labelling. Although translocation of N across tree-rings is a well-known phenomenon (Elhani *et al.*

2003, Hart & Classen 2003), the magnitude of this effect is beyond what has been reported so far and may be species-specific. Previous investigations on the mobility of N within the tree stem observed movement into older tree-rings, and also into needles formed before the label application, but did not analyse long enough tree-ring chronologies to determine the extent of the movement in this direction (Elhani *et al.* 2003, Nömmik 1966, Schleppi *et al.* 1999). Our investigation also indicates that the presence of the $^{15}$N-label in N addition trees decreases to background levels beyond the heartwood-sapwood boundary. This supports previous hypotheses that the tree re-assimilates mobile N compounds throughout the physiologically-active sapwood, but not beyond the boundary of the heartwood (Sheppard & Thompson 2000). The low mobility of N beyond this boundary might be due to the fact that the N is transported through the xylem, the cells of which are no longer living within the heartwood (Mead & Preston 1994). The potential contamination of tree-ring $\delta^{15}$N by the presence of wood-decomposing fungi (Kohzu *et al.* 1999) can be eliminated by the fact that control trees did not show any enrichment in $^{15}$N, since all samples were oven-dried to remove any chance of decomposition.

The difference in the behaviour of the $^{15}$N label before and after the labelling year implies that different processes are involved for each respective movement. The gradual decline with time of the $^{15}$N-label following the application of the label demonstrates that the tree actively metabolises N from previously formed tree rings towards younger tree-rings (Hart & Classen 2003), as well as from the remaining label found in the soil. The sharper decrease in tree-ring $\delta^{15}$N values in rings formed before the labelling application, together with the fact that $\delta^{15}$N values of these tree-ring years still remain significantly higher than in the control trees, indicates that the movement into older tree-rings is caused more by a diffusion process than by active tree metabolism.

The increase in N concentrations of the N addition trees relative to the control trees can be attributed to the increased N availability, even though there was no apparent increase in tree growth. This implies that growth is limited by site-specific factors other than N availability, possibly low phosphorous availability or anaerobic soil conditions at Alptal (Schleppi *et al.* 1999). Additionally, the increase in tree-ring N concentration with tree age, seen in both N addition and control trees, is probably caused by the re-allocation of mobile N compounds to the youngest tree-rings (Merrill & Cowling 1966, Poulson *et al.* 1995). The storage of the $^{15}$N-label throughout the sapwood of the tree stem may also partially explain the 'missing' N sink that has been linked with long-term $^{15}$N-label investigations in forest ecosystems, for example at Alptal (Krause *et al.* 2012b).

### *Carbon*

In contrast to nitrogen, there was no significant effect of the labelling or the increased N availability on tree-ring $\delta^{13}$C values. Previous studies (Guerrieri *et al.* 2011) have noted an increase in relative $\delta^{13}$C due to increased photosynthetic activity/decreased stomatal conductance as a result of the fertilizing effect of elevated N. However, in accordance with other studies (Choi *et al.* 2005, Elhani *et al.* 2005), the lack of such an increase in our study implies that N availability is not the primary limiting factor to photosynthetic activity and wood production at the Alptal site. This hypothesis is supported by the apparent lack of increased tree growth in the N addition trees relative to the control, as well as by a previous dual isotope investigation at the same site (Krause *et al.* 2012a). Increases in tree-ring $\delta^{13}$C values in response to increased N may be suppressed by unfavourable site-specific characteristics, such as deficiencies in foliage phosphorous and magnesium concentrations, as well as the inhibition of fine root system dynamics caused by anaerobic soil conditions (Schleppi *et al.* 1999, Schleppi *et al.* 1998). Having already corrected the tree-ring $\delta^{13}$C values for the well-documented decrease in atmospheric $\delta^{13}$C (McCarroll *et al.* 2009), there must also be site-specific conditions contributing to the slight decrease in tree-ring $\delta^{13}$C values with time, for example a possible change in the stand leaf area index through time (Krause *et al.* 2012a).

Rather unexpected observations are the higher C concentrations in control trees relative to the N addition trees (0.73% on average). This may partly be a result of changes in the proportions of earlywood and latewood between the tree-rings of the N addition and control trees (Krause *et al.* 2012a). Earlywood has higher C concentrations relative to latewood due to the higher lignin content, which itself has higher C concentrations than cellulose (Lamlom & Savidge 2003). Thus, changes in the proportion of earlywood to latewood following fertilization would also alter the C concentrations of the entire tree-ring.

### *Effect of extraction*

#### *Nitrogen*

The extraction procedure had no significant effect on tree-ring $\delta^{15}N$ values from either the N addition or control trees. The slight difference in the absolute values of the samples may be the result of the physiological process of re-assimilation that discriminates against the heavier $^{15}N$ isotope, making the mobile N compounds depleted in $^{15}N$, and thus their removal causes a minor increase in remaining $^{15}N$. It may also be caused by the extraction procedure itself discriminating against the heavier $^{15}N$, removing slightly more of the lighter $^{14}N$ than the heavier $^{15}N$. In addition, the extraction had no significant effect on tree-ring N concentration, which implies that the compounds removed by the extraction procedure may have had relatively low N concentrations (Doucet *et al.* 2011, Sheppard & Thompson 2000). Interestingly, the extraction procedure had similar effects on the tree-ring N concentrations, regardless of the higher concentrations of N in the N addition tree-rings. The 7.8% weight lost in extracted samples is similar to the ~ 8% total weight lost when a longer version of the extraction procedure is used (18hrs rather than 4hrs) (Beghin *et al.* 2011).

In addition to the insignificance between the absolute isotope values of extracted and un-extracted samples, the strong correlation between the two in the N addition trees implies that trends are also very similar both before and after extraction. On the other hand, the correlation did not exist between the extracted and un-extracted wood samples from control trees, which is most probably a result of the lack of variation in $\delta^{15}N$ values between the tree-rings of control trees. The maximum difference between extracted and un-extracted samples between 1991-2008 was 1.14‰, which is just under twice the analytical precision of the repeated analyses of standards of the isotope analysis of $\delta^{15}N$.

*Carbon*

Tree-ring $\delta^{13}C$ values were unaffected by the extraction procedure, with no significant differences between either the absolute values or the correlations between extracted and un-extracted samples from both N addition and control trees. It has been previously suggested that, rather than using extracted cellulose, whole wood material can be used in $\delta^{13}C$ tree-ring studies after a simple solvent extraction (Taylor *et al.* 2008, Harlow *et al.* 2006). Our results, however, concur with previous studies (Loader *et al.* 2003) that suggest that this removal of extractives may not be required for certain species before the analysis of tree-ring $\delta^{13}C$ in dendroclimatological studies.

The extraction did, however, cause a decrease in C concentrations in N addition trees, although the opposite effect was observed for control trees. While this is a rather unexpected occurrence, it is not without precedence (Harlow *et al.* 2006). It might be due to the removal of other compounds during the extraction procedure, resulting in an increase of the proportion of C remaining in samples after extraction. As stated previously, the fertilization experiment may have caused changes in the proportions of earlywood and latewood between the N addition and control trees (Lamlom & Savidge 2003), and therefore the tree-ring chemical composition (Harlow *et al.* 2006). Changes in the chemical composition of the tree-ring between the N addition and control trees could also result in a change in the type/amount of compounds removed during the extraction procedure.

## Conclusion

Our results indicate that the extraction of mobile N compounds through the rapid extraction procedure from tree-rings of Norway spruce is unnecessary prior to their use in dendrochemical studies. This supports previous investigations that have studied a variety of extraction techniques on a number of different species (*Picea abies*, *Fagus sylvatica* and *Pinus thunbergii)* (Caceres *et al.* 2011, Doucet *et al.* 2011, Hart & Classen 2003). The translocation of the $^{15}N$ label through the stem sapwood over several decades, however, has serious implications for the use of nitrogen isotopes in Norway spruce wood material in understanding temporal and spatial changes in tree response to changing environmental conditions or when used as a proxy for the N status of trees.

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## References

Beghin, R., Cherubini, P., Battipaglia, G., Siegwolf, R., Saurer, M. & Bovio, G. (2011) Tree-ring growth and stable isotopes ($^{13}$C and $^{15}$N) detect effects of wildfires on tree physiological processes in *Pinus sylvestris* L. *Trees - Structure and Function,* **25,** 627-636.

Bukata, A. R. & Kyser, T. K. (2005) Response of the nitrogen isotopic composition of tree-rings following tree-clearing and land-use change. *Environmental Science & Technology,* **39,** 7777-7783.

Bukata, A. R. & Kyser, T. K. (2007) Carbon and nitrogen isotope variations in tree-rings as records of perturbations in regional carbon and nitrogen cycles. *Environmental Science & Technology,* **41,** 1331-1338.

Caceres, L. L. M., Mizota, C., Yamanaka, T. & Nobori, Y. (2011) Effects of pre-treatment on the nitrogen isotope composition of Japanese black pine (*Pinus thunbergii*) tree-rings as affected by high N input. *Rapid Communications in Mass Spectrometry,* **25,** 3298-3302.

Choi, W.-J., Lee, S.-M., Chang, S. X. & Ro, H.-M. (2005) Variations of $\delta^{13}$C and $\delta^{15}$N in *Pinus densiflora* tree-rings and their relationship to environmental changes in Eastern Korea. *Water, Air, & Soil Pollution,* **164,** 173-187.

Cook, E. R. & Kairiukstis, L. A. (1990) *Methods of dendrochronology - applications in the environmental sciences,* Dordrecht, NL, Kluwer Academic Publishers, pp 394.

Doucet, A., Savard, M. M., Begin, C. & Smirnoff, A. (2011) Is wood pre-treatment essential for tree-ring nitrogen concentration and isotope analysis? *Rapid Communications in Mass Spectrometry,* **25,** 469-475.

Doucet, A., Savard, M. M., Bégin, C. & Smirnoff, A. (2012) Tree-ring $\delta^{15}$N values to infer air quality changes at regional scale. *Chemical Geology,* **320–321,** 9-16.

Elhani, S., Guehl, J. M., Nys, C., Picard, J. F. & Dupouey, J. L. (2005) Impact of fertilization on tree-ring $\delta^{15}$N and $\delta^{13}$C in beech stands: a retrospective analysis. *Tree Physiol.,* **25,** 1437-1446.

Elhani, S., Lema, B. F., Zeller, B., Brechet, C., Guehl, J. M. & Dupouey, J. L. (2003) Inter-annual mobility of nitrogen between beech rings: a labelling experiment. *Annals of Forest Science,* **60,** 503-508.

Emmett, B. A. (1999) The impact of nitrogen on forest soils and feedbacks on tree growth. *Water Air and Soil Pollution,* **116,** 65-74.

Fritts, H. C. (1976) *Tree rings and climate,* London, UK, Academic Press, pp 567.

Guerrieri, R., Mencuccini, M., Sheppard, L. J., Saurer, M., Perks, M. P., Levy, P., Sutton, M. A., Borghetti, M. & Grace, J. (2011) The legacy of enhanced N and S deposition as revealed by the combined analysis of $\delta^{13}$C, $\delta^{18}$O and $\delta^{15}$N in tree rings. *Global Change Biology,* **17,** 1946-1962.

Guerrieri, R., Siegwolf, R., Saurer, M., Ripullone, F., Mencuccini, M. & Borghetti, M. (2010) Anthropogenic $NO_X$ emissions alter the intrinsic water-use efficiency ($WUE_i$) for *Quercus cerris* stands under Mediterranean climate conditions. *Environmental Pollution,* **158,** 2841-2847.

Harlow, B. A., Marshall, J. D. & Robinson, A. P. (2006) A multi-species comparison of δ13C from whole wood, extractive-free wood and holocellulose. *Tree Physiology,* **26,** 767-774.

Hart, S. C. & Classen, A. T. (2003) Potential for assessing long-term dynamics in soil nitrogen availability from variations in $\delta^{15}$N of tree rings. *Isotopes in Environmental and Health Studies,* **39,** 15-28.

Hättenschwiler, S., Schweingruber, F. H. & Körner, C. (1996) Tree ring responses to elevated $CO_2$ and increased N deposition in *Picea abies*. *Plant, Cell & Environment,* **19,** 1369-1378.

Holmes, R. L. (1983) Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin,* **43,** 69-78.

Kohzu, A., Yoshioka, T., Ando, T., Takahashi, M., Koba, K. & Wada, E. (1999) Natural $^{13}$C and $^{15}$N abundance of field-collected fungi and their ecological implications. *New Phytol.,* **144,** 323-330.

Krause, K., Cherubini, P., Bugmann, H. & Schleppi, P. (2012a) Growth enhancement of *Picea abies* trees under long-term, low-dose N addition is due to morphological more than to physiological changes. *Tree Physiology,* **32,** 1471-1481.

Krause, K., Providoli, I., Currie, W., Bugmann, H. & Schleppi, P. (2012b) Long-term tracing of whole catchment $^{15}$N additions in a mountain spruce forest: measurements and simulations with the TRACE model. *Trees - Structure and Function,* **26,** 1683-1702.

Lamlom, S. H. & Savidge, R. A. (2003) A reassessment of carbon content in wood: variation within and between 41 North American species. *Biomass and Bioenergy,* **25,** 381-388.

Leuenberger, M. (2007) To what extent can ice core data contribute to the understanding of plant ecological developments of the past? In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 211-233.

Lévy, G., Bréchet, C. & Becker, M. (1996) Element analysis of tree rings in pedunculate oak heartwood: an indicator of historical trends in the soil chemistry, related to atmospheric deposition. *Ann. For. Sci.,* **53,** 685-696.

Lim, M. T. & Cousens, J. E. (1986) The internal transfer of nutrients in a Scots pine stand II. The patterns of transfer and the effects of nitrogen availability. *Forestry,* **59,** 17-27.

Loader, N. J., McCarroll, D., Gagen, M., Robertson, I. & Jalkanen, R. (2007) Extracting Climatic Information from Stable Isotopes in Tree Rings. In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, Amsterdam, 25-48.

Loader, N. J., Robertson, I. & McCarroll, D. (2003) Comparison of stable carbon isotope ratios in the whole wood, cellulose and lignin of oak tree-rings. *Palaeogeography, Palaeoclimatology, Palaeoecology,* **196,** 395-407.

McCarroll, D., Gagen, M. H., Loader, N. J., Robertson, I., Anchukaitis, K. J., Los, S., Young, G. H. F., Jalkanen, R., Kirchhefer, A. & Waterhouse, J. S. (2009) Correction of tree ring stable carbon isotope chronologies for changes in the carbon dioxide content of the atmosphere. *Geochimica et Cosmochimica Acta,* **73,** 1539-1547.

Mead, D. J. & Preston, C. M. (1994) Distribution and retranslocation of $^{15}$N lodgepole pine over eight growing seasons. *Tree Physiology,* **14,** 389-402.

Merrill, W. & Cowling, E. B. (1966) Role of nitrogen in wood deterioration: amounts and distribution of nitrogen in tree stems. *Canadian Journal of Botany,* **44,** 1555-1580.

Millard, P. (1996) Ecophysiology of the internal cycling of nitrogen for tree growth. *Zeitschrift für Pflanzenernährung und Bodenkunde,* **159,** 1-10.

Nömmik, H. (1966) The uptake and translocation of fertilizer $^{15}$N in young trees of Scots pine and Norway spruce. *Studia Forestalia Suecica,* **35,** 1-18.

Poulson, S. R., Chamberlain, C. P. & Friedland, A. J. (1995) Nitrogen isotope variation of tree-rings as a potential indicator of environmental change. *Chemical Geology,* **125,** 307-315.

Rinn, F. (1996) *TSAP-Reference manual. Version 3.5,* Heidelberg, Germany, Rinntech, pp 262.

Salifu, K. F. & Timmer, V. R. (2003) Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. *Soil Sci. Soc. Am. J.,* **67,** 309-317.

Saurer, M., Cherubini, P., Ammann, M., De Cinti, B. & Siegwolf, R. (2004) First detection of nitrogen from $NO_X$ in tree rings: a $^{15}N/^{14}N$ study near a motorway. *Atmospheric Environment,* **38,** 2779-2787.

Saurer, M. & Siegwolf, R. T. W. (2007) Human impacts on tree-ring growth reconstructed from stable isotopes. In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 49-62.

Schleppi, P., Bucher-Wallin, L., Siegwolf, R., Saurer, M., Muller, N. & Bucher, J. B. (1999) Simulation of increased nitrogen deposition to a montane forest ecosystem: partitioning of the added $^{15}$N. *Water, Air, & Soil Pollution,* **116,** 129-134.

Schleppi, P., Hagedorn, F. & Providoli, I. (2004) Nitrate Leaching from a Mountain Forest Ecosystem with Gleysols Subjected to Experimentally Increased N Deposition. In: Wieder, R. K., Novák, M. & Vile, M. (eds.) *Biogeochemical Investigations of Terrestrial, Freshwater, and Wetland Ecosystems across the Globe*. Springer Netherlands, 453-467.

Schleppi, P., Muller, N., Feyen, H., Papritz, A., Bucher, J. B. & Flühler, H. (1998) Nitrogen budgets of two small experimental forested catchments at Alptal, Switzerland. *Forest Ecology and Management,* **101,** 177-185.

Seftigen, K., Moldan, F. & Linderholm, H. (2012) Radial growth of Norway spruce and Scots pine: effects of nitrogen deposition experiments. *European Journal of Forest Research,* **132,** 1-10.

Sheppard, P. R. & Thompson, T. L. (2000) Effect of extraction pretreatment on radial variation of nitrogen concentration in tree rings. *J. Environ. Qual.,* **29,** 2037-2042.

Solberg, S., Andreassen, K., Clarke, N., Tørseth, K., Tveito, O. E., Strand, G. H. & Tomter, S. (2004) The possible influence of nitrogen and acid deposition on forest growth in Norway. *Forest Ecology and Management,* **192,** 241-249.

Solberg, S., Dobbertin, M., Reinds, G. J., Lange, H., Andreassen, K., Fernandez, P. G., Hildingsson, A. & de Vries, W. (2009) Analyses of the impact of changes in atmospheric deposition and climate on forest growth in European monitoring plots: A stand growth approach. *Forest Ecology and Management,* **258,** 1735-1750.

Taylor, A. M., Renée Brooks, J., Lachenbruch, B., Morrell, J. J. & Voelker, S. (2008) Correlation of carbon isotope ratios in the cellulose and wood extractives of Douglas-fir. *Dendrochronologia,* **26,** 125-131.

Turner, J. (1977) Effect of nitrogen availability on nitrogen cycling in a Douglas-fir stand. *Forest Science,* **23,** 307-316.

Weber, P., Bol, R., Dixon, L. & Bardgett, R. D. (2008) Large old trees influence patterns of $\delta^{13}C$ and $\delta^{15}N$ in forests. *Rapid Communications in Mass Spectrometry,* **22,** 1627-1630.

## Figures

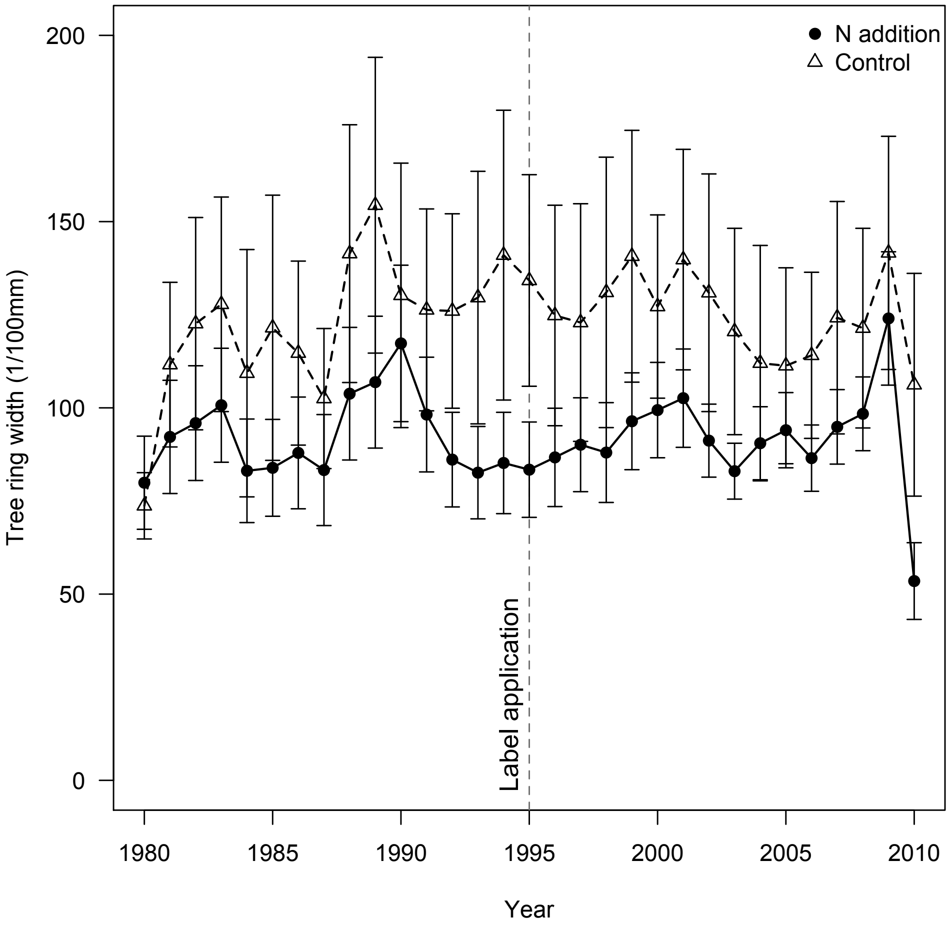

*Fig. 1* Tree-ring widths of N addition and control tree between 1980 and 2010 (bars represent ± S.E.; n = 10). The vertical dashed line represents the start of the labelling/N addition experiment (1995/96).

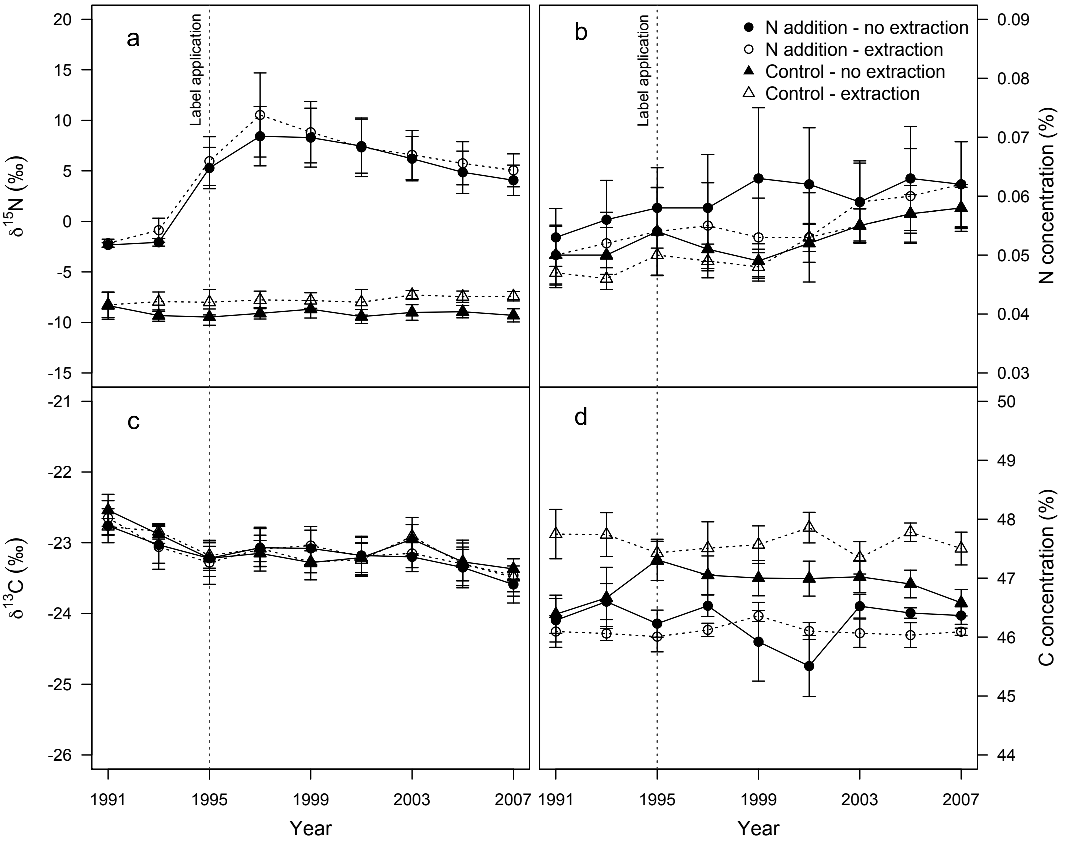

*Fig. 2* (a) $\delta^{15}$N, (b) N concentration, (c) $\delta^{13}$C, and (d) C concentration of N addition and control tree from both extracted and un-extracted samples. (bars represent ± S.E.; n = 10).

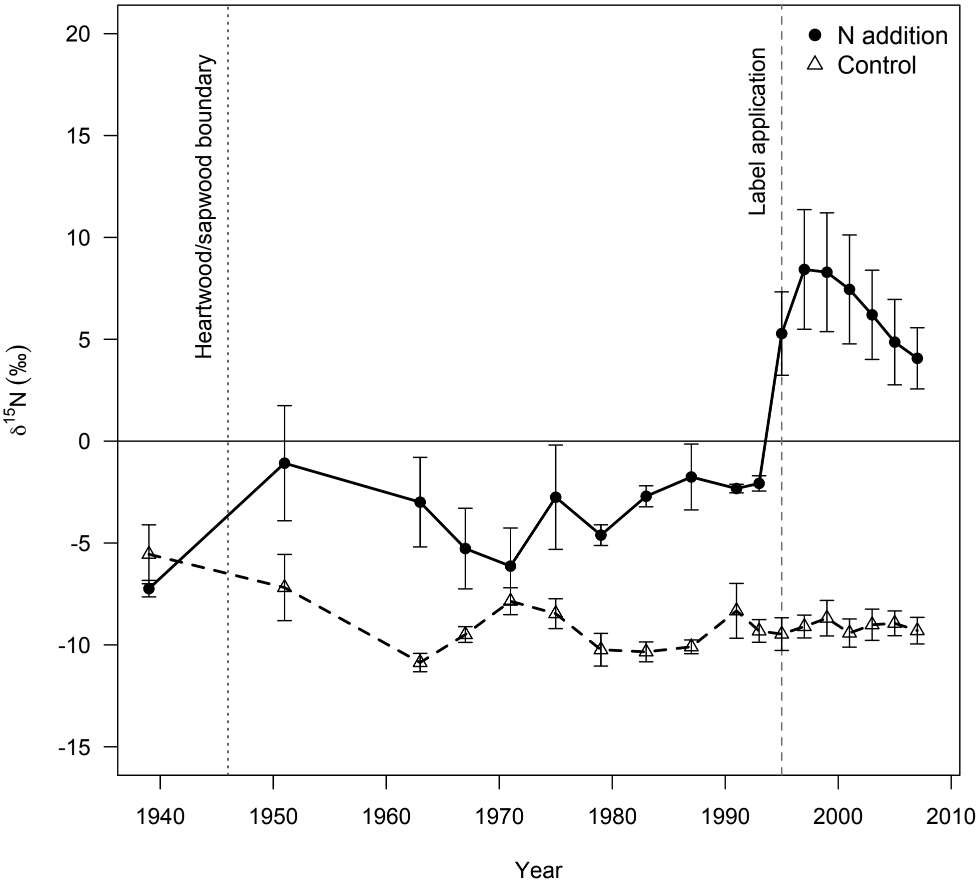

*Fig. 3* Un-extracted tree-ring $\delta^{15}N$ from the N addition and control trees between 1939 and 2008 (bars represent ± S.E.; 1963-2008, n = 10; 1939-1952, n = 8).

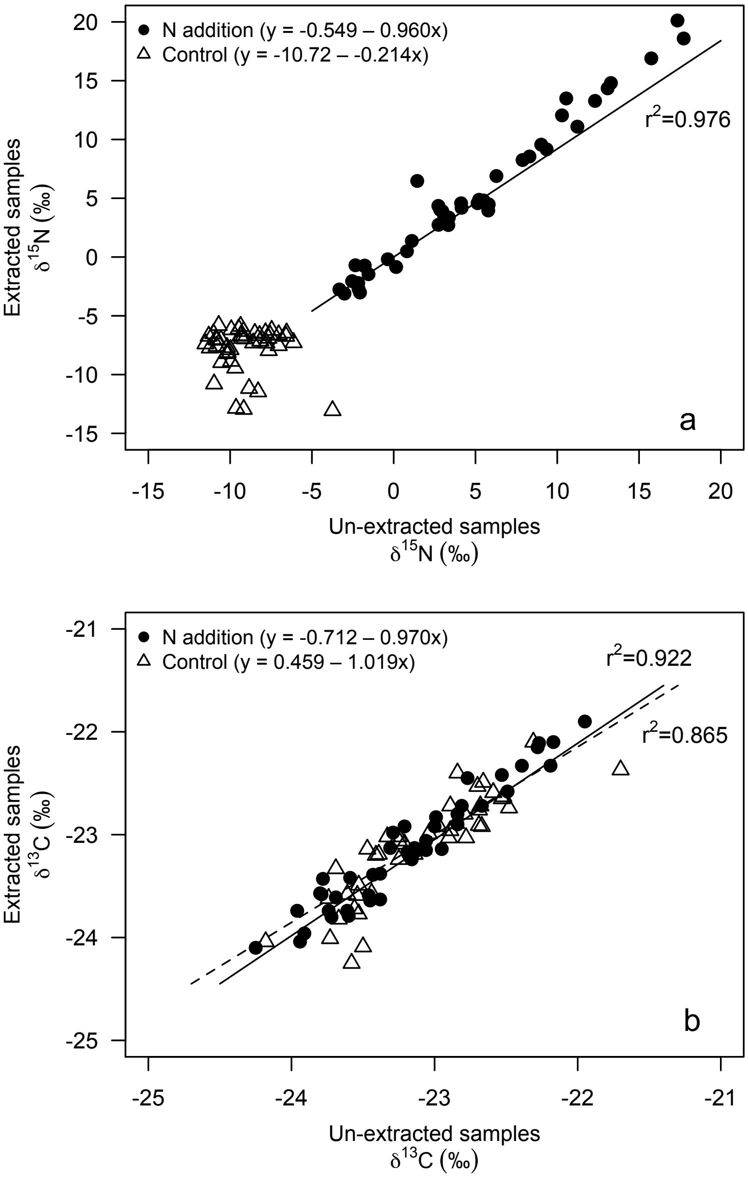

*Fig. 4* The regression between un-extracted and extracted tree-ring samples of both (a) $\delta^{15}N$ and (b) $\delta^{13}C$ from the N addition and control trees.

# Chapter 2

## Can tree-ring $\delta^{15}$N be used as a proxy for foliar $\delta^{15}$N in European beech and Norway spruce?

Tomlinson, G.[1], Buchmann, N.[2], Siegwolf, R. T. W.[3], Weber, P.[1], Thimonier, A.[1], Graf Pannatier, E.[1], Schmitt, M.[1], Schaub, M.[1], Waldner, P.[1]

[1]WSL, Swiss Federal Institute for Forest, Snow and Landscape Research, CH-8903 Birmensdorf, Switzerland
[2]Department of Environmental Systems Science, ETH Zurich, CH-8092 Zurich, Switzerland
[3]Ecosystem Fluxes Group, Paul Scherrer Institute (PSI), CH-5232 Villigen, Switzerland

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## Abstract

Currently it is unclear whether stable nitrogen isotope signals of tree-rings are related to those in foliage, and whether they can be used to infer tree responses to environmental changes. We studied foliar and tree-ring nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L.) from six long-term forest monitoring sites in Switzerland together with data on N deposition and soil N availability, as well as a drought response index over the last two decades. For both species, tree-ring $\delta^{15}N$ and $\delta^{13}C$ values were less negative compared to foliar $\delta^{15}N$ and $\delta^{13}C$ values, most likely due to recycling and reallocation of N within the tree and fractionation processes associated with the transport of sucrose and the formation of tree-rings, respectively. Temporal trends recorded in foliar $\delta^{15}N$ was not reflected in tree-ring $\delta^{15}N$, with much higher variations in tree-rings compared to foliage. Soil N availability and N deposition were partially able to explain changes in foliar $\delta^{13}C$, while there were no significant correlations between environmental variables and either tree-ring or foliar $\delta^{15}N$. Our results suggest an uncoupling between the N isotopic composition of tree-rings and foliage. Consequently, tree-ring $\delta^{15}N$ values are inappropriate proxies of foliar $\delta^{15}N$ values under low to moderate N deposition loads. Furthermore, at such low levels of deposition, tree-ring $\delta^{15}N$ values are not recommended as archives of tree responses to soil C/N or bulk N deposition.

## Introduction

Forest ecosystems are experiencing changes in climate and other environmental conditions on both regional and global scales (de Vries *et al.* 2014, Settele *et al.* 2014). Atmospheric $CO_2$ concentrations, air temperature and precipitation are highly influential in controlling forest productivity, whilst forest stands themselves influence climate through the fixation and release of $CO_2$ or through climate cooling via evapotranspiration (Settele *et al.* 2014). At the same time, the increasing use of nitrogen (N) fertilizers, coupled with increased emissions of nitrogen oxides ($NO_X$) from fossil fuel combustion are causing large-scale changes in the global N cycle (Galloway *et al.* 2008). In N-limited forest ecosystems, increased N inputs can cause a short-term increase in tree growth (Solberg *et al.* 2009). In N-saturated ecosystems, however, further mineral N inputs (as ammonium or nitrate) will lead to soil acidification (de Vries *et al.* 2014), resulting in reduced tree productivity and even tree mortality (Aber *et al.* 1998, Wallace *et al.* 2007). Further effects of N saturation include decreased vegetation biodiversity and increased susceptibility to disease (Erisman *et al.* 2011, Gilliam 2006). While it is clear that forest ecosystems will undergo significant changes throughout the 21st century, it is difficult to quantify forest responses to predicted environmental changes

The assessment of stable isotopic compositions in tree-rings and foliage is a valuable tool for assessing the influence of environmental changes on forest ecosystems (Dawson *et al.* 2002). Carbon and nitrogen stable isotopic compositions are determined by (*1*) isotopic composition of the respective source, (*2*) climatic and environmental conditions, and (*3*) tree physiological and biochemical processes. As a result, they are often used within these three areas of research. There are a number of studies that have investigated the relationship between climate and C isotopic composition of both tree-rings and foliage (e.g. Eilmann *et al.* 2010, Di Matteo *et al.* 2014). A number of studies have also used a dual-isotope approach based on both carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope ratios of tree-rings and foliage to better understand the physiological processes that alter isotopic compositions (Scheidegger *et al.* 2000, Saurer & Siegwolf 2007). In addition, there have been a number of investigations that described the relationships between the isotopic composition in tree-rings and foliage for both C (Marshall *et al.* 2008) and O (Jäggi *et al.* 2003). The mechanistic processes that determine intra-tree variation of $\delta^{13}C$ have been particularly well documented and investigated (Cernusak *et al.* 2009). Foliar $\delta^{15}N$ values have also been used to assess site-specific environmental conditions, such as N emissions (Ammann *et al.* 1999), soil N (Garten 1993) and changes in the regional N cycle (Pardo *et al.* 2006). However, there are few studies that investigate tree-ring $\delta^{15}N$. This may be due to the fact that N concentrations in tree-rings are low (relative to C in tree-rings and N in foliage) and related methodological difficulties (Savard 2010). Low tree-ring N concentrations also result in the movement of mobile N compounds between tree-rings (Tomlinson *et al.* 2014). These N recycling processes within the tree play an important role in meeting the N demand from sinks within, potentially masking N deposition signals and the impact of added N on tree-ring $\delta^{15}N$ values (Kolb & Evans 2002, Evans 2001). While some studies point out the potential of tree-ring $\delta^{15}N$ as a proxy for various forest N inputs (Savard 2010), the relationship between tree-ring and foliar $\delta^{15}N$ is still poorly understood. It is also not clear whether this relationship is species-specific, and whether changes in climate and/or site-specific N conditions affect tree-ring and foliar N (and C) isotopic compositions in the same fashion. Furthermore, it is unclear whether the isotope ratios of tree-rings or of foliage better reflect site-specific environmental conditions such as water or N availability. The assessment of the relationship between tree-ring and foliar $\delta^{15}N$ values would determine the potential use of the much longer chronologies of tree-ring $\delta^{15}N$ values as proxies for foliar $\delta^{15}N$ values in environmental investigations.

Long-term monitoring programs are essential in both understanding and testing how forest ecosystems respond to environmental changes, particularly of climate and N availability. In this dual-isotope investigation, we combined chronologies of both tree-ring and foliar $\delta^{13}C$ and $\delta^{15}N$ of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L.) with environmental data collected from six long-term monitoring sites within Switzerland. Our objectives were to: (*1*) determine whether intra-tree $\delta^{15}N$ and $\delta^{13}C$ variations differ between *Fagus sylvatica* and *Picea abies*, (*2*) investigate differences in the inter-annual variability of both $\delta^{15}N$ and $\delta^{13}C$ between foliage and tree-rings, and the influence of selected environmental variables on $\delta^{15}N$ tree isotopic composition, and (*3*) determine the potential of tree-ring $\delta^{15}N$ as a proxy for foliar $\delta^{15}N$.

## Methods

### *Site description*

Switzerland is one of approximately thirty European countries currently participating in the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (www.icp-forests.net). The 19 Swiss sites included in the programme are also part of the Swiss Long-Term Forest

Ecosystem Research Programme (LWF) and the European Long-Term Ecosystem Research network (LTER-Europe) (Cherubini & Innes 2000, Schaub *et al.* 2011). These 19 sites are distributed across Switzerland, spanning a wide range of geographical/altitudinal gradients and cover various forest ecosystem types. Based on species distribution, the availability of archived foliar samples for both *Fagus sylvatica* and *Picea abies*, as well as the environmental data available (*Table 1*), six LWF sites (Alptal, Beatenberg, Bettlachstock, Lausanne, Othmarsingen, and Vordemwald) were selected for this study. All measurements were conducted in accordance with the ICP Forests Programme (ICP-Forests 2010).

### *Climate, nitrogen deposition and soil data*

At each LWF site, there is one meteorological station within the forested site and another one in an open field nearby, within a radius of 2km. In Alptal, meteorological data from the station Erlenhöhe, located in the hydrological research catchment Erlenbach (operated by WSL), were used. Variables measured at these meteorological stations include precipitation (measured at 1.5 m height), air temperature (2 m height) and relative humidity (2 m height), global short-wave solar radiation (3 m height), and wind speed (4.6 m height). The values from the open field station were verified by comparing against corresponding values from the forest meteorological station and from the closest MeteoSwiss meteorological station (www.meteoswiss.ch). Non-plausible and missing values were replaced by values either from the forest meteorological LWF station or the MeteoSwiss station after applying regression analysis that was established from periods with plausible values. For precipitation, the values of the open field station were also checked against the values from deposition volume measurements and subsequently replaced or scaled as appropriate.

Bulk precipitation, from which bulk N deposition was determined, was continuously sampled using three replicated, custom-made funnel-type polyethylene collectors (replaced by bucket-type collectors during the winter). These collectors were positioned 1.5 m above ground in the open fields where the meteorological stations were situated. In Alptal, bulk precipitation measurements were carried out with only one funnel-type polyethylene collector, connected with a tube to a sample bottle placed beneath the soil surface. The content of each of these collectors was collected biweekly and brought to the lab where it was stored at 2°C prior to chemical analyses. Bulk N deposition values were used in this investigation, as total N deposition values were not available for all sites. However, the similar temporal trends between bulk N deposition and total N deposition (where available) meant that we were able to make conclusions based on bulk N deposition measurements during our analyses. A detailed description of the sampling, N analysis, quality assurance and control procedures of atmospheric deposition calculations can be found in Thimonier *et al.* (2005) for LWF and Kloeti *et al.* (1989) for Alptal.

Soil matric potentials were measured using tensiometers at five different depths: 15, 30, 50, 80, and 130 cm, with eight replicates per depth and site, every two weeks at all sites (with the exception of Alptal) (Graf Pannatier *et al.* 2012). At Alptal, the ground water level was measured instead of soil matric potential because of high levels of precipitation, shallow soils and an impermeable under-layer. The C/N ratios of the forest floor (Oe and Oa layers) or, when these organic horizons were missing of the upper 5 cm of mineral soil, were determined once during the period 1994–1999 at each of the six sites. A detailed description of the sampling and analysis of soil samples can be found in Walthert *et al.* (2003).

*Drought response index*

A drought response index (*DRI*) was calculated for each site and day as the difference between potential evapotranspiration (*PET*) and measured precipitation (*P*), with:

$$DRI = PET - P \qquad \text{eq. 1}$$

*PET* was calculated with the Penman-Monteith approach (Monteith 1965) as implemented in the CoupModel (Jansson & Karlberg 2004), using the parameter settings of Gustafsson *et al.* (2004) for forests. Soil matric potential measurements were used to verify the PET model. Daily DRI values were totalled over the entire growing-season (1st April-31st October) to obtain annual growing-season values between 1997-2011 (or 1998-2011, depending on data availability) that were subsequently used in the analyses.

### *Tree sampling*

Foliage has been sampled biennially since 1997 at each of the LWF sites, with the exception of Vordemwald, for which samples were only taken in 2009. Samples were collected from the upper third of the tree crown from five dominant trees in the surrounding buffer zone of the LWF site. Sampling was undertaken during summer for *F. sylvatica* before the onset of autumnal leaf yellowing or senescence, and during autumn for *P. abies* after the onset of the dormancy period of coniferous species (Thimonier *et al*. 2010).

For tree-ring samples, ten dominant trees were selected adjacent to each LWF site, as close as possible to the trees from which foliage samples had been taken, in terms of distance and site topography. Trees were selected adjacent to the LWF site due to the fact that the coring of trees from within the site is prohibited under the guidelines set out by the ICP-Forests program. This is with the exception of *P. abies* at Vordemwald, from which it was possible to extract cores from the same trees from which foliage samples had been taken in 2009 as well as from an additional five trees adjacent to the LWF site. Two cores were extracted from each tree at a height of approximately 1.5 m in 2012 using a 0.5 cm diameter increment borer. Cores were air-dried and planed using a custom-made core microtome (WSL, Switzerland) to improve the visibility of individual tree-rings without contamination of wood material between tree-ring years.

### *Dendrochronological analyses*

Annual tree-rings were identified and measured from bark to pith, with a resolution of 0.01mm using the TSAPWIN tree-ring software (Version 3.5) connected to a LINTAB3 measuring system (F. Rinn S.A., Heidelberg, Germany). The tree-rings were subsequently cross-dated, both visually by comparing individual chronologies to site mean chronologies and by using standard methods (Cook & Kairiukstis 1990, Fritts 1976). The tree-ring chronologies were statistically verified using the TSAP and COFECHA routines (Holmes 1983), before being detrended with a 30 year smoothing spline using the ARSTAN program (Version 44h2, Cook (1985) to remove tree-ring biases caused by tree age. Although chronologies were found to date back to 1725, we considered 1997-2011 only, as this was the time period for which archived foliage samples were available.

*Sample preparation and isotope analyses*

Biennial foliar samples (leaves and current-year needles) were dried at 65°C, after which the weight of 100 leaves or 1000 needles was determined. The samples taken since 1997 have been stored in a micro-climate controlled archive at the WSL. In 2012, the archived foliage samples of each of the five trees were pooled to produce one composite sample per site and sampling year, and subsequently milled and homogenised using a MM200 centrifugal ball mill (Retsch, Haan, Germany). This was with the exception of needle samples taken from *P. abies* at Vordemwald in 2009 that were prepared and analysed at an individual tree level. The milled material was weighed into tin capsules (1.8-2.0mg for *F. sylvatica* leaves and 3.5-3.8 mg for *P. abies* needles, according to species differences in foliage N concentration) for stable isotope analysis. The $\delta^{13}C$ and $\delta^{15}N$ were measured simultaneously through combustion under excess oxygen in an elemental analyser (EA3000, EuroVector, Milano, Italy) interfaced to a continuous flow stable isotope mass spectrometer (Delta V, Thermo Fisher, Bremen, Germany), with a precision of 0.2 and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Five trees (ten cores) from each site were selected for C and N tree-ring isotope analyses. Based on the findings of Tomlinson *et al.* (2014), where no significant changes in isotopic composition of either C or N were found after the extraction of mobile compounds, we decided to use whole wood material. Single tree-rings were separated with a razor blade under a microscope, and then pooled for each individual tree-ring year per site. These samples were milled and homogenised using the MM200 centrifugal ball mill. The resulting wood material was weighed into tin capsules (0.5-0.55mg) for $\delta^{13}C$ analysis (site n = 15, total n = 105). For the analysis of wood $\delta^{15}N$, we applied the '10 year split pool approach', whereby wood samples are pooled for three consecutive tree-ring years, with an individual year sample every 10th year (e.g. Kress *et al.* (2010); site n = 6, total n = 35). This ensures sufficient wood material for the $\delta^{15}N$ analysis, which requires much more material due to very low N concentrations. This approach also allows the assessment of annual inter-site variance every 10th year. Samples were pooled by combining 10 mg of material from each tree-ring year (e.g. 1998-2000, 2002-2004 etc.), and homogenized using a vortexer (Select Bioproducts, Edison, USA). For *P. abies* from Vordemwald, the tree-ring samples were pooled differently between 2008-2011, as wood material was sampled from the same trees from which foliar samples were taken in 2009. The wood material from each tree at this site was measured individually (rather than all five trees pooled), whilst the tree-rings were pooled every two years between 2008 and 2011 (i.e. 2008/2009 and 2010/2011). All wood material prepared for $\delta^{15}N$ analysis was then weighed into tin capsules (~15mg). Both the C and N samples were analysed by combustion under excess oxygen in an elemental analyser (EA 1110, Carlo Erba, Milano, Italy) connected in continuous-flow mode to an isotope ratio mass spectrometer (Delta-S, Finnigan, Bremen, Germany), with a precision of 0.1 and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. A blank (empty tin capsule) was also measured after each N sample due to the high C/N ratios in wood material (approx. 500:1).

Isotope values are expressed in the δ-notation as relative deviations from the international standards; Vienna Pee Dee Belemnite for carbon ($\delta^{13}C = ((^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1)$; [‰]), and atmospheric $N_2$ for nitrogen ($\delta^{15}N = ((^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1)$; [‰]). The tree-ring $\delta^{13}C$ was corrected for the Suess effect, caused by the production of $^{13}C$-depleted $CO_2$ emissions during fossil fuel combustion. This was achieved by using records of past atmospheric $\delta^{13}C$ obtained from ice cores and from historical $\delta^{13}C$ recorded at Mauna Loa (Francey *et al.* 1999, Leuenberger 2007). For both tree-ring and foliar $\delta^{13}C$ values, we used these Suess-corrected values for all further analyses.

### *Data analyses*

### *Intra-tree and inter-species variation*

All statistical analyses were performed using the statistical software program *R* (version 3.1.3). We applied paired t-tests to investigate whether there were significant differences in the isotopic composition of C and N between tree-rings and foliage within each tree species. We investigated the relationship between tree-ring and foliage isotope ratios of both C and N with linear regression. For C, both tree-ring and foliar $\delta^{13}C$ isotope data used in these models matched the time resolution of the foliage data (i.e. 1997, 1999, 2001 etc.; n = 8/site), with the exception of Vordemwald. Here, no foliage data were available for *F. sylvatica*, but individual tree-ring and foliar results for the five *P. abies* trees measured in 2009 were used. For all sites, as tree-ring $\delta^{15}N$ samples had been pooled prior to analyses, the foliage samples were averaged accordingly to match the tree-ring samples (site n = 6, with the exception of Vordemwald). Un-paired t-tests were applied to determine species differences in tree-ring $\delta^{13}C$, tree-ring $\delta^{15}N$, foliar $\delta^{13}C$ and foliar $\delta^{15}N$ isotopic composition.

### *Environmental effects*

Linear regression models were used to test for temporal trends in environmental variables between 1997 and 2011. Linear mixed-effects models were used to investigate the effects of environmental variables (explanatory variables) on both tree-ring and foliage isotopic composition and tree growth. Initial analyses on annual tree-ring $\delta^{13}C$ values demonstrated a lack of independency between tree-ring years, and subsequently it was decided to average the results of the tree-ring $\delta^{13}C$ analyses to match the pooling of the tree-ring $\delta^{15}N$ (i.e. 1998-2000, 2001, 2002-2004, 2005-2007, 2008-2010, 2011). Data for the environmental variables were averaged to match the tree-ring data (i.e. 10-year split pool approach) or biannually selected to match the foliar data (every second year between 1997 and 2011). Tree-ring width (TRW), tree-ring $\delta^{15}N$, foliar $\delta^{15}N$, tree-ring $\delta^{13}C$, and foliar $\delta^{13}C$ were assessed using individual linear mixed-effects models. In these models, species (*F. sylvatica* or *P. abies*), soil C/N, annual growing-season DRI ($DRI_{Apr\text{-}Oct}$), annual bulk deposition of mineral N (N deposition), year and site (as a random factor; n = 6) were included as explanatory variables in a complete model.

The significance of each explanatory variable was tested by removing each one in turn from the complete model. An Analysis of Variance (ANOVA) was subsequently used to determine whether there was a significant difference between the original model and the model lacking the particular explanatory parameter, i.e. to determine whether this variable significantly contributed to explaining the remaining variability.

## Results

### *Intra-tree relationships and inter-species differences*

Tree-ring $\delta^{15}$N values were significantly less negative than foliar $\delta^{15}$N values for both *F. sylvatica* and *P. abies* ($p \leq 0.001$; *Table 2*). Moreover, $\delta^{15}$N of tree-rings was not related to that of foliage for either species ($p = 0.80$ and 0.55 for *F. sylvatica* and *P. abies*, respectively) (*Fig. 1b*). Tree-ring $\delta^{13}$C was also significantly less negative than foliar $\delta^{13}$C for both tree species ($p \leq 0.001$; *Table 2*). $\delta^{13}$C of tree-rings and foliage of both species were positively related to each other, with highly significant relationships for *P. abies* ($p \leq 0.001$), but less so for *F. sylvatica* ($p = 0.05$) (*Fig. 1a*).

Overall, $\delta^{15}$N of tree-rings and foliage of *F. sylvatica* were significantly ($p \leq 0.001$) less negative compared to those of *P. abies* (by 1.58‰ and 1.85‰, respectively; *Table 2*). In contrast, tree-ring $\delta^{13}$C of *P. abies* was significantly less negative than that of *F. sylvatica* (~1.98‰; $p \leq 0.001$), showing a similar pattern as foliar $\delta^{13}$C, which was also significantly less negative in *P. abies* compared to *F. sylvatica* (1.54‰; $p \leq 0.001$) (*Table 2*).

### *Temporal variations and environmental drivers*

Between 1997 and 2011, no significant temporal trends in either $\delta^{13}C$ or $\delta^{15}N$ in tree-rings were observed for any of the sites (*Table 3*; *Fig. 2a*, *2c*). However, large among-site variations were present for both $\delta^{13}C$ in tree-rings (with maximum differences of 3.0‰ and 1.6‰ for *F. sylvatica* and *P. abies*, respectively) and $\delta^{15}N$ in tree-rings (maximum differences of 5.0‰ and 5.8‰ for *F. sylvatica* and *P. abies*, respectively). In contrast, both $\delta^{13}C$ and $\delta^{15}N$ in foliage significantly decreased between 1997 and 2011, by up to 1.5‰ for $\delta^{13}C$ and 1.8‰ for $\delta^{15}N$ (*Table 3*; *Fig. 2b*, *2d*). At the same time, variations of both foliar $\delta^{13}C$ and $\delta^{15}N$ among sites for the same species were lower than seen in tree-rings. Standardized TRW (corrected for tree age) also decreased significantly between 1997-2011 (*Table 3*; *Fig. 3a*), in contrast to $DRI_{Apr\text{-}Oct}$ ($p = 0.65$; *Fig. 3b*). Bulk N deposition also decreased significantly within this period ($p \leq 0.001$; *Fig. 3c*). Foliar $\delta^{13}C$ values were affected by these environmental factors and became less negative with increasing soil C/N values and increasing N deposition (*Table 3*). Conversely, tree-ring $\delta^{13}C$ values and both $\delta^{15}N$ values in tree-rings and foliage were not affected by any of the environmental factors considered (*Table 3*).

## Discussion

### *Intra-tree and inter-species isotopic variations*

Tree-rings showed less negative $\delta^{15}N$ values relative to foliage for both species (~1.0‰). Bukata & Kyser (2007) found the same pattern for a number of *Quercus* and *Betula* species (with differences of about 0.5‰). In our investigation, we found no significant relationship between $\delta^{15}N$ in tree-rings and foliage. Furthermore, N concentrations within the tree stem were particularly low compared to those in foliage for both *F. sylvatica* (average values of 0.12‰ and 2.36%, respectively) and *P. abies* (0.06‰ and 1.13%, respectively). This marked difference in N concentration between the tree stem and the foliage highlights the greater need for N in the foliage than in the tree-rings. Tree foliage is a large N sink within a tree as N is a key component of the photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in leaves and needles (Takashima *et al.* 2004). With low tree N concentrations and a high foliar N demand, N recycling within the tree is an essential physiological process to ensure that all N demands within the tree are met, primarily in the foliage. This process of N recycling can occur within the foliage, before leaf abscission in deciduous trees or between different age-classes of needles in conifers (Gebauer & Schulze 1991, Kolb & Evans 2002). Further N recycling processes occur within the tree stem (Tomlinson *et al.* 2014) and between the tree stem and foliage. Notably, foliar $\delta^{15}N$ values measured in our investigation showed very little variation compared to $\delta^{15}N$ in tree-rings from the same site. Similarly, Gerhart & McLauchlan (2014) highlighted a lack of a consistent correlation between tree-ring $\delta^{15}N$ and tree-ring N concentrations, as often seen in the foliage. They attributed the lack of a relationship between tree-ring $\delta^{15}N$ and tree-ring N concentrations to non-environmental processes, such as N recycling to fulfill the high N demands in the foliage. Recycling of N will most likely result in more enriched (less negative) $\delta^{15}N$ in the source tissues since molecules containing heavier isotopes are discriminated against and are left behind, a pattern indeed observed in tree-rings in our study.

The comparison of $\delta^{15}N$ between species revealed less negative $\delta^{15}N$ values in tree-rings and foliage for *F. sylvatica* than for *P. abies* (by 1.6‰ and 1.9‰, respectively). These differences might be due to a substantial difference in the biochemical composition of the foliage between the two species. For example, *P. abies* allocates far less N to compounds used in photosynthesis than *F. sylvatica*, instead allocating more N (in relative terms) to cell wall structures to increase foliage toughness and longevity (Takashima *et al.* 2004). The two species also differ in rooting depth (*Table 1*), which could result in beech taking up N also from deeper soil N pools than spruce. $\delta^{15}N$ values are known to increase with increasing soil depth due to fractionation during mineralisation and litter decomposition (Gebauer & Schulze 1991, Högberg 1997). As a result, differences in N uptake from different soil depths may explain the higher $\delta^{15}N$ for beech compared to spruce. Furthermore, differences in mycorrhizal relationships may influence species-specific $\delta^{15}N$ values, as mycorrhizae typically transfer $^{15}N$-depleted N to the tree (Craine *et al.* 2009). Different species-specific uptake preferences for nitrate and ammonium may also contribute to species differences in both tree-ring and foliar $\delta^{15}N$. However, the uptake of ammonium is known to be significantly greater than the uptake of nitrate for both tree species (Gessler *et al.* 1998). Furthermore, we do not have any data on the isotopic composition of either soil nitrate or ammonium at any of our sites. There was, however, no effect of site-specific soil C/N values (which are inversely correlated with nitrification rates; Bengtsson *et al.* (2003)) on either tree-ring or foliar $\delta^{15}N$ values.

Tree-ring $\delta^{13}C$ was less negative than foliar $\delta^{13}C$ by 0.91‰ on average. A multi-species review by Badeck *et al.* (2005) also found less negative $\delta^{13}C$ values in woody stems relative to foliage (by 1.9‰). Other studies have suggested this intra-tree variation to primarily being the result of (*1*) differences in the biochemical composition between tree-rings and foliage, and (*2*) the growth of tree-rings and foliage occurring during periods of different environmental conditions and thus photosynthetic discrimination (Badeck *et al.* 2005, Cernusak *et al.* 2009). Our investigation found a significant positive correlation between $\delta^{13}C$ values of tree-rings and foliage for *P. abies* but not so for *F. sylvatica*. Species-specific differences between evergreen and deciduous tree $\delta^{13}C$ values have generally been attributed to differences in tree physiology, primarily to lower stomatal conductance and higher intrinsic water-use efficiency of *P. abies* relative to *F. sylvatica* (Brooks *et al.* 1997, Cernusak *et al.* 2009). Moreover, the intra-tree difference between $\delta^{13}C$ values of tree-rings vs. foliage was nearly twice as large for *P. abies* than for *F. sylvatica* (1.13‰ and 0.69‰, respectively). This can additionally be attributed to species-specific differences in wood biochemical composition. Softwoods, such as *P.abies,* typically have lower lignin and higher cellulose concentrations than hardwoods, such as *F. sylvatica*. This is significant, given that cellulose is often $^{13}C$-enriched and lignin $^{13}C$-depleted relative to the organic mass of the measured plant component (Badeck *et al.* 2005, Saka 2001).

### *Temporal trends and environmental influences*

Tree-ring $\delta^{15}N$ did not show any significant temporal trends, whilst foliage $\delta^{15}N$ significantly decreased over the measurement period by about 0.9‰. Internal N recycling and a smaller sink strength than growing photosynthesizing tissues might explain the lack of temporal trends in the $\delta^{15}N$ of tree-rings. A temporal decrease of up to 4‰ in foliar $\delta^{15}N$ has previously been observed by Högberg *et al.* (1992) in current-year foliage between 1966 and 1989. These decreasing $\delta^{15}N$ values over time have been suggested to be the result of changes in atmospheric N isotopic composition, which is strongly dependent on emission sources (Ammann *et al.* 1999, Saurer *et al.* 2004). However, there are no long-term datasets of the N isotopic signature of atmospheric N deposition available that would allow us to determine whether this was the case at our study sites.

Similarly, we found no significant temporal trends in the $\delta^{13}C$ values of tree-rings for this relatively short time period. Foliar $\delta^{13}C$ values, however, became more negative over the measurement period for both species, even after correcting for the declining atmospheric $^{13}C/^{12}C$ caused by the production of $^{13}C$-depleted fossil fuel emissions (McCarroll *et al.* 2009). While none of the environmental variables considered provided an explanation for the variation in tree-ring $\delta^{13}C$, both soil C/N and N deposition were partially able to explain the site differences in foliar $\delta^{13}C$ (*Table 3*). Less negative foliar $\delta^{13}C$ values were found at sites with higher soil C/N values (i.e. decreasing soil N availability), while foliar $\delta^{13}C$ also became less negative with increasing N deposition. Investigating the water-use efficiency of poplar leaves under different soil N and atmospheric $NO_2$ regimes, Siegwolf *et al.* (2001) also found a decrease in foliar $\delta^{13}C$ with increasing soil N availability and decreasing $NO_2$ exposure. In that study, the effect of decreasing $\delta^{13}C$ in foliage with increasing soil N availability was attributed to an increase in stomatal conductance. Increasing $\delta^{13}C$ values with increasing $NO_2$, however, was attributed to an increase in $CO_2$ assimilation, but with a more moderate increase in stomatal conductance. In our study, the lack of soil C/N or N deposition affecting tree-ring $\delta^{13}C$ and TRW suggests that both spatial and temporal variations in N availability were not strong enough to significantly alter either tree-ring $\delta^{13}C$ or TRW.

### *Are tree-ring $\delta^{15}N$ values suitable proxies for foliar $\delta^{15}N$ values?*

Our analyses for the period 1997 to 2011 clearly demonstrated a significant uncoupling between tree-ring and foliar $\delta^{15}N$, suggesting that tree-ring $\delta^{15}N$ represents an inappropriate proxy for foliar $\delta^{15}N$ or $\delta^{15}N$ in atmospheric N deposition. Contrary to our results, Bukata & Kyser (2007) reported a significant positive correlation between the $\delta^{15}$N values in both tissues for two *Quercus* species. However, their analyses included foliar and tree-ring samples from one year only (2006) and thus did not include a time series of foliar and tree-ring isotopic composition, such as in our investigation. The lack of a time series in the investigation of Bukata & Kyser (2007) becomes significant when N recycling processes within the tree are taken into account. While all foliage samples analyzed in our investigation were current year foliage samples (subsequently archived), all tree-ring samples were collected together during one sampling procedure in 2012 (i.e. the tree-rings from 1997 were already 15 years old). Consequently, the tree-rings included in our investigation would have been subjected to N recycling processes to meet the strong N demand from sinks within the foliage (and younger tree-rings). The fact that N can be assimilated into the tree from both the soil and directly from the atmosphere also contributes to this uncoupling effect, as direct foliar uptake of atmospheric reactive N has previously been suggested to contribute to 16% of plant N demand (Vallano & Sparks 2007). This uncoupling process is further supported by: (*1*) the fact that foliar $\delta^{15}$N values significantly decreased over our measurement period, while there was no significant difference in tree-ring $\delta^{15}$N, (*2*) the much greater variation in tree-ring $\delta^{15}$N relative to foliar $\delta^{15}$N, and (*3*) the large differences in N concentrations between tree-rings and foliage in both *F. sylvatica* and *P. abies*.

Furthermore, our analyses demonstrated that tree-ring $\delta^{15}$N values were not affected by various site-specific environmental conditions over a 15-year period, such as soil N availability or changing rates of bulk N deposition. This is in contrast to a number of previous investigations that have shown that tree-ring $\delta^{15}$N values might be able to provide information on anthropogenic sources of forest N inputs (Elhani *et al.* 2005, Guerrieri *et al.* 2009, McLauchlan & Craine 2012, Saurer *et al.* 2004, Savard *et al.* 2009, Sun *et al.* 2010). For example, a number of these studies demonstrated a gradual decrease in tree-ring $\delta^{15}$N values over time, thought to be due to changes in the isotopic composition of atmospheric N (e.g. McLauchlan & Craine 2012, Poulson *et al.* 1995, Savard *et al.* 2009). Unlike our investigation, however, many of these studies sampled trees that were either exposed to high levels of N deposition

(Guerrieri *et al.* 2009, Saurer *et al.* 2004, Sun *et al.* 2010) or from N fertilization experiments that simulated high doses of N deposition (Elhani *et al.* 2005, Guerrieri *et al.* 2011). Furthermore, a recent review by Gerhart & McLauchlan (2014) on the potential application and limitations of using wood $\delta^{15}N$ to study terrestrial nutrient cycles, highlighted the speculative nature of the causes of these temporal trends of tree-ring $\delta^{15}N$. This is primarily due to a lack of both long- and short-term data on the isotopic composition of deposited N, and the large regional and seasonal variability of the isotopic signature of N deposition (Gerhart & McLauchlan 2014). Moreover, the high bidirectional mobility of N within the tree stem of *P. abies,* demonstrated by Tomlinson *et al.* (2014) for a period of 70 years, suggests that any potential $^{15}N$-deposition signal within the tree-ring $\delta^{15}N$ chronology must be interpreted with great care. This movement of N within the tree stem also contributes to the large inter-annual variability in tree-ring $\delta^{15}N$ seen in our investigation, a systematic feature found within many investigations on tree-ring $\delta^{15}N$. Thus, the results of our investigation have shown that, over a 15-year period and at levels of low to moderate N deposition, physiological processes within the tree mask any environmental signals that were present within the tree-ring $\delta^{15}N$ chronology.

In conclusion, using tree-ring $\delta^{15}N$ as a proxy for foliar $\delta^{15}N$ for both *F. sylvatica* and *P. abies* is inappropriate for site conditions with low to moderate N deposition. Furthermore, the large variation in tree-ring $\delta^{15}N$ found for the same species and among sites make them also unsuitable as archives for the nitrogen isotopic composition of low to moderate atmospheric N deposition, most likely due to internal N recycling processes and/or differences in tree N assimilation. As a consequence of such recycling processes, particularly within the tree stem, any potential N deposition signal – once present in tree-ring N isotopic composition – will unfortunately not be preserved within a given tree-ring year.

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## References

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L. & Fernandez, I. (1998) Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited. *Bioscience,* **48,** 921-934.

Ammann, M., Siegwolf, R., Pichlmayer, F., Suter, M., Saurer, M. & Brunold, C. (1999) Estimating the uptake of traffic-derived $NO_2$ from $^{15}N$ abundance in Norway spruce needles. *Oecologia,* **118,** 124-131.

Badeck, F.-W., Tcherkez, G., Nogués, S., Piel, C. & Ghashghaie, J. (2005) Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a widespread phenomenon. *Rapid Communications in Mass Spectrometry,* **19,** 1381-1391.

Bengtsson, G., Bengtson, P. & Månsson, K. F. (2003) Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry,* **35,** 143-154.

Brooks, J. R., Flanagan, L. B., Buchmann, N. & Ehleringer, J. R. (1997) Carbon isotope composition of boreal plants: functional grouping of life forms. *Oecologia,* **110,** 301-311.

Bukata, A. R. & Kyser, T. K. (2007) Carbon and nitrogen isotope variations in tree-rings as records of perturbations in regional carbon and nitrogen cycles. *Environmental Science & Technology,* **41,** 1331-1338.

Cernusak, L. A., Tcherkez, G., Keitel, C., Cornwell, W. K., Santiago, L. S., Knohl, A., Barbour, M. M., Williams, D. G., Reich, P. B., Ellsworth, D. S., Dawson, T. E., Griffiths, H. G., Farquhar, G. D. & Wright, I. J. (2009) Why are non-photosynthetic tissues generally $^{13}C$ enriched compared with leaves in C3 plants? Review and synthesis of current hypotheses. *Functional Plant Biology,* **36,** 199-213.

Cherubini, P. & Innes, J. L. (2000) Switzerland: The Swiss Long-Term Forest Ecosystem Research Programme. In: Gosz, J. R., French, C., Sprott, P. & White, M. (eds.) *The International Long Term Ecological Research Network, 2000 Perspectives from Participating Networks*. U.S. Long Term Ecological Research Network Office, University of New Mexico, Albuquerque, NM, 56-59.

Cook, E. R. (1985) *A time series approach to tree-ring standardization,* Tucson, Arizona, USA, University of Arizona, pp 171.

Cook, E. R. & Kairiukstis, L. A. (1990) *Methods of dendrochronology - applications in the environmental sciences,* Dordrecht, NL, Kluwer Academic Publishers, pp 394.

Craine, J. M., Elmore, A. J., Aidar, M. P. M., Bustamante, M., Dawson, T. E., Hobbie, E. A., Kahmen, A., Mack, M. C., McLauchlan, K. K., Michelsen, A., Nardoto, G. B., Pardo, L. H., Penuelas, J., Reich, P. B., Schuur, E. A. G., Stock, W. D., Templer, P. H., Virginia, R. A., Welker, J. M. & Wright, I. J. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist,* **183,** 980-992.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H. & Tu, K. P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics,* **33,** 507-559.

de Vries, W., Du, E. & Butterbach-Bahl, K. (2014) Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. *Current Opinion in Environmental Sustainability,* **9–10,** 90-104.

Di Matteo, G., Perini, L., Atzori, P., De Angelis, P., Mei, T., Bertini, G., Fabbio, G. & Scarascia-Mugnozza, G. (2014) Changes in foliar carbon isotope composition and seasonal stomatal conductance reveal adaptive traits in Mediterranean coppices affected by drought. *Journal of Forestry Research,* **25,** 839-845.

Eilmann, B., Buchmann, N., Siegwolf, R., Saurer, M., Cherubini, P. & Rigling, A. (2010) Fast response of Scots pine to improved water availability reflected in tree-ring width and $\delta^{13}$C. *Plant, Cell & Environment,* **33,** 1351-1360.

Elhani, S., Guehl, J. M., Nys, C., Picard, J. F. & Dupouey, J. L. (2005) Impact of fertilization on tree-ring $\delta^{15}$N and $\delta^{13}$C in beech stands: a retrospective analysis. *Tree Physiol.,* **25,** 1437-1446.

Erisman, J. W., van Grinsven, H., Grizzetti, B., Bouraoui, F., Powlson, D. & Reis, S. (2011) The European nitrogen problem in a global perspective. In: Sutton, M. A., Britton, C., Erisman, J. W., Billen, G., Bleeker, A., Greenfelt, P., van Grinsven, H. & Grizzetti, B. (eds.) *The European Nitrogen Assessment.* Cambridge University Press, Cambridge, 9-31.

Evans, R. D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science,* **6,** 121-126.

Francey, R. J., Allison, C. E., Etheridge, D. M., Trudinger, C. M., Enting, I. G., Leuenberger, M., Langenfelds, R. L., Michel, E. & Steele, L. P. (1999) A 1000-year high precision record of $\delta^{13}$C in atmospheric $CO_2$. *Tellus B,* **51,** 170-193.

Fritts, H. C. (1976) *Tree rings and climate,* London, UK, Academic Press, pp 567.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S. P. & Sutton, M. A. (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science,* **320,** 889-892.

Garten, C. T. (1993) Variation in foliar $^{15}$N abundance and the availability of soil nitrogen on Walker Branch watershed. *Ecology,* **74,** 2098-2113.

Gebauer, G. & Schulze, E. D. (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. *Oecologia,* **87,** 198-207.

Gerhart, L. & McLauchlan, K. (2014) Reconstructing terrestrial nutrient cycling using stable nitrogen isotopes in wood. *Biogeochemistry,* **120,** 1-21.

Gessler, A., Schneider, S., Von Sengbusch, D., Weber, P., Hanemann, U., Huber, C., Rothe, A., Kreutzer, K. & Rennenberg, H. (1998) Field and laboratory experiments on net uptake of nitrate and ammonium by the roots of spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. *New Phytologist,* **138,** 275-285.

Gilliam, F. S. (2006) Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. *Journal of Ecology,* **94,** 1176-1191.

Graf Pannatier, E., Dobbertin, M., Heim, A., Schmitt, M., Thimonier, A., Waldner, P. & Frey, B. (2012) Response of carbon fluxes to the 2003 heat wave and drought in three mature forests in Switzerland. *Biogeochemistry,* **107,** 295-317.

Guerrieri, M. R., Siegwolf, R. T. W., Saurer, M., Jaggi, M., Cherubini, P., Ripullone, F. & Borghetti, M. (2009) Impact of different nitrogen emission sources on tree physiology as assessed by a triple stable isotope approach. *Atmospheric Environment,* **43,** 410-418.

Guerrieri, R., Mencuccini, M., Sheppard, L. J., Saurer, M., Perks, M. P., Levy, P., Sutton, M. A., Borghetti, M. & Grace, J. (2011) The legacy of enhanced N and S deposition as revealed by the combined analysis of $\delta^{13}$C, $\delta^{18}$O and $\delta^{15}$N in tree rings. *Global Change Biology,* **17,** 1946-1962.

Gustafsson, D., Waldner, P. A. & Stähli, M. (2004) Factors governing the formation and persistence of layers in a subalpine snowpack. *Hydrological Processes,* **18,** 1165-1183.

Högberg, P. (1997) Tansley Review No. 95 $^{15}$N natural abundance in soil-plant systems. *New Phytologist,* **137,** 179-203.

Högberg, P., Tamm, C. O. & Högberg, M. (1992) Variations in $^{15}$N abundance in a forest fertilization trial: Critical loads of N, N saturation, contamination and effects of revitalization fertilization. *Plant and Soil,* **142,** 211-219.

Holmes, R. L. (1983) Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin,* **43,** 69-78.

ICP-Forests. 2010. Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests, UNECE, ICP Forests Programme Co-ordinating Centre, Hamburg. ISBN: 978-3-926301-03-1. Available: http://www.icp-forests.org/Manual.htm

Jäggi, M., Saurer, M., Fuhrer, J. & Siegwolf, R. (2003) Seasonality of $\delta^{18}$O in needles and wood of *Picea abies*. *New Phytologist,* **158,** 51-59.

Jansson, P. E. & Karlberg, L. (2004) COUP manual: Coupled heat and mass transfer model for soil-plant-atmosphere systems. *Technical manual for the CoupModel***,** 1-453.

Kloeti, P., Keller, H. & Guecheva, M. (1989) Effects of forest canopy on throughfall precipitation chemistry. Proceedings of a conference held in Baltimore, Baltimore. IAHS Publication, International Association of Hydrological Sciences, 203-209.

Kolb, K. J. & Evans, R. D. (2002) Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. *New Phytologist,* **156,** 57-64.

Kress, A., Saurer, M., Siegwolf, R. T. W., Frank, D. C., Esper, J. & Bugmann, H. (2010) A 350 year drought reconstruction from Alpine tree ring stable isotopes. *Global Biogeochemical Cycles,* **24**.

Leuenberger, M. (2007) To what extent can ice core data contribute to the understanding of plant ecological developments of the past? In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 211-233.

Marshall, J. D., Brooks, J. R. & Lajtha, K. (2008) Sources of variation in the stable isotopic composition of plants. In: Michener, R. & Lajtha, K. (eds.) *Stable Isotopes in Ecology and Environmental Science*. Blackwell Publishing Ltd, 22-60.

McCarroll, D., Gagen, M. H., Loader, N. J., Robertson, I., Anchukaitis, K. J., Los, S., Young, G. H. F., Jalkanen, R., Kirchhefer, A. & Waterhouse, J. S. (2009) Correction of tree ring stable carbon isotope chronologies for changes in the carbon dioxide content of the atmosphere. *Geochimica et Cosmochimica Acta,* **73,** 1539-1547.

McLauchlan, K. K. & Craine, J. M. (2012) Species-specific trajectories of nitrogen isotopes in Indiana hardwood forests, USA. *Biogeosciences,* **9,** 867-874.

Monteith, J. (1965) Evaporation and environment. 19th Symp. Soc. Exp. Biol. 205-234.

Pardo, L., Templer, P., Goodale, C., Duke, S., Groffman, P., Adams, M., Boeckx, P., Boggs, J., Campbell, J., Colman, B., Compton, J., Emmett, B., Gundersen, P., Kjønaas, J., Lovett, G., Mack, M., Magill, A., Mbila, M., Mitchell, M., McGee, G., McNulty, S., Nadelhoffer, K., Ollinger, S., Ross, D., Rueth, H., Rustad, L., Schaberg, P., Schiff, S., Schleppi, P., Spoelstra, J. & Wessel, W. (2006) Regional assessment of N saturation using foliar and root $\delta\ ^{15}$N. *Biogeochemistry,* **80,** 143-171.

Poulson, S. R., Chamberlain, C. P. & Friedland, A. J. (1995) Nitrogen isotope variation of tree-rings as a potential indicator of environmental change. *Chemical Geology,* **125,** 307-315.

Saka, S. (2001) Chemical composition and distribution. In: Hon, D. N.-S. & Shiraishi, N. (eds.) *Wood and cellulosic chemistry - second edition, revised and expanded*. Marcel Dekker, New York, 51-81.

Saurer, M., Cherubini, P., Ammann, M., De Cinti, B. & Siegwolf, R. (2004) First detection of nitrogen from $NO_X$ in tree rings: a $^{15}$N/$^{14}$N study near a motorway. *Atmospheric Environment,* **38,** 2779-2787.

Saurer, M. & Siegwolf, R. T. W. (2007) Human impacts on tree-ring growth reconstructed from stable isotopes. In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 49-62.

Savard, M. M. (2010) Tree-ring stable isotopes and historical perspectives on pollution - An overview. *Environmental Pollution,* **158,** 2007-2013.

Savard, M. M., Begin, C., Smirnoff, A., Marion, J. & Rioux-Paquette, E. (2009) Tree-ring nitrogen isotopes reflect anthropogenic $NO_X$ emissions and climatic effects. *Environmental Science & Technology,* **43,** 604-609.

Schaub, M., Dobbertin, M., Kräuchi, N. & Dobbertin, M. (2011) Preface—long-term ecosystem research: understanding the present to shape the future. *Environmental Monitoring and Assessment,* **174,** 1-2.

Scheidegger, Y., Saurer, M., Bahn, M. & Siegwolf, R. (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia,* **125,** 350-357.

Settele, J., Scholes, R., Betts, R., Bunn, S., Leadley, P., Nepstad, D., Overpeck, J. T. & Taboada, M. A. (2014) Terrestrial and inland water systems. In: Field, C. B., Barros, V. R., Dokken, D. J., Mach, K. J., Mastrandrea, M. D., Bilir, T. E., Chatterjee, M., Ebi, K. L., Estrada, Y. O., Genova, R. C., Girma, B., Kissel, E. S., Levy, A. N., MacCracken, S., Mastrandrea, P. R. & White, L. L. (eds.) *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 271-359.

Siegwolf, R. T. W., Matyssek, R., Saurer, M., Maurer, S., Günthardt-Goerg, M. S., Schmutz, P. & Bucher, J. B. (2001) Stable isotope analysis reveals differential effects of soil nitrogen and nitrogen dioxide on the water use efficiency in hybrid poplar leaves. *New Phytologist,* **149,** 233-246.

Solberg, S., Dobbertin, M., Reinds, G. J., Lange, H., Andreassen, K., Fernandez, P. G., Hildingsson, A. & de Vries, W. (2009) Analyses of the impact of changes in atmospheric deposition and climate on forest growth in European monitoring plots: A stand growth approach. *Forest Ecology and Management,* **258,** 1735-1750.

Sun, F., Kuang, Y., Wen, D., Xu, Z., Li, J., Zuo, W. & Hou, E. (2010) Long-term tree growth rate, water use efficiency, and tree ring nitrogen isotope composition of *Pinus massoniana* L. in response to global climate change and local nitrogen deposition in Southern China. *Journal of Soils and Sediments,* **10,** 1453-1465.

Takashima, T., Hikosaka, K. & Hirose, T. (2004) Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. *Plant, Cell & Environment,* **27,** 1047-1054.

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

Thimonier, A., Schmitt, M., Waldner, P. & Rihm, B. (2005) Atmospheric deposition on Swiss long-term forest ecosystem research (LWF) plots. *Environmental Monitoring and Assessment,* **104,** 81-118.

Tomlinson, G., Siegwolf, R. T. W., Buchmann, N., Schleppi, P., Waldner, P. & Weber, P. (2014) The mobility of nitrogen across tree-rings of Norway spruce (*Picea abies* L.) and the effect of extraction method on tree-ring $\delta^{15}$N and $\delta^{13}$C values. *Rapid Communications in Mass Spectrometry,* **28,** 1258-1264.

Vallano, D. M. & Sparks, J. P. (2007) Foliar $\delta^{15}$N values as indicators of foliar uptake of atmospheric nitrogen pollution. In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 93-109.

Wallace, Z. P., Lovett, G. M., Hart, J. E. & Machona, B. (2007) Effects of nitrogen saturation on tree growth and death in a mixed-oak forest. *Forest Ecology and Management,* **243,** 210-218.

Walthert, L., Blaser, P., Lüscher, P., Luster, J. & Zimmermann, S. 2003. Langfristige Waldökosystem-Forschung LWF in der Schweiz, Kernprojekt

Bodenmatrix: Ergebnisse der ersten Erhebung 1994-1999. Available: http://dx.doi.org/10.3929/ethz-a-004500705

# Tables

*Table 1* Main site characteristics, climate, deposition and soil (Walthert *et al*. 2003) data from the six long-term forest monitoring sites of the LWF.

| | Sites | | | | | |
|---|---|---|---|---|---|---|
| Site | Alptal | Beatenberg | Bettlachstock | Lausanne | Othmarsingen | Vordemwald |
| Abbreviation | ALP | BEA | BET | LAU | OTH | VOR |
| Main tree species | *Picea abies* | *Picea abies* | *Fagus sylvatica* | *Fagus sylvatica* | *Fagus sylvatica* | *Abies alba* |
| Forest type | Coniferous | Coniferous | Mixed broad-leaved | Broad-leaved | Broad-leaved | Mixed coniferous |
| Region | Prealps | Prealps | Jura | Central Plateau | Central Plateau | Central plateau |
| Altitude a.s.l. (m) | 1160 | 1511 | 1149 | 807 | 484 | 480 |
| Orientation | NW | SW | S | NE | S | NW |
| Mean slope (%) | 23 | 33 | 66 | 7 | 27 | 14 |
| Mean annual temperature [°C] | 5.3 | 4.7 | 6.0 | 8.6 | 8.6 | 8.4 |
| Mean annual precipitation [mm] | 2129 | 1725 | 1454 | 1062 | 1045 | 1106 |
| Mean annual bulk N deposition [kg/ha/yr] | 12.0 | 7.4 | 7.0 | 9.7 | 8.0 | 9.7 |
| Mean growing season (Apr-Oct) drought response index [mm] | -1044.8 | -366.1 | -179.0 | 81.5 | -25.2 | 81.5 |
| Soil type | Mollic Gleysol | Podzol | Rendzic Leptosol | Dystric Cambisol | Haplic Acrisol | Dystric Planosol |
| Soil C/N ratio | 17 | 28 | 18 | 15 | 17 | 21 |
| Rooting depth [cm] | 40 | 55 | > 120 | 240 | > 190 | 60 |

*Table 2* Average tree-ring and foliar $\delta^{15}N$ and $\delta^{13}C$ values (‰; ± standard deviation (n)) of *F. sylvatica* and *P. abies* as well as the intra-tree isotopic differences and differences between species (‰; ± standard deviation) (*, **, *** for $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively).

| | | *F. sylvatica* | *P. abies* | Species difference |
|---|---|---|---|---|
| $\delta^{15}N$ | Tree-ring | -5.98 ± 1.44 (24) | -7.56 ± 1.36 (18) | 1.58 ± 1.98*** |
| | Foliage | -6.85 ± 0.34 (24) | -8.70 ± 0.47 (17) | 1.85 ± 0.58*** |
| | Intra-tree difference | 0.87 ± 1.28*** | 1.14 ± 1.43*** | |
| $\delta^{13}C$ | Tree-ring | -25.69 ± 0.70 (60) | -23.71 ± 0.37 (45) | -1.98 ± 0.79*** |
| | Foliage | -26.38 ± 0.50 (24) | -24.84 ± 0.88 (17) | -1.54 ± 1.01*** |
| | Intra-tree difference | 0.69 ± 0.58*** | 1.13 ± 0.86*** | |

*Table 3* Summary of the significance of explanatory variables in explaining variation in tree-ring width (TRW), foliage and tree-ring $\delta^{15}N$ chronologies, and foliage and tree-ring $\delta^{13}C$ chronologies from the linear mixed-effect models (*, ** or *** for $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively). Explanatory variables considered were year, species, soil C/N, bulk deposition mineral N (N deposition) and growing-season drought response index ($DRI_{Apr\text{-}Oct}$). T-, p-, and $R^2$ values given with significant variables marked in bold.

| Variable | Tree-ring width (TRW) | | Foliar $\delta^{15}N$ | | Tree-ring $\delta^{15}N$ | | Foliar $\delta^{13}C$ | | Tree-ring $\delta^{13}C$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | t-value | p-value | t-value | p-value | t-value | p-value | t-value | p-value | t-value | p-value |
| Year | **-2.301** | **0.016*** | **-2.538** | **0.008**** | 0.331 | 0.878 | **-3.114** | **0.002**** | 0.295 | 0.787 |
| Species | -0.589 | 0.464 | **-9.074** | **< 0.001***** | -1.685 | 0.053 | **5.005** | **< 0.001***** | **16.858** | **< 0.001***** |
| Soil C/N | **0.173** | 0.818 | -0.442 | 0.628 | -0.874 | 0.203 | **-1.81** | **0.026*** | -1.075 | 0.196 |
| N deposition | **1.321** | 0.084 | 0.214 | 0.818 | -0.429 | 0.08 | **-1.374** | **0.021*** | 0.381 | 0.251 |
| $DRI_{Apr\text{-}Oct}$ | **-1.372** | 0.091 | -1.451 | 0.117 | -0.232 | 0.432 | -0.125 | 0.932 | 1.407 | 0.135 |
| $R^2$ | 0.14 | | 0.91 | | 0.21 | | 0.74 | | 0.75 | |

# Figures

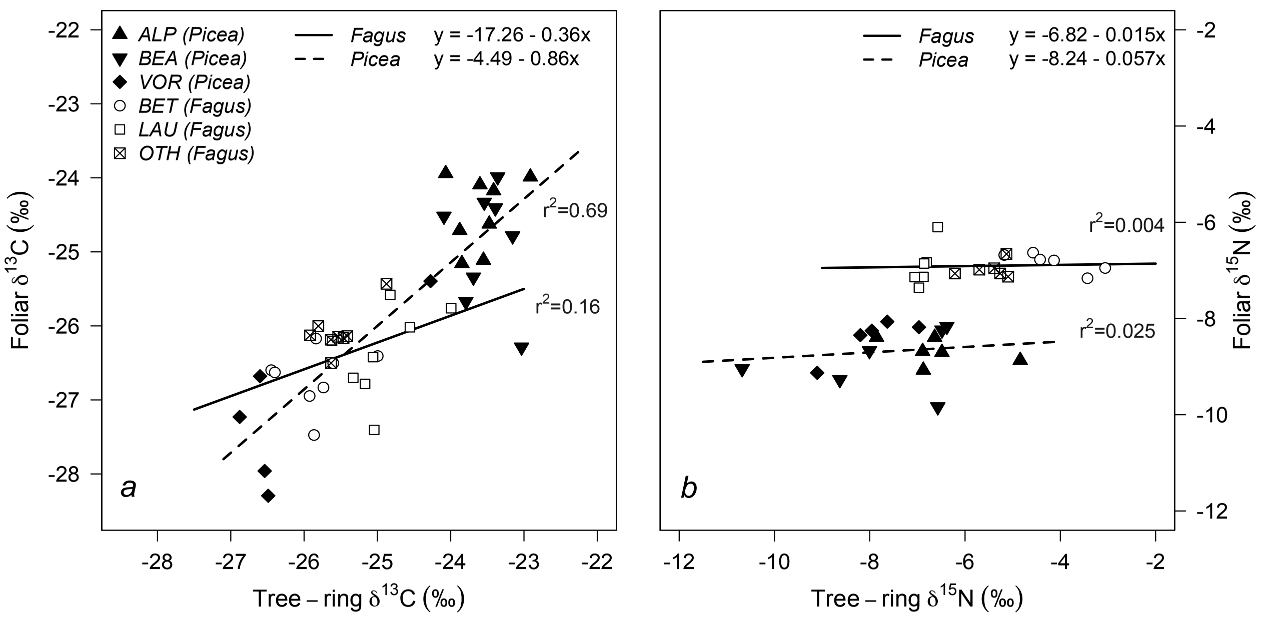

*Fig. 1* Relationships between (a) tree-ring and foliar $\delta^{13}C$, and (b) tree-ring and foliar $\delta^{15}N$ for *P. abies* (dashed line) and *F. sylvatica* (solid line). *F. sylvatica* of Vordemwald is excluded due to lack of foliage data.

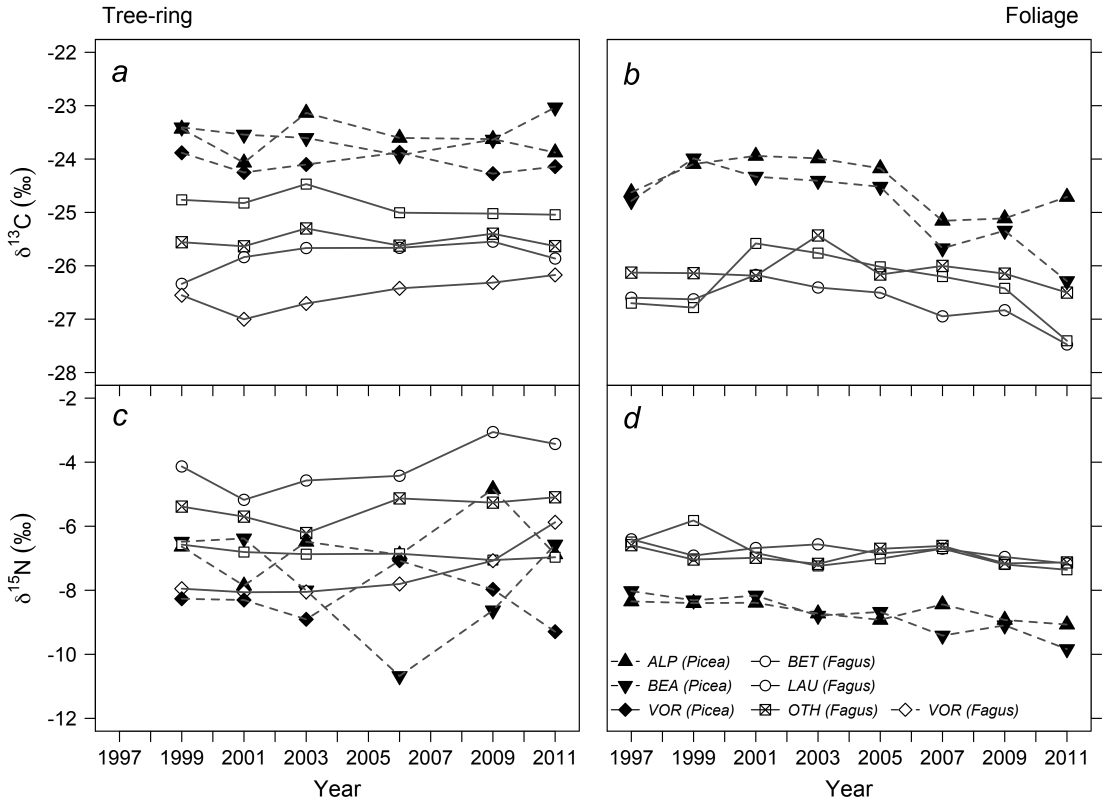

*Fig. 2* Chronologies of (a) tree-ring $\delta^{13}$C, (b) foliar $\delta^{13}$C, (c) tree-ring $\delta^{15}$N, and (d) foliar $\delta^{15}$N for both *P. abies* (dashed lines) and *F. sylvatica* (solid lines) between 1997 and 2011 for six sites across Switzerland. Annual tree-ring $\delta^{13}$C data have been averaged to match the tree-ring $\delta^{15}$N data (i.e. the 10-year split pool approach; 1998-2000, 2001, 2002-2004, 2005-2007, 2008-2010, 2011).

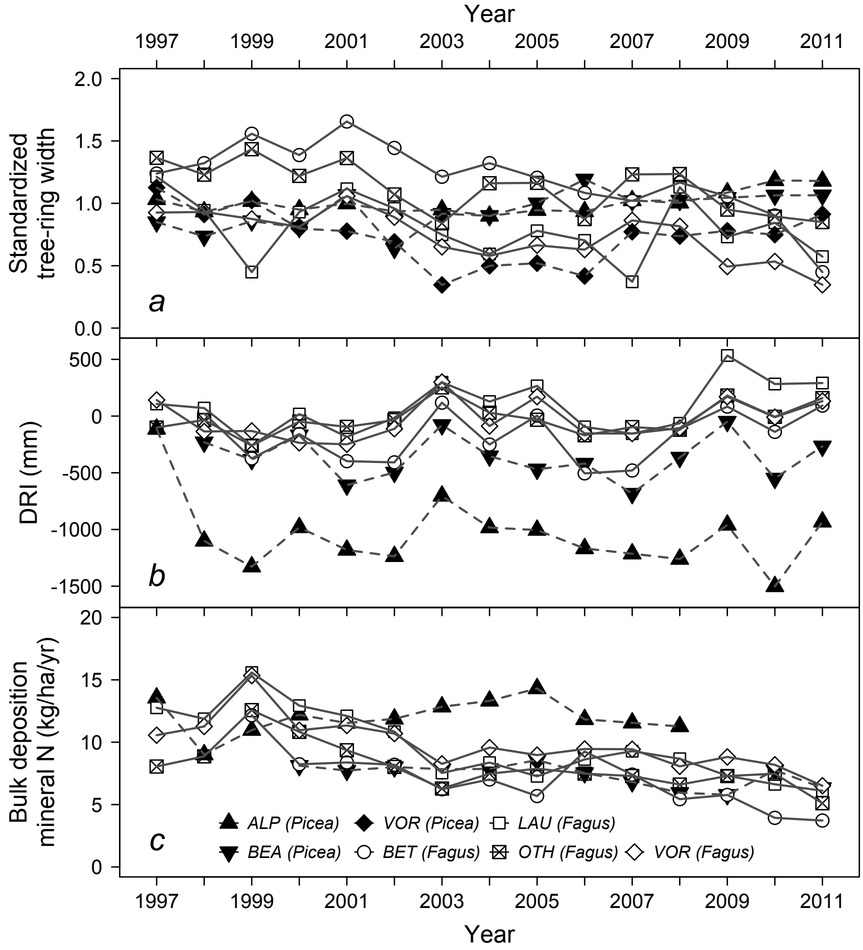

*Fig. 3* Chronologies of (a) standardised tree-ring widths, (b) growing-season (April-October) drought response index (DRI), and (c) bulk deposition mineral N for six *P. abies* (dashed lines) and *F. sylvatica* sites (solid lines) sites between 1997 and 2011.

# Chapter 3

## Environmental drivers of tree growth and water-use efficiency of *Fagus sylvatica* (L.) and *Picea abies* (L.) Karst across Switzerland in the 20th century: Climate vs N deposition

Tomlinson, G.[1], Waldner, P.[1], Siegwolf, R.[2], Rihm, B.[3], Klesse, S.[1], Weigt, R.[2] & Buchmann, N.[4]

[1]WSL, Swiss Federal Institute for Forest, Snow and Landscape Research, CH-8903 Birmensdorf, Switzerland

[2]Ecosystem Fluxes Group, Paul Scherrer Institute (PSI), CH-5232 Villigen, Switzerland

[3]Meteotest, Fabrikstrasse 14, CH-3012 Bern, Switzerland

[4]Department of Environmental Systems Science, ETH Zurich, CH-8092 Zurich, Switzerland

## Abstract

How trees have responded to changing climate vs. nitrogen (N) deposition is difficult to assess, since long-term N deposition data are scarce. Therefore, we first tested the accuracy of modelled N deposition and then assessed the response of tree-ring width (TRW) and basal area increment (BAI) of European beech and Norway spruce to environmental change at eight sites across Switzerland and of intrinsic water-use efficiency ($WUE_i$; based on stable isotopes) for a mixed forest (Lägeren) during 1901-2010. TRW generally increased with precipitation but decreased with temperature, highlighting the importance of water availability for growth, while BAI increased with N deposition at all eight sites. At Lägeren, BAI was not affected by climate, but increased significantly with N deposition, thereby increasing tree water demand. $WUE_i$ increased significantly with N deposition (during 1960-1990) and summer temperatures (particularly after 2000). The combination of high BAI and $WUE_i$ during decades with high N deposition strongly suggests greater susceptibility of tree growth to water stress, particularly with predicted increases in temperature.

## Introduction

The continued emission of large amounts of greenhouse gases, primarily carbon dioxide ($CO_2$) from fossil fuel combustion, is causing fundamental changes in temperature on both global and regional scales (Hartmann *et al.* 2013). Concurrently, increased fertilizer use and fossil fuel combustion throughout the 20th century have caused a large increase in the global availability of reactive nitrogen (N) compounds (Galloway *et al.* 2008). Atmospheric transport of nitrous oxides ($NO_x$) and reduced nitrogen ($NH_y$) has profound effects on local and global N cycles, particularly for N-limited ecosystems (Erisman *et al.* 2011). In Switzerland, temperatures have increased at more than twice the global average during the 20th century, with a particularly sharp increase between 1980 and 2000 (Rebetez 1999), while atmospheric concentrations of $NO_x$ and ammonia ($NH_3$) increased significantly between 1950 and 1990 (EKL 2005). Technological advancements, however, have led to a recent decrease in $NO_x$ emissions since its peak in the 1980s (Winiwarter *et al.* 2011).

Forests play an important role in global C sequestration (Pan *et al.* 2011), particularly since increased atmospheric $CO_2$ concentrations are thought to have a species-specific fertilizing effect on tree growth, although the occurrence and size of this effect is still highly debated (Lindner *et al.* 2010, Bader *et al.* 2013). The effects of different temperature and precipitation regimes on tree growth in Europe are also species-specific and can be highly site-specific (Babst *et al.* 2013). Initially, the increased availability of N also had a fertilizing effect on N-limited tree growth in many temperate and boreal forest ecosystems, increasing forest C sequestration (Magnani *et al.* 2007, Pretzsch *et al.* 2014). However, once N inputs exceed N demands and the ecosystem is no longer able to retain the excess N, nutrient imbalances in the forest soil can lead to soil acidification and increased N leaching rates, reducing tree growth and, in some cases, resulting in tree mortality and forest decline (Erisman *et al.* 2011, Wallace *et al.* 2007). The response of tree growth to increased N availability is a subject of on-going research due to the complex interactions between atmospheric N deposition, soil N pools, and root and canopy N uptake (De Schrijver *et al.* 2008, de Vries *et al.* 2008, Magnani *et al.* 2007). The influence of these different interacting environmental factors on tree growth is difficult to disentangle due to counter-acting effects of a wide variety of site-specific conditions (Solberg *et al.* 2009). For example, while temperate continental forest ecosystems experienced increased growth due to increased N availability in the past (de Vries *et al.* 2014), they are predicted to suffer from decreased water availability and increased drought stress throughout the 21st century, maybe even becoming a C source by the end of the century (Lindner *et al.* 2010). Thus, it is essential to further develop our understanding of the influence of past environmental changes on tree growth in order to predict how forest ecosystems will be affected by future changes in the environment.

Annual tree-ring width chronologies, together with records of site-specific environmental conditions, are particularly useful in observing the effects of changing environmental conditions on both an individual tree and a forest stand level. Tree-rings respond to and preserve climate information with such accuracy that they can be used to reliably reconstruct long-term temperature records dating back centuries (Fritts 1976). Together with tree-ring width chronologies, records of tree-ring stable isotopic composition can be used to better understand the tree physiological responses to changing climatic conditions (Cernusak & English 2015). The carbon isotopic composition ($\delta^{13}C$) of tree-rings is a sensitive proxy for water availability and can provide historical records of intrinsic water-use efficiency ($WUE_i$) (Farquhar *et al.* 1982), defined as the ratio of net photosynthesis to transpiration, and is affected by changes in climate, atmospheric $CO_2$ concentrations, air pollution and nutrient availability (Saurer & Siegwolf 2007, Keenan *et al.* 2013). Thus, it can be used to investigate the effect of water availability and N deposition levels on tree physiology (Savard 2010). However, a majority of the investigations on the potential fertilizing effect of N on tree growth either (a) span a short period since long time series of measured N deposition are not available (Solberg *et al.* 2009), or (b) are manipulation experiments that involve the experimental addition of high doses of N. Thus, the assessment of a potential long-term anthropogenic-driven fertilization effect on tree growth throughout the 20th century is still difficult.

Based on a combination of tree-ring width measurements, environmental data and modelled N deposition for eight sites within Switzerland, as well as tree-ring $\delta^{13}C$ values from one mixed forest site, the aims of this study were to: (1) assess the accuracy of modelled decadal N deposition spanning the 20th century, (2) identify environmental drivers of tree growth in Swiss forests between 1901 and 2011, and (3) use $WUE_i$ estimates to investigate changes in tree physiology due to changes in N deposition, temperature and precipitation between 1900 and 2010.

## Methods

### *Site description*

This investigation used data from six Swiss Long-term Forest Ecosystem Research (LWF) sites, run by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), and two Swiss sites from the National Air Pollution Monitoring Network (NABEL), run by the Federal Office for the Environment (FOEN) and the Swiss Federal Laboratories for Materials Science and Technology (Empa). These eight sites span a wide elevation range (from 432 to 1511 m) and incorporate two main tree species, European beech (*Fagus sylvatica* (L.)) and Norway spruce (*Picea abies* (L.) Karst). The main site characteristics and abbreviations of the eight sites used in this study are shown in *Table 1*.

Switzerland is one of approximately thirty European countries currently participating in the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effect on Forests (ICP Forests), a programme that includes over 500 intensive monitoring plots across Europe (Fischer *et al*. 2012). Six LWF sites were selected for this study based on availability of tree-ring chronologies for the two tree species *Fagus sylvatica* and *Picea abies* as well as environmental data.

The National Air Pollution Monitoring Network was initiated in 1978, and further extended in 1990/91 to include a total of 16 monitoring stations within Switzerland across a gradient of varying air pollution levels. These sites record levels of selected gaseous pollutants, suspended particulates, volatile organic compounds, and open field wet deposition at various time intervals, ranging from every half an hour to up to once a year. Two NABEL sites (Dübendorf and Payerne) were included in this investigation.

### *Meteorological data*

Monthly temperature and precipitation records were obtained for each of the eight sites between 1901 and 2012 from the publicly available observational Climate Research Unit (CRU) TS 3.2 worldwide dataset, in which climate observations were constructed from meteorological stations on a 0.5° x 0.5° grid covering the global land surface (Mitchell & Jones 2005). From this dataset, we produced seasonal and annual averages and sums for temperature and precipitation, respectively.

### *Nitrogen deposition*

#### *Estimated N deposition (based on the canopy budget model)*

Total deposition was estimated from bulk and throughfall deposition measurements on an annual basis using the canopy budget model developed by Ulrich (1983) and extended by Draaijers & Erisman (1995), whereby throughfall fluxes represent total deposition plus or minus canopy exchanges, i.e. leaching or uptake, according to Thimonier *et al.* (2010). Deposition data are available for LAU, OTH and VOR from 1997 to 2013 and for BEA from 1999 to 2013.

*Modelled N deposition*

Atmospheric N deposition was estimated using a pragmatic modelling approach that combines monitoring data, spatial interpolation methods, emission inventories, statistical dispersion models and inferential deposition models (updated from Thimonier *et al.* 2005). The following variables were included in this combined model approach: wet deposition of nitrate ($NO_3^-$) and ammonium ($NH_4^+$), gaseous ammonia ($NH_3$), nitrogen dioxide ($NO_2$) and nitric acid ($HNO_3$) as well as dry deposition of particulate $NH_4^+$ and $NO_3^-$ (secondary aerosols). Wet deposition was obtained by multiplying mean annual concentrations of soluble N compounds in precipitation with precipitation rate. Dry deposition of gases and aerosols was calculated on the basis of monitored and modelled air concentrations. For $NH_3$ and $NO_2$ , the air concentrations were calculated applying an emission-dispersion approach. Emissions and concentrations of $NH_3$ were mapped on a grid of 100 m by 100 m (Thöni *et al.* 2004). The velocity of deposition depends on meteorological conditions, the reactivity of the pollutant and the surface roughness. For calculating the $NH_3$ deposition of beech stands, a typical average deposition velocity of 22 mm $s^{-1}$ was used. Emissions and concentrations of $NO_2$ were mapped on a grid of 200 m by 200 m, based on emission maps and statistical dispersion models (FOEN 2011). For the deposition velocity of $NO_2$ , a value of 3.0 mm $s^{-1}$ was used. The concentration field for $HNO_3$ was derived from maps of air humidity, temperature, ozone concentrations and $NO_2$ concentrations, applying an empirical relationship (UNECE 2005). For $HNO_3$ a deposition velocity of 15 mm $s^{-1}$ was used. The concentration of particulate $NO_3^-$ and $NH_4^+$ was derived from modelled PM10 maps (SAEFL 2003), and deposition velocities of aerosols were in a range of 1.5–3.0 mm

$s^{-1}$ depending on altitude. Deposition was calculated with this combined approach for the years 1990, 2000, 2005 and 2010 using existing input maps.

Deposition in the period 1880-1980 was calculated for every ten years using a gridded dataset of historic deposition rates (CCE (2012); on the basis of Schöpp *et al.* (2003)). To estimate historic N deposition, separate depositions for $NH_y$ and $NO_x$ of the year 2000 were used; a reduction factor derived from the gridded dataset was subsequently applied (e.g. $NH_y$ deposition in 1880/$NH_y$ deposition in 2000).

### *Tree sampling*

A total of ten dominant trees were selected from each site from as close to the deposition measurement stations as possible. Two stem cores were extracted at breast height (approximately 1.2 m) from each tree in either 2012 or 2013 using a 0.5 cm diameter increment borer. Cores were air-dried and planed using a custom-made core microtome (WSL, Switzerland) to improve the visibility of the individual tree-rings without contamination of wood material between tree-ring years.

### *Dendrochronological analysis*

Annual tree-rings were identified and tree-ring width (TRW) measured from bark to pith to a resolution of 0.01mm using the TSAPWIN tree-ring software (Version 3.5) connected to a LINTAB3 measuring system (F. Rinn S.A., Heidelberg, Germany). For each site, samples were visually and statistically crossdated with COFECHA (Holmes 1983) and quality checked following standard dendrochronological procedures (Fritts 1976). The TRW series were subsequently detrended with a spline with a 50% frequency-response threshold of 30 years (Cook & Peters 1981) to remove biased trends caused by tree age, and averaged to a site chronology using Tukey's biweight robust mean. Although chronologies were found to date back to 1725, the time series included in this study are either from 1901-2011 (for climate-growth correlations) or 1900-2010 (for deposition-growth correlations). TRW measurements were additionally used to calculate the tree basal area increment

(BAI) at each of the eight sites between 1900 and 2010 to account for long-term productivity changes of tree growth:

$$BAI = \pi({R_t}^2 - {R_{t-1}}^2) \tag{1}$$

where $R$ is the tree radius and $t$ the year the ring was formed.

*Sample preparation and isotope analysis*

Five trees (ten cores) from the mixed forest site Lägeren (LAE) were selected for $\delta^{13}$C tree-ring analysis. Tree-rings were separated with a razor blade under a microscope, transferred into Teflon filter bags (Ankom Technology, Macedon, NY, USA) and underwent a cellulose extraction that consisted of three steps: (1) the removal of lignin by $NaClO_2$ in an ultrasonic bath for 24 hours (24 g sodium chlorite and 8 ml in acetic acid in 2 L water, solution renewed every three hours followed by an overnight solution with 72 g $NaClO_2$ at 70°C), (2) the removal of fats, resins, oils, tannins and hemicellulose by washing in distilled water and soaking in a sodium hydroxide solution (150g NaOH in 1L water) for 45 minutes, and (3) the neutralization with HCl solution (3%) for 45 minutes in an ultrasonic bath and washing in distilled water until neutral pH was achieved. The cellulose material was dried at 50°C in a cabinet drier for 1 day before being transferred to Eppendorf caps, soaked in distilled water for 2 days for subsequent homogenization by ultrasound (Hielscher Ultrasonics GmbH, Teltow, Germany), and dried by lyophilisation. The cellulose samples were weighed (0.5-1.0 mg) into silver capsules for further simultaneous measurement of $\delta^{13}C/^{12}C$ and $\delta^{18}O/^{16}O$ by pyrolysis at 1450°C (PYRO-cube, Elementar, Hanau, Germany) and subsequent IRMS (Delta Plus XP; Thermo Finnigan Mat, Bremen, Germany) with a precision of ±0.15‰ for $\delta^{18}$O and ±0.12‰ for $\delta^{13}$C. The $\delta^{13}$C signal was corrected for a dampening of the signal due to memory effects from pyrolysis by applying a linear correction as determined from double measurements with pyrolysis and combustion of a subset according to Woodley *et al*. (2012) with the following equation:

$$\delta^{13}\mathrm{C}_{\mathrm{corr}} = 1.219\ \delta^{13}\mathrm{C}_{\mathrm{pyro}} + 5.3545 \tag{2}$$

where $\delta^{13}C_{corr}$ represents the corrected values, and $\delta^{13}C_{pyro}$ refer to pyrolysis measurements (after correction with internal standards).. Isotope values are expressed in the $\delta$-notation as relative deviations from the international standard, V-PDB ($\delta^{13}C = ((^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{standard}) - 1)$; [‰]). The tree-ring $\delta^{13}C$ values were corrected for the Suess effect resulting from the combustion of fossil fuels (Keeling 1979). For this correction, records of past atmospheric $\delta^{13}C$ obtained from ice cores and from historical records from Mauna Loa (Leuenberger 2007, White & Vaughn 2015) were applied.

### *Intrinsic water-use efficiency ($WUE_i$)*

The intrinsic water-use efficiency ($WUE_i$) values were calculated from tree-ring $\delta^{13}C$ chronologies between 1850 and 2012 (*Appendix, Fig. 1*). The isotope discrimination of $\delta^{13}C$ ($\Delta$) during photosynthesis can be defined as:

$$\Delta = a\,\frac{c_a - c_i}{c_a} + b\,\frac{c_i}{c_a} = a + (b - a)\frac{c_i}{c_a} \tag{3}$$

where $a$ is the fractionation occurring due to diffusion in air (~4.4‰), $b$ is the net fractionation during carboxylation (27‰), and $c_i$ and $c_a$ are the $CO_2$ concentrations of the intercellular spaces and atmospheric air, respectively (Farquhar *et al.* 1982). The discrimination can also be defined as:

$$\Delta = \frac{\delta^{13}C_a - \delta^{13}C_{sample}}{1 + \delta^{13}C_{sample}} \approx \delta^{13}C_a - \delta^{13}C_{sample} \tag{4}$$

where $\delta^{13}C_a$ is the isotope value of atmospheric $CO_2$ and $\delta^{13}C_{sample}$ that of the sample, in our case tree-rings. Using equations (3) and (4) in combination, $c_i$ can be calculated as:

$$c_i = c_a\frac{\delta^{13}C_a - \delta^{13}C_{sample} - a}{b - a} \tag{5}$$

Finally, intrinsic water-use efficiency ($\mathrm{WUE_i}$) can be defined as the ratio of photosynthesis (*A*) to the conductance for water vapour ($g_{H2O}$):

$$\mathrm{WUE_i} = \frac{\mathrm{A}}{\mathrm{g_{H2O}}} = \frac{(\mathrm{c_a} - \mathrm{c_i})}{1.6} \qquad (6)$$

Combining equations (5) and (6) allows us to determine $\mathrm{WUE_i}$ as:

$$\mathrm{WUE_i} = \frac{\mathrm{c_a}}{1.6}\,\frac{\mathrm{b} - \delta^{13}\mathrm{C_a} - \delta^{13}\mathrm{C_{sample}}}{\mathrm{b} - \mathrm{a}} \qquad (7)$$

*Data analysis*

All statistics and figures based on the results of this investigation were performed using the statistical software program R (version 3.1.3). A paired t-test, together with a linear regression model, was used to investigate the relationship between inorganic modelled N deposition and estimated N deposition (based on the canopy budget model of Ulrich 1983). Both estimated N deposition and modelled N deposition values were 5-year average values from 1998-2002, 2003-2007 and 2008-2012.

Climate-growth correlations were assessed based on Pearson correlation coefficients between the TRW chronologies and monthly CRU meteorological data for each site between 1901 and 2011 (*Appendix, Fig. 2*), as some sites lacked tree-ring width data for 2012. Based on these results, it was decided to use the following environmental variables for further analyses: mean annual temperature (MAT), summer average temperature (June, July and August ($\mathrm{T_{JJA}}$)), previous summer average temperature ($\mathrm{T_{pJJA}}$), sum of annual precipitation ($\mathrm{P_{sum}}$), sum of summer precipitation ($\mathrm{P_{JJA}}$) and sum of previous summer precipitation ($\mathrm{P_{pJJA}}$). The degrees of freedom for each correlation were 108, as each correlation covered a total of 110 years (df = n–2), and subsequently the critical value for the significance of each correlation coefficient was approximately 0.195. Correlation analyses between BAI (5-year moving average) and modelled N deposition (linearly interpolated decadal values) were also performed between 1900 and 2010. BAI was used for this part of the analyses, as the standardized TRW does not give information of the long-term environmental effects on tree growth.

The variability of environmental and tree physiological variables at LAE was assessed using a principal component analysis (PCA) on annual values between 1901 and 2010, as modelled N deposition values were available to 2010 only, while climate data extended as far back as 1901. Decadal values of modelled N deposition were linearly interpolated to obtain annual values. Variables considered in this analysis were: basal area increment (BAI), intrinsic water-use efficiency ($WUE_i$), year, modelled N deposition, $P_{sum}$, $P_{JJA}$, $P_{pJJA}$, MAT, $T_{JJA}$ and $T_{pJJA}$. This analysis was supported by both multiple regression analyses and a number of linear regression models performed between decadal (5-year average) values of BAI or $WUE_i$ with decadal modelled N deposition and decadal 5-year average values of $P_{JJA}$ and $T_{JJA}$ for the period 1900-2010 (as climate-growth correlations demonstrated tree growth at LAE to be more strongly influenced by $P_{pJJA}$ and $T_{pJJA}$ than by $P_{sum}$ or MAT). For the year 1900, average $P_{JJA}$ and $T_{JJA}$ values were calculated using values from 1901 and 1902 only, as climate data extended as far back as 1901.

## Results

### *Modelled nitrogen deposition*

There was a significant positive relationship between the estimated N deposition (based on Ulrich 1983) and modelled N deposition values ($p < 0.001$; *Fig. 1*), with an offset of 4.31 kg N ha $yr^{-1}$, while the paired t-test showed that modelled N deposition was consistently significantly higher than the estimated N deposition ($t = 13.84$, $p < 0.001$) at each of the four sites included in the analysis.

### *Environmental drivers of tree growth in the 20th century*

At all eight sites, deposition had the largest positive effect on tree growth (using BAI measurements), while response of standardized TRW to changes in precipitation and temperature regimes varied among each of the eight sites (*Fig. 2*).

The common trends in TRW among sites, however, include positive correlations with precipitation and negative correlations with temperature. Five of the eight sites significantly increased in TRW with increases in one or more of the precipitation variables, with the strongest correlation found at the lowest beech site DUB with $P_{JJA}$ ($r = 0.42$). None of the sites included in this investigation showed a strong negative relationship with increasing precipitation. Five sites showed a negative response with increases in one of the temperature variables, the strongest negative response being at the mixed forest site LAE with $T_{pJJA}$ ($r = -0.34$), while temperature had a significantly positive effect on TRW at just one site (the spruce site BEA, situated above 1500m). TRW both increased significantly with precipitation and decreased significantly with temperature at four of the sites (the beech sites DUB, LAE and LAU, and the spruce site ALP), demonstrating the strong influence of water demand and availability on TRW at these sites. There were no consistent species-specific differences in TRW in response to changing climatic conditions, with sites of both species showing different growth patterns in response to changes in precipitation and temperature.

*Physiological response to changing environmental conditions*

At the mixed forest site LAE, $T_{JJA}$ increased significantly between 1901 and 2011 ($p < 0.001$), with peak values occurring at the start of the 21st century (*Fig. 3a*), while no long-term changes in $P_{JJA}$ values were observed ($p = 0.70$). Modelled N deposition increased significantly between 1901 and 1980 ($p<0.001$), particularly between 1950 and 1980, before decreasing again (*Fig. 3b*). Both BAI ($p<0.001$) and $WUE_i$ ($p<0.001$) significantly increased at LAE throughout the 20th century, with peak values of BAI occurring between 1950 and 1990 (*Fig. 3c*, *3d*).

The first principal component (PC) of the PCA was mainly represented by year, modelled N deposition and temperature (PC1), while the secondary axis (PC2) was primarily determined by precipitation (*Fig. 4*). Based on temporal changes in long-term environmental conditions throughout the 20th century, three groups could be separated: 1901-1950, 1951-1990 and 1991-2010, i.e. before, during and after the rise in N deposition during the second half of the 20th century (*Fig. 3b*). Those environmental variables most strongly representing PC1 and PC2 for BAI and $WUE_i$, i.e. modelled N deposition, $T_{JJA}$, and $P_{JJA}$, explained 70% and 79% of the variation in BAI and $WUE_i$, respectively (*Table 2*), with modelled N deposition clearly dominating the observed variation at this site. Additionally, the increase and subsequent peak in modelled N deposition between 1950 and 1990 corresponded to some of the highest values of BAI and $WUE_i$ within the measured time period (1950-1990; *Fig. 5a*). While there was no significant relationship between $P_{JJA}$ and either BAI or $WUE_i$ (*Fig. 5b*), $WUE_i$ increased significantly with $T_{JJA}$ ($r^2 = 0.37$; $p = 0.035$), but there was no significant relationship between BAI and $T_{JJA}$ (*Fig. 5c*).

## Discussion

### *Modelled nitrogen deposition*

Modelled N deposition was positively related to, but consistently higher than, estimated N deposition (based on Ulrich 1983) at the four sites used for this analysis. Modelled N deposition values were calculated using a combination of measured concentration values (notably wet deposition values of nitrate ($NO_3^-$) and ammonium ( $NH_4^+$ )) together with various interpolation methods, emission inventories, dispersion models and deposition models for dry deposition. Wet deposition values are directly dependent on precipitation values, while the dry deposition values are dependent on reactive N concentrations in the atmosphere and deposition velocities. The model has no method of incorporating forest edge effects into these modelled values, which may overestimate dry N deposition at forest borders and thus contribute to the offset. Conversely, while the model may have overestimated N deposition, it is equally probable that N deposition calculated using the canopy budget model (Ulrich 1983) has been underestimated. In particular, direct canopy uptake of N is difficult to calculate reliably and thus difficult to incorporate within the canopy budget model. In addition, stemflow was neglected and snowfall can contribute to the underestimation of precipitation, while microbial activity within the sample collectors can reduce N concentrations within the sample, thereby reducing N deposition calculated using the canopy budget model (Ulrich 1983).

Importantly, however, there was a significant positive relationship between modelled and estimated N deposition, meaning that while modelled N deposition values may not absolutely reflect the amount of N entering the forest ecosystem, the model does capture changes and trends in N deposition through time. In addition, the total amount of N deposited in Switzerland calculated by the model used in this study is similar to total N emissions estimated by atmospheric transport models for the long-range transport of N (EKL 2005). Consequently, despite potential methodological biases in both approaches, modelled N deposition was used in analysing how trees have responded to environmental changes throughout the 20th century.

### *Environmental drivers of tree growth*

Climate drivers of tree growth varied among the eight sites included in this investigation, but TRW generally responded positively to increases in precipitation and negatively to increases in temperature, particularly for *F. sylvatica* sites. This is in agreement with a number of previous investigations, studying the effect of climate on the growth on a variety of tree species across Europe (Lebourgeois *et al.* 2005, Scharnweber *et al.* 2011). Temperature and precipitation are closely related in both being strong drivers of water availability, and thus, it is not surprising that some sites showed both positive and negative correlations of TRW with precipitation and temperature, respectively. The main mechanism through which low water availability (caused by high temperatures and/or low precipitation) reduces tree growth is through decreases in stomatal conductance to reduce foliar water loss through transpiration, thus also indirectly reducing tree $CO_2$ uptake. Certainly, our results suggest that climate drivers of tree growth in Switzerland are more site-specific than species-specific, with no clear trends emerging within each species. At the four sites that precipitation and temperature both significantly affected tree growth (ALP, DUB, LAE and LAU), the climate-growth effects were temporally consistent within each site (i.e. summer temperature and summer precipitation, or previous summer temperature and previous summer precipitation).

The one site that showed an increase in TRW with temperature was also the highest site in this study, situated at 1511m. This result is similar to those from a European-wide investigation on climate drivers of tree growth that found an increase in TRW with temperature at high altitude sites, such as at the central Alpine tree-line (Babst *et al.* 2013). Moreover, an investigation by Jolly *et al.* (2005) found the increase in temperature during the European heat wave of 2003 resulted in increased growth at high altitude sites, attributed to an increase in the snow-free growing season length. Thus, our results suggest that increased temperatures during the transition times (i.e. from winter to spring and autumn to winter) for tree growth at this site are beneficial, and that tree productivity, and to a large extent also C sequestration in this forest, may benefit from temperature increases associated with climate change at such high elevation sites.

BAI increased significantly with increasing N deposition at all sites (*Fig. 2, Appendix, Fig. 3*). This is in agreement with a number of investigations that found N deposition to be a significant driver of past forest growth in Central Europe (Pretzsch *et al.* 2014, Solberg *et al.* 2009, de Vries & Posch 2011). While BAI curves tend to level off with time due to age effects, our BAI chronologies (with the exception of ALP and DUB) showed marked decreases within the same 20 year period in the most recent decades, even though stands differed in age by as much as 160 years. Some sites also appear to have a lagged response to changing N deposition, which could be the result of site-specific slow soil organic N turnover and subsequent tree assimilation (Krause *et al.* 2012b). The decrease in BAI in recent decades may have resulted from direct or indirect effects of changing N availability, such as decreased photosynthetic rates with decreasing N, or nutrient imbalances leading to e.g. phosphorus limitations (Braun *et al.* 2010).

*Tree physiology under changing environmental conditions*

TRW at the mixed forest site LAE was significantly affected by $P_{pJJA}$ (positively) and $T_{pJJA}$ (negatively) during the 20th century, evidence that water availability and vapour pressure deficit (VPD) during dry spells are important drivers of tree growth for the dominant *F. sylvatica* at this site. This is even more relevant given that $T_{JJA}$ at

LAE increased significantly during the 20th century, while there was no significant change in the amount of $P_{JJA}$ at this site, and both temperature and the frequency of drought events are predicted to further increase in Central Europe in the 21st century (Kovats *et al.* 2014). Thus, tree growth of *F. sylvatica* at LAE might be impaired under future climate conditions. Furthermore, results from both the PCA and multiple regression analyses also showed that the increase in N deposition during the 20th century (but also the subsequent decrease after 1980) influenced both tree BAI and $WUE_i$ at LAE. High values of BAI and $WUE_i$ occurred between 1960 and 1990 during the rapid increase in N deposition at this site (*Fig. 5a*). While $T_{JJA}$ and $P_{JJA}$ had no significant effect on BAI at LAE, $WUE_i$ also increased significantly with $T_{JJA}$ during the 20th century, which is ultimately the result of a concomitant increase in VPD (Day 2000). Interestingly, high $WUE_i$ were observed at high $T_{JJA}$ (1990-2010) but also at lower $T_{JJA}$ (1970-1980; *Fig. 5c*), suggesting two different mechanisms controlling $WUE_i$ over time at LAE, namely N deposition and summer temperature-driven VPD.

The fact that BAI at LAE was highest between 1960 and 1990, and not in the most recent decades, implies that N deposition, rather than rising $CO_2$ concentrations or changes in climate, was the most significant driver of this increase in tree growth (*Fig. 5a*, *Table 2*). Increased tree growth under elevated N deposition is caused by either increased leaf-level photosynthetic capacity or by increased tree leaf area and in turn increased canopy-level photosynthesis rate (Krause *et al.* 2012a). However, this increase in growth of the tree canopy also leads to increases in canopy-level transpiration rates, resulting in increased water loss through the foliage (Brooks & Coulombe 2009, Krause *et al.* 2012a). To counteract this high water loss, stomatal conductance is often reduced, leading to increased $WUE_\mathrm{i}$, particularly if leaf-level photosynthesis is high due to an N fertilization effect. Such high $WUE_\mathrm{i}$ was exactly what we observed in our study during 1960-1990 (*Fig. 5a*). However, studying the effects of $NO_x$ emissions on the $WUE_\mathrm{i}$ of *Quercus cerris*, Guerrieri *et al.* (2010) found that increases in tree $WUE_\mathrm{i}$ with increased N deposition were caused by an increase in photosynthesis, whilst stomatal conductance was similar in trees before and after an exposure to high levels of N. Without a decrease in stomatal conductance, water stress would be greater due to higher rates of water loss through transpiration. Overall, our results agree with previous investigations suggesting that, among changes in atmospheric $CO_2$ concentrations and climate, increasing levels of N deposition have been responsible for increases in tree $WUE_i$ during the 20th century (Duquesnay *et al.* 1998, Leonardi *et al.* 2012). Fertilization experiments by Betson *et al.* (2007) with *Pinus sylvestris* in Sweden, and by Brooks & Coulombe (2009) with *Pseudotsuga menziesii* in North America, support the notion that levels of N availability can increase water stress for trees, represented as high $WUE_i$ values for *Fagus sylvatica* under high N deposition at LAE. However, N deposition levels at LAE started decreasing in the 1980s, thus easing this N-induced water stress in the more recent decades.

Moreover, the increase in 20th century summer temperature ($T_{JJA}$) also caused an increase in tree $WUE_i$ albeit less significant than the effect of N deposition on $WUE_i$. This was most likely through decreased stomatal conductance in response to temperature-induced increases in transpiration rates, driven by VPD, although increasing atmospheric $CO_2$ concentrations have the same effect (Keenan *et al.* 2013). However, due to lower BAI during the recent decades compared to 1960-1990, we conclude that the impact of atmospheric $CO_2$ concentrations on stomatal conductance and thus tree growth was less than that of temperature (and N deposition). Although physiological responses to the environment are complex and include many direct and indirect effects (Leonardi *et al.* 2012), our observations at LAE demonstrate that tree growth *of F. sylvatica* increased under elevated N deposition, resulting in an inevitable increase in tree water demand, and in turn in higher $WUE_i$. Our results also suggest that the primary environmental driver of $WUE_i$ has shifted from N deposition during the period 1960-1990 to temperature (and VPD) in the more recent decades (*Fig. 5a, 5c*). Although N deposition decreased since the 1990s in Switzerland, the underlying mechanisms of changes in BAI and $WUE_i$ suggest that trees at the Lägeren site might become more susceptible to water stress and dry spells in the future, when temperatures are predicted to increase, despite decreasing N deposition.

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## References

Babst, F., Poulter, B., Trouet, V., Tan, K., Neuwirth, B., Wilson, R., Carrer, M., Grabner, M., Tegel, W., Levanic, T., Panayotov, M., Urbinati, C., Bouriaud, O., Ciais, P. & Frank, D. (2013) Site- and species-specific responses of forest growth to climate across the European continent. *Global Ecology and Biogeography,* **22,** 706-717.

Bader, M. K. F., Leuzinger, S., Keel, S. G., Siegwolf, R. T. W., Hagedorn, F., Schleppi, P. & Körner, C. (2013) Central European hardwood trees in a high-$CO_2$ future: synthesis of an 8-year forest canopy $CO_2$ enrichment project. *Journal of Ecology,* **101,** 1509-1519.

Betson, N. R., Johannisson, C., Löfvenius, M. O., Grip, H., Granström, A. & Högberg, P. (2007) Variation in the $\delta^{13}$C of foliage of *Pinus sylvestris* L. in relation to climate and additions of nitrogen: analysis of a 32-year chronology. *Global Change Biology,* **13,** 2317-2328.

Braun, S., Thomas, V. F. D., Quiring, R. & Flückiger, W. (2010) Does nitrogen deposition increase forest production? The role of phosphorus. *Environmental Pollution,* **158,** 2043-2052.

Brooks, J. R. & Coulombe, R. (2009) Physiological responses to fertilization recorded in tree rings: isotopic lessons from a long-term fertilization trial. *Ecological Applications,* **19,** 1044-1060.

CCE (2012) Swiss depositions 1880–2020 of Sulphur and Nitrogen on the EMEP 50km-Grid: historic, natural background and Gothenburg protocol. Data supplied by the UNECE Coordination Center for Effects (CCE) [Online]. Available: http://wge-cce.org [Accessed 9th July 2015].

Cernusak, L. A. & English, N. B. (2015) Beyond tree-ring widths: stable isotopes sharpen the focus on climate responses of temperate forest trees. *Tree Physiology,* **35,** 1-3.

Cook, E. R. & Peters, K. (1981) The smoothing spline: a new approach to standardizing forest interior tree-ring width series for dendroclimatic studies. *Tree-ring bulletin,* **41,** 45-53.

Day, M. E. (2000) Influence of temperature and leaf-to-air vapor pressure deficit on net photosynthesis and stomatal conductance in red spruce (*Picea rubens*). *Tree Physiology,* **20,** 57-63.

De Schrijver, A., Verheyen, K., Mertens, J., Staelens, J., Wuyts, K. & Muys, B. (2008) Nitrogen saturation and net ecosystem production. *Nature,* **451,** E1-E1.

de Vries, W., Du, E. & Butterbach-Bahl, K. (2014) Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. *Current Opinion in Environmental Sustainability,* **9–10,** 90-104.

de Vries, W. & Posch, M. (2011) Modelling the impact of nitrogen deposition, climate change and nutrient limitations on tree carbon sequestration in Europe for the period 1900–2050. *Environmental Pollution,* **159,** 2289-2299.

de Vries, W., Solberg, S., Dobbertin, M., Sterba, H., Laubhahn, D., Reinds, G. J., Nabuurs, G.-J., Gundersen, P. & Sutton, M. A. (2008) Ecologically implausible carbon response? *Nature,* **451,** E1-E3.

Draaijers, G. P. J. & Erisman, J. W. (1995) A canopy budget model to assess atmospheric deposition from throughfall measurements. *Water, Air, & Soil Pollution,* **85,** 2253-2258.

Duquesnay, A., Bréda, N., Stievenard, M. & Dupouey, J. L. (1998) Changes of tree-ring $\delta^{13}C$ and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant, Cell & Environment,* **21,** 565-572.

EKL (2005) Stickstoffhaltige Luftschadstoffe in der Schweiz. Status-Bericht der Eidgenössischen Kommission für Lufthygiene (EKL). Bundesamt für Umwelt, Wald und Landschaft (BUWAL, Ed.), Schriftenreihe Umwelt Nr. 384, Bern. 168 S. [Online]. Available:

http://www.bafu.admin.ch/publikationen/publikation/00557 [Accessed 9th July 2015].

Erisman, J. W., van Grinsven, H., Grizzetti, B., Bouraoui, F., Powlson, D. & Reis, S. (2011) The European nitrogen problem in a global perspective. In: Sutton, M. A., Britton, C., Erisman, J. W., Billen, G., Bleeker, A., Greenfelt, P., van Grinsven, H. & Grizzetti, B. (eds.) *The European Nitrogen Assessment*. Cambridge University Press, Cambridge, 9-31.

Farquhar, G. D., O'Leary, M. H. & Berry, J. A. (1982) On the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves. *Australian Journal of Plant Physiology,* **9,** 121-137.

Fischer, R., Waldner, P., Carnicer, J., Coll, M., Dobbertin, M., Ferretti, M., Hansen, K., Kindermann, G., Lasch-Born, P., Lorenz, M., Marchetto, A., Meining, S., Nieminen, T., Penuelas, J., Rautio, P., Reyer, C., Roskams, P. & Sanchez, G. (2012) *The condition of forests in Europe. 2012 Executive Report,* Hamburg, ICP Forests, pp 24.

FOEN (2011) $NO_2$ ambient concentrations in Switzerland. Modelling results for 2005, 2010, 2015. Federal Office for the Environment, Bern. Environmental studies no. 1123: pp. 68 [Online]. Available: http://www.bafu.admin.ch/publikationen/publikation/01634/index.html?lang=en [Accessed 9th July 2015].

Fritts, H. C. (1976) *Tree rings and climate,* London, UK, Academic Press, pp 567.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S. P. & Sutton, M. A. (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science,* **320,** 889-892.

Guerrieri, R., Siegwolf, R., Saurer, M., Ripullone, F., Mencuccini, M. & Borghetti, M. (2010) Anthropogenic $NO_X$ emissions alter the intrinsic water-use efficiency ($WUE_i$) for *Quercus cerris* stands under Mediterranean climate conditions. *Environmental Pollution,* **158,** 2841-2847.

Hartmann, D. L., Klein Tank, A. M. G., Rusticucci, M., Alexander, L. V., Brönnimann, S., Charabi, Y., Dentener, F. J., Dlugokencky, E. J., Easterling, D. R., Kaplan, A., Soden, B. J., Thorne, P. W., Wild, M. & Zhai, P. M. (2013) Observations: Atmosphere and Surface. In: Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V. & Midgley, P. M. (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA., 159-254.

Holmes, R. L. (1983) Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin,* **43,** 69-78.

Jolly, W. M., Dobbertin, M., Zimmermann, N. E. & Reichstein, M. (2005) Divergent vegetation growth responses to the 2003 heat wave in the Swiss Alps. *Geophysical Research Letters,* **32,** L18409.

Keeling, C. D. (1979) The Suess effect: $^{13}$Carbon-$^{14}$Carbon interrelations. *Environment International,* **2,** 229-300.

Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P. & Richardson, A. D. (2013) Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature,* **499,** 324-327.

Kovats, R. S., Valentini, R., Bouwer, L. M., Georgopoulou, E., Jacob, D., Martin, E., Rounsevell, M. & Soussana, J.-F. (2014) Europe. In: Barros, V. R., Field, C. B., Dokken, D. J., Mastrandrea, M. D., Mach, K. J., Bilir, T. E., Chatterjee, M., Ebi, K. L., Estrada, Y. O., Genova, R. C., Girma, B., Kissel, E. S., Levy, A. N., MacCracken, S., Mastrandrea, P. R. & White, L. L. (eds.) *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1267-1326.

Krause, K., Cherubini, P., Bugmann, H. & Schleppi, P. (2012a) Growth enhancement of *Picea abies* trees under long-term, low-dose N addition is due to morphological more than to physiological changes. *Tree Physiology,* **32,** 1471-1481.

Krause, K., Providoli, I., Currie, W., Bugmann, H. & Schleppi, P. (2012b) Long-term tracing of whole catchment $^{15}$N additions in a mountain spruce forest: measurements and simulations with the TRACE model. *Trees - Structure and Function,* **26,** 1683-1702.

Lebourgeois, F., Bréda, N., Ulrich, E. & Granier, A. (2005) Climate-tree-growth relationships of European beech (*Fagus sylvatica* L.) in the French Permanent Plot Network (RENECOFOR). *Trees,* **19,** 385-401.

Leonardi, S., Gentilesca, T., Guerrieri, R., Ripullone, F., Magnani, F., Mencuccini, M., Noije, T. V. & Borghetti, M. (2012) Assessing the effects of nitrogen deposition and climate on carbon isotope discrimination and intrinsic water-use efficiency of angiosperm and conifer trees under rising $CO_2$ conditions. *Global Change Biology,* **18,** 2925-2944.

Leuenberger, M. (2007) To what extent can ice core data contribute to the understanding of plant ecological developments of the past? In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 211-233.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J., Seidl, R., Delzon, S., Corona, P., Kolström, M., Lexer, M. J. & Marchetti, M. (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management,* **259,** 698-709.

Magnani, F., Mencuccini, M., Borghetti, M., Berbigier, P., Berninger, F., Delzon, S., Grelle, A., Hari, P., Jarvis, P. G., Kolari, P., Kowalski, A. S., Lankreijer, H., Law, B. E., Lindroth, A., Loustau, D., Manca, G., Moncrieff, J. B., Rayment, M., Tedeschi, V., Valentini, R. & Grace, J. (2007) The human footprint in the carbon cycle of temperate and boreal forests. *Nature,* **447,** 849-851.

Mitchell, T. D. & Jones, P. D. (2005) An improved method of constructing a database of monthly climate observations and associated high-resolution grids. *International Journal of Climatology,* **25,** 693-712.

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., Phillips, O. L., Shvidenko, A., Lewis, S. L., Canadell, J. G., Ciais, P., Jackson, R. B., Pacala, S. W., McGuire, A. D., Piao, S., Rautiainen, A., Sitch, S. & Hayes, D. (2011) A large and persistent carbon sink in the world's forests. *Science,* **333,** 988-993.

Pretzsch, H., Biber, P., Schütze, G., Uhl, E. & Rötzer, T. (2014) Forest stand growth dynamics in Central Europe have accelerated since 1870. *Nat Commun,* 5. DOI: 10.1038/ncomms5967.

Rebetez, M. (1999) Twentieth century trends in droughts in southern Switzerland. *Geophysical Research Letters,* **26,** 755-758.

SAEFL (2003) Modelling of PM10 and PM2.5 Ambient Concentrations in Switzerland 2000 and 2010. Environmental Documentation. Swiss Agency for the Environment, Forests and Landscape (SAEFL), Bern. [Online]. Available: http://www.bafu.admin.ch/publikationen/publikation/00258/index.html?lang=en [Accessed 9th July 2015].

Saurer, M. & Siegwolf, R. T. W. (2007) Human impacts on tree-ring growth reconstructed from stable isotopes. In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 49-62.

Savard, M. M. (2010) Tree-ring stable isotopes and historical perspectives on pollution - An overview. *Environmental Pollution,* **158,** 2007-2013.

Scharnweber, T., Manthey, M., Criegee, C., Bauwe, A., Schröder, C. & Wilmking, M. (2011) Drought matters – Declining precipitation influences growth of *Fagus sylvatica* L. and *Quercus robur* L. in north-eastern Germany. *Forest Ecology and Management,* **262,** 947-961.

Schöpp, W., Posch, M., Mylona, S. & Johansson, M. (2003) Long-term development of acid deposition (1880–2030) in sensitive freshwater regions in Europe. *Hydrol. Earth Syst. Sci.,* **7,** 436-446.

Solberg, S., Dobbertin, M., Reinds, G. J., Lange, H., Andreassen, K., Fernandez, P. G., Hildingsson, A. & de Vries, W. (2009) Analyses of the impact of changes in atmospheric deposition and climate on forest growth in European monitoring plots: A stand growth approach. *Forest Ecology and Management,* **258,** 1735-1750.

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

Thimonier, A., Schmitt, M., Waldner, P. & Rihm, B. (2005) Atmospheric deposition on Swiss long-term forest ecosystem research (LWF) plots. *Environmental Monitoring and Assessment,* **104,** 81-118.

Thöni, L., Brang, P., Braun, S., Seitler, E. & Rihm, B. (2004) Ammonia monitoring in Switzerland with passive samplers: Patterns, determinants and comparison with modelled concentrations. *Environmental Monitoring and Assessment,* **98,** 93-107.

Ulrich, B. (1983) Interaction of forest canopies with atmospheric constituents: $SO_2$, alkali and earth alkali cations and chloride. In: Ulrich, B. & Pankrath, J. (eds.) *Effects of accumulation of air pollutatns in forest ecosystems*. Reidel, Dordrecht, 33-45.

UNECE (2005) Technical Report of ICP Materials. UNECE Working Group on Effects, EB.AIR/WG.1/2005/7 [Online]. Available: http://www.unece.org/index.php?id=5189 - / [Accessed 9th July 2015].

Wallace, Z. P., Lovett, G. M., Hart, J. E. & Machona, B. (2007) Effects of nitrogen saturation on tree growth and death in a mixed-oak forest. *Forest Ecology and Management,* **243,** 210-218.

White, J. W. C. & Vaughn, B. H. (2015) University of Colorado, Institute of Arctic and Alpine Research (INSTAAR), Stable isotopic composition of atmospheric carbon dioxide ($^{13}$C and $^{18}$O) from the NOAA ESRL Carbon Cycle Cooperative Global Air Sampling Network, 1990-2013, Version: 2013-04-05 [Online]. Available: ftp://aftp.cmdl.noaa.gov/data/trace_gases/co2c13/flask/surface/ [Accessed 9th July 2015].

Winiwarter, W., Hettelingh, J.-P., Bouwman, A. F., de Vries, W., Erisman, J. W., Galloway, J., Klimont, Z., Leach, A., Leip, A., Pallière, C., Schneider, U. A., Spranger, T., Sutton, M. A., Svirejeva-Hopkins, A., van der Hoek, K. W. & Witzke, P. (2011) Future scenarios of nitrogen in Europe. In: Sutton, M. A., Britton, C., Erisman, J. W., Billen, G., Bleeker, A., Greenfelt, P., van Grinsven, H. & Grizzetti, B. (eds.) *The European Nitrogen Assessment*. Cambridge University Press, Cambridge, 551-569.

Woodley, E. J., Loader, N. J., McCarroll, D., Young, G. H. F., Robertson, I., Heaton, T. H. E., Gagen, M. H. & Warham, J. O. (2012) High-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry: simultaneous measurement of the stable isotopes of oxygen and carbon in cellulose. *Rapid Communications in Mass Spectrometry,* **26,** 109-114.

## Tables

*Table 1* Main site characteristics of the eight sites included in this investigation.

| Site | Abbreviation | Program | Altitude (m) | Mean slope (%) | Mean annual temperature (°C) | Mean annual precipitation (mm) | Soil type | Main tree species |
|---|---|---|---|---|---|---|---|---|
| Alptal | ALP | LWF | 1160 | 23 | 5.3 | 2129 | Mollic Gleysol | *Picea abies* |
| Beatenberg | BEA | LWF | 1511 | 33 | 4.7 | 1725 | Podzol | *Picea abies* |
| Dübendorf | DUB | NABEL | 432 | 30 | 8.5 | 1190 | Luvisol | *Fagus sylvatica* |
| Lägeren | LAE | LWF | 680 | 37 | 8.3 | 1100 | Rendzic Leptosol | *Fagus sylvatica* |
| Lausanne | LAU | LWF | 807 | 7 | 8.6 | 1062 | Dystic Cambisol | *Fagus sylvatica* |
| Payerne | PAY | NABEL | 489 | 0 | 8.5 | 910 | Stagnic Luvisol | *Fagus sylvatica* |
| Othmarsingen | OTH | LWF | 484 | 27 | 8.6 | 1045 | Haplic Acrisol | *Fagus sylvatica* |
| Vordemwald | VOR | LWF | 480 | 14 | 8.4 | 1106 | Dystric Planosol | *Abies alba* |

*Table 2* T-values (and p-values) of multiple regression analyses for both basal area increment (BAI) and water-use efficiency (WUEi) using decadal values (5-year averages) between 1900 and 2010 at the mixed forest site Lägeren (*, ** or *** if $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively). R-squared values are given, while significant relationships are shown in bold.

| Variable | Basal area increment (BAI) | Water-use efficiency ($WUE_i$) |
|---|---|---|
| Modelled N deposition (N) | **4.05 (0.003**)** | **3.96 (0.004**)** |
| Summer temperature ($T_{JJA}$) | 0.37 (0.72) | **2.72 (0.026*)** |
| Summer precipitation ($P_{JJA}$) | 0.14 (0.90) | -0.13 (0.90) |
| $R^2$ | 0.70 | 0.79 |
| Overall | BAI = -697.7 - 74.9 (**N****) – 72.1 ($T_{JJA}$) – 0.60 ($P_{JJA}$) | $WUE_i$ = -15.2 – 0.67 (**N****) – 4.82 (**$T_{JJA}$***) – 0.005 ($P_{JJA}$) |

## Figures

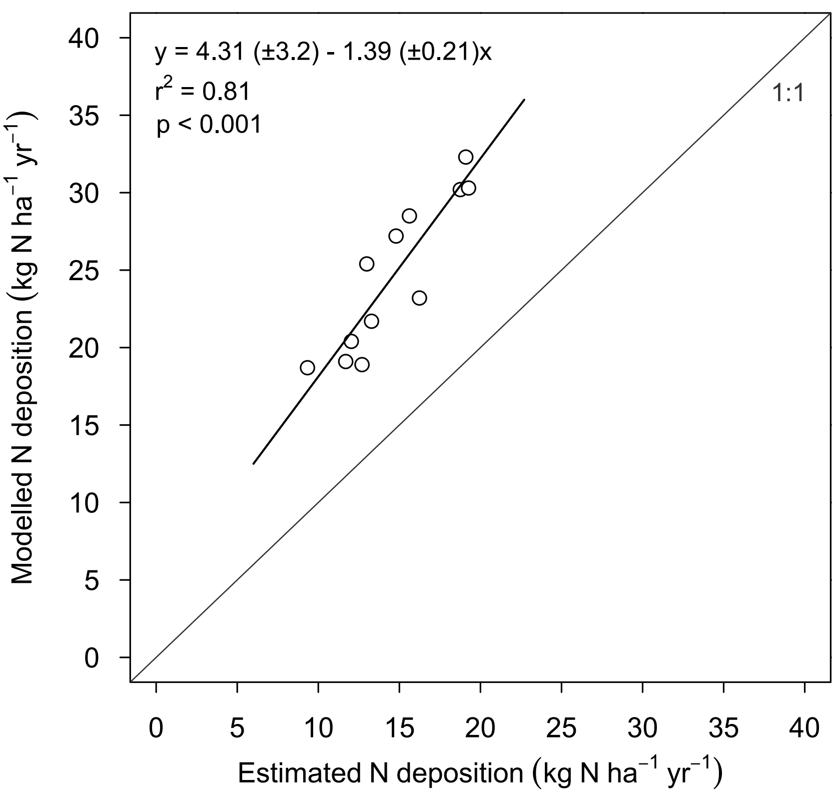


*Fig. 1* The relationship between estimated N deposition (based on the canopy budget model of Ulrich (1983)) and modelled N deposition at four LWF sites (BEA, LAU, OTH and VOR; *Table 1*). Both estimated and modelled N deposition are represented by 5-year averages (e.g. 1998-2002, 2003-2007 and 2008-2012) with standard error also given.

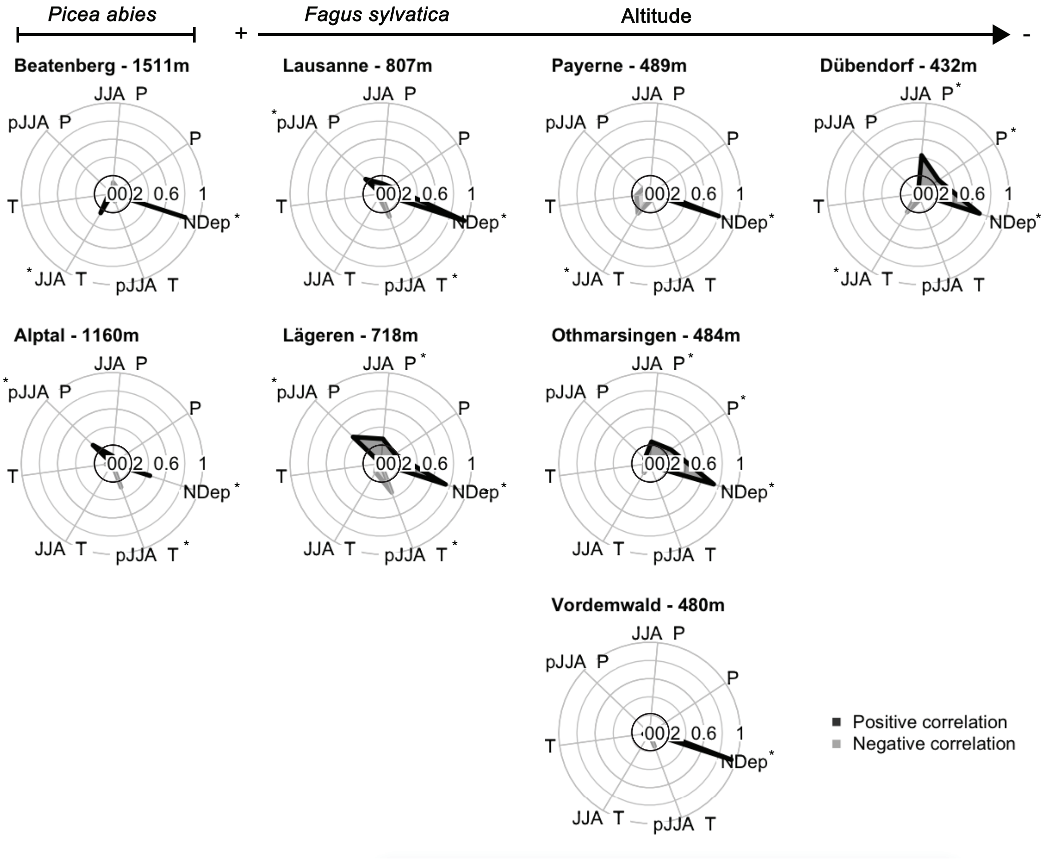


*Fig. 2* Radial plots between selected environmental variables (based on *Appendix, Fig. 2*) and standardised tree-ring width (TRW) at each of the eight sites for the period 1901-2011. For deposition-growth correlations, however, basal area increment was used instead of standardised TRW for the period 1901-2010 to incorporate long-term changes in tree growth. Positive and negative correlations are plotted on the same axis, but with different grey scales. All sites are *F. sylvatica* except for two *P. abies* sites (Beatenberg and Alptal). Climate data were taken from the publicly available observational Climate Research Unit (CRU) TS 3.2 dataset, in which climate observations were constructed from meteorological stations on a 0.5° x 0.5° grid covering the global land surface (Mitchell & Jones 2005). *T* and *P* represent average annual temperature and total annual precipitation, respectively, while *NDep* represents modelled N deposition. *JJA* denotes summer values (June, July and August), while *pJJA* denotes previous year summer values. Significant correlations (< 0.2, $p \leq 0.05$) are indicated with an asterisk (*).

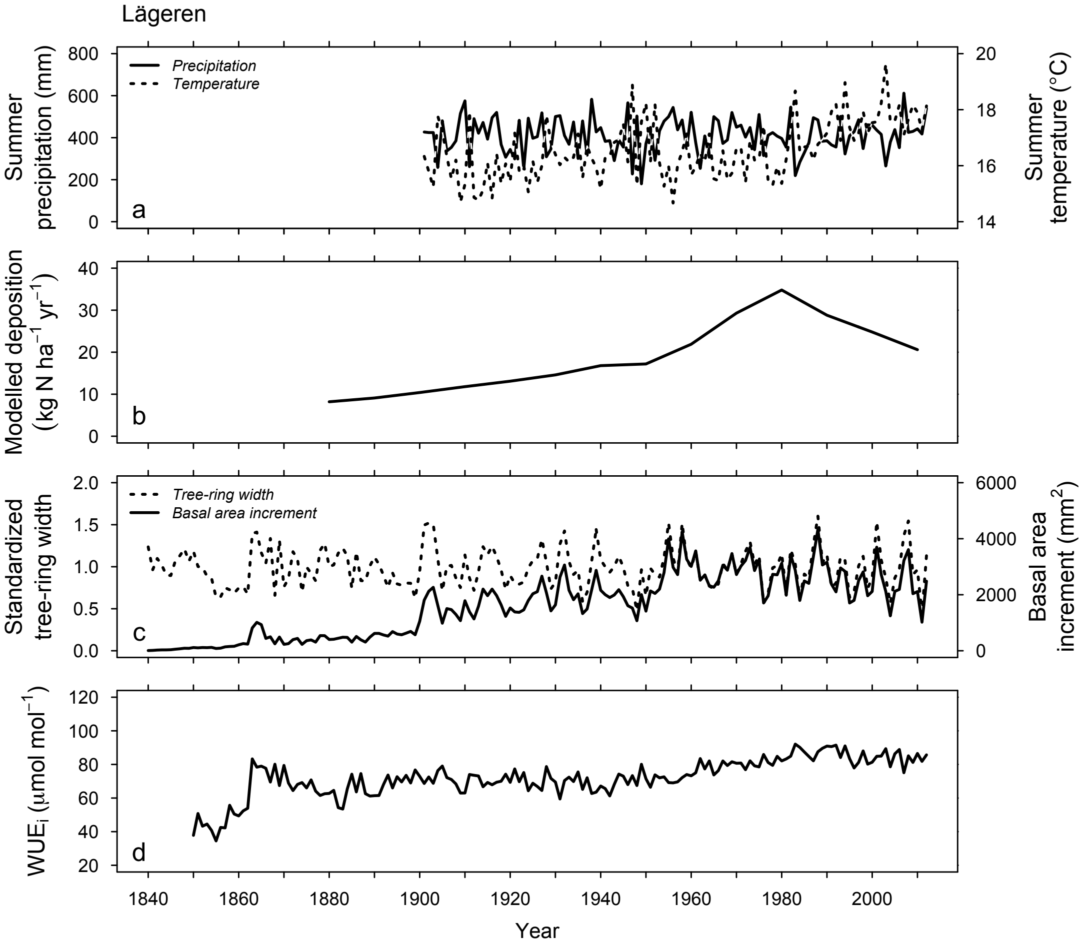


*Fig. 3* Long-term datasets of (*a*) summer (JJA) precipitation and summer temperature (1901-2012), (*b*) modelled N deposition (1880-2010), (c) standardised tree-ring width and basal area increment (1840-2012), as well as (*d*) intrinsic water-use efficiency (WUEi; 1850-2012) at the mixed forest site Lägeren.

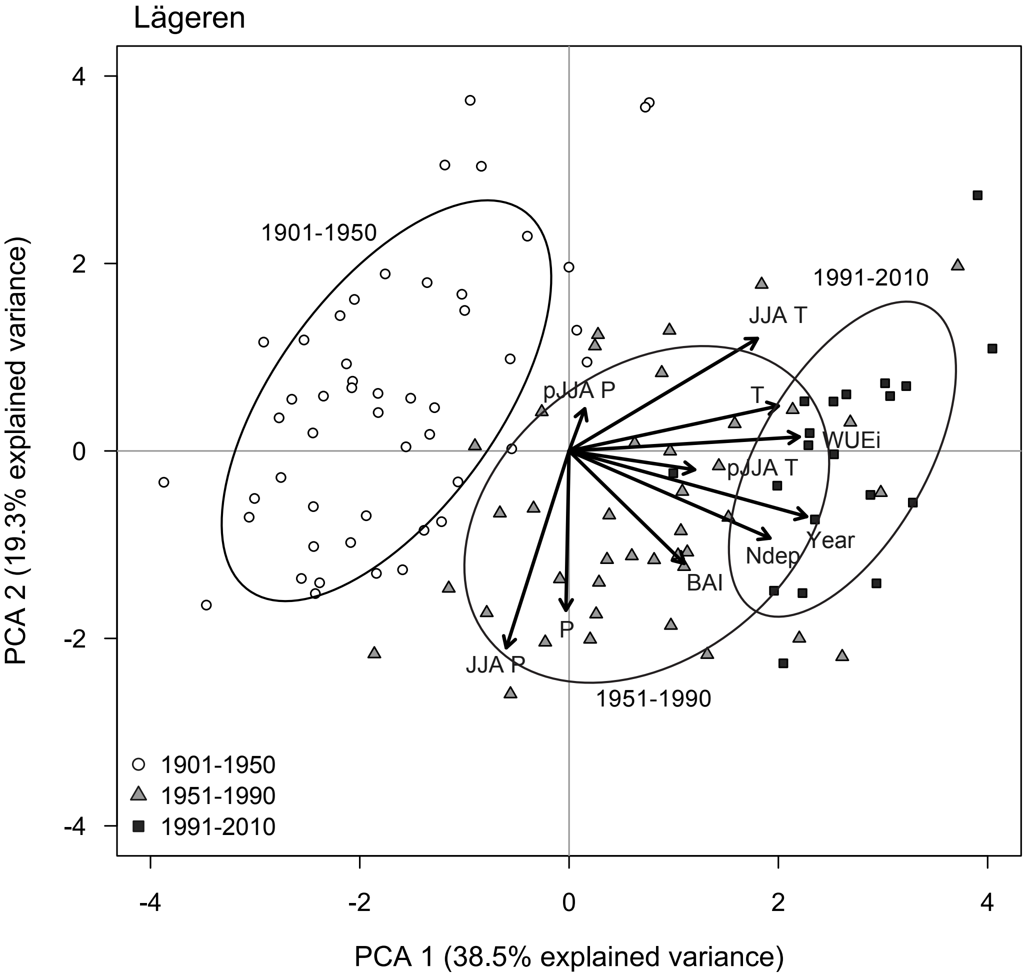


*Fig. 4* Principal component analysis using basal area increment (*BAI*), intrinsic water-use efficiency (*WUEi*), modelled N deposition (*Ndep*), year (*Year*), mean annual temperature (*T*), total annual precipitation (*P*), average summer temperature (*JJA T*) and summer precipitation (*JJA P*), and previous summer average temperature (*pJJA T*) and previous summer precipitation (*pJJA P*), at the mixed-forest site Lägeren. The first principal component (*PCA 1*) has been plotted against the second principal component (*PCA 2*). Three temporal groups were determined, and then subsequently fitted on the plot through the statistical program: 1901-1950, 1951-1990 and 1991-2010 (i.e. before, during and after the 20th century increase in N deposition; *Fig. 3b*)

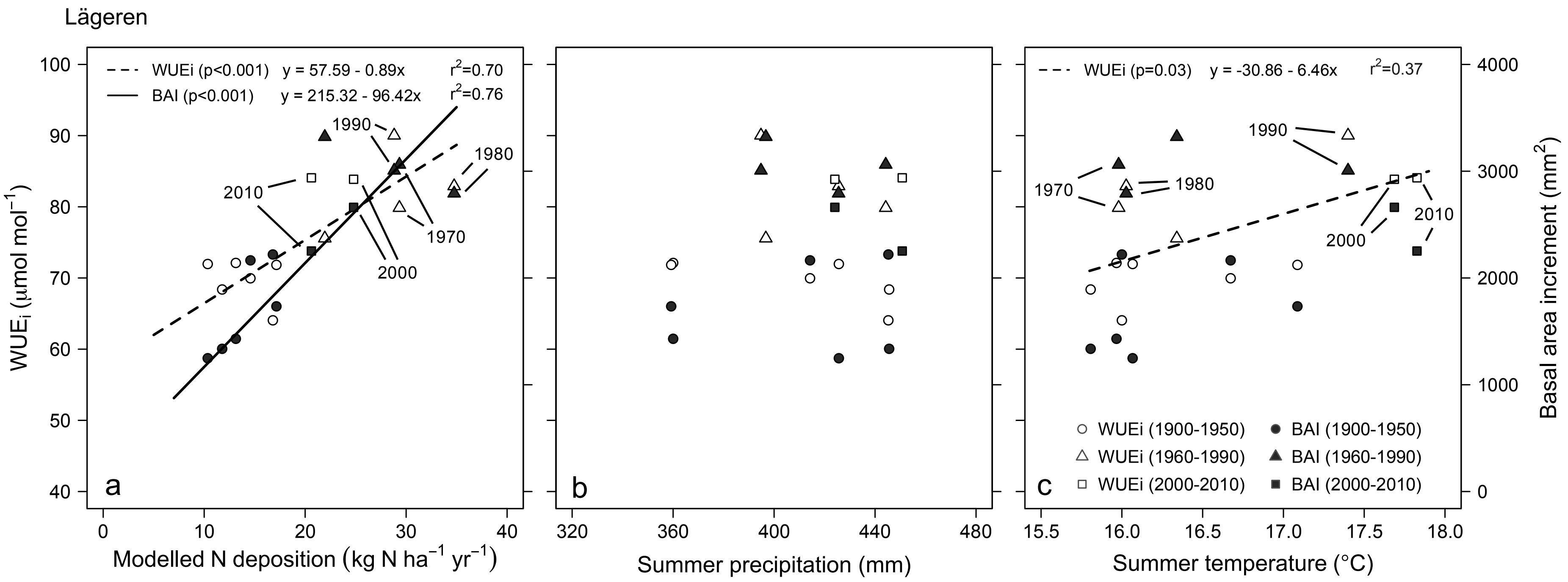
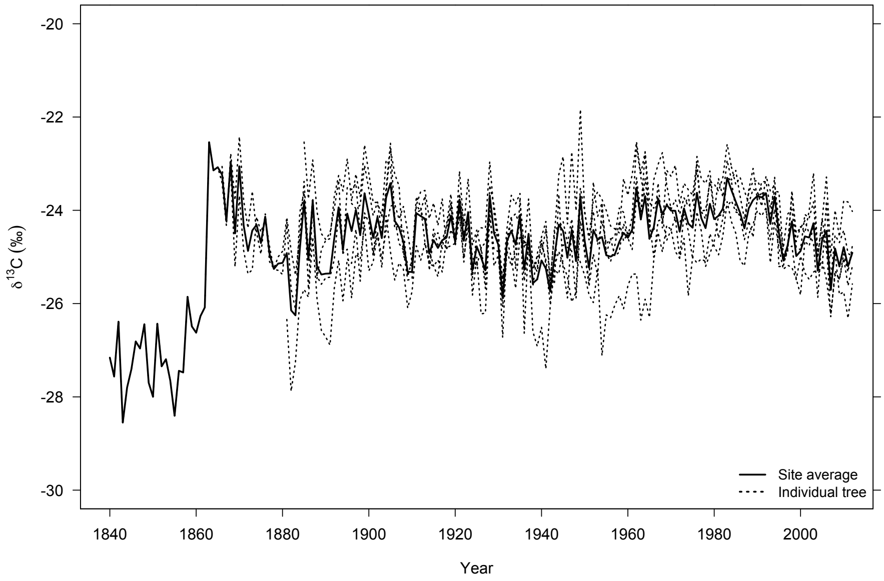
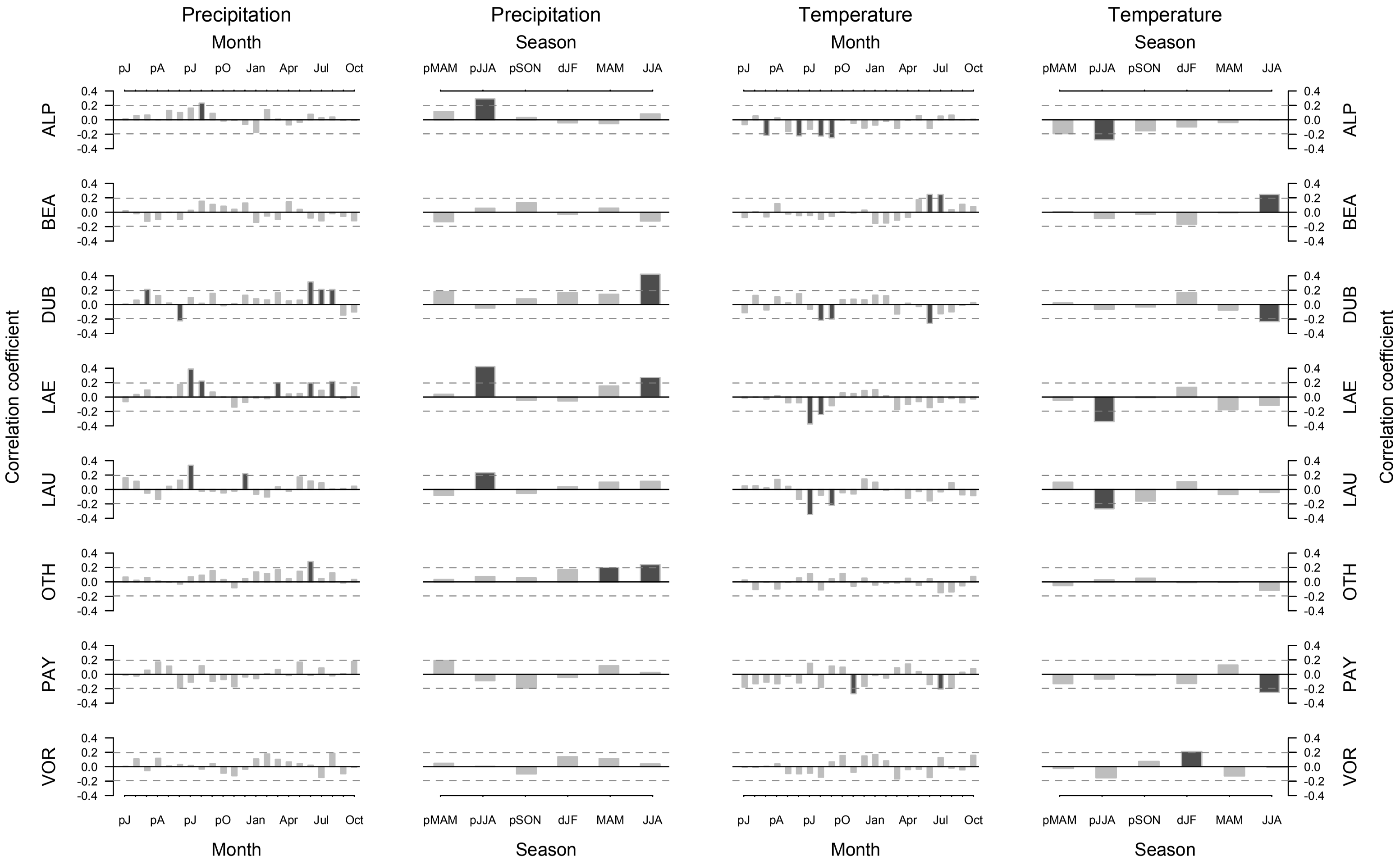


*Fig. 5* The relationships of both decadal WUEi and BAI with (*a*) modelled N deposition, (*b*) summer precipitation (*JJA P*), and (*c*) summer temperature (*JJA T*) at the mixed forest site Lägeren for the years 1900-2010 (*, ** or *** if $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$, respectively). Only significant relationships are shown. All values indicated are 5-year averages (i.e. 1968-1972, 1978-1982, 1988-1992, 1998-2002 and 2008-2012).

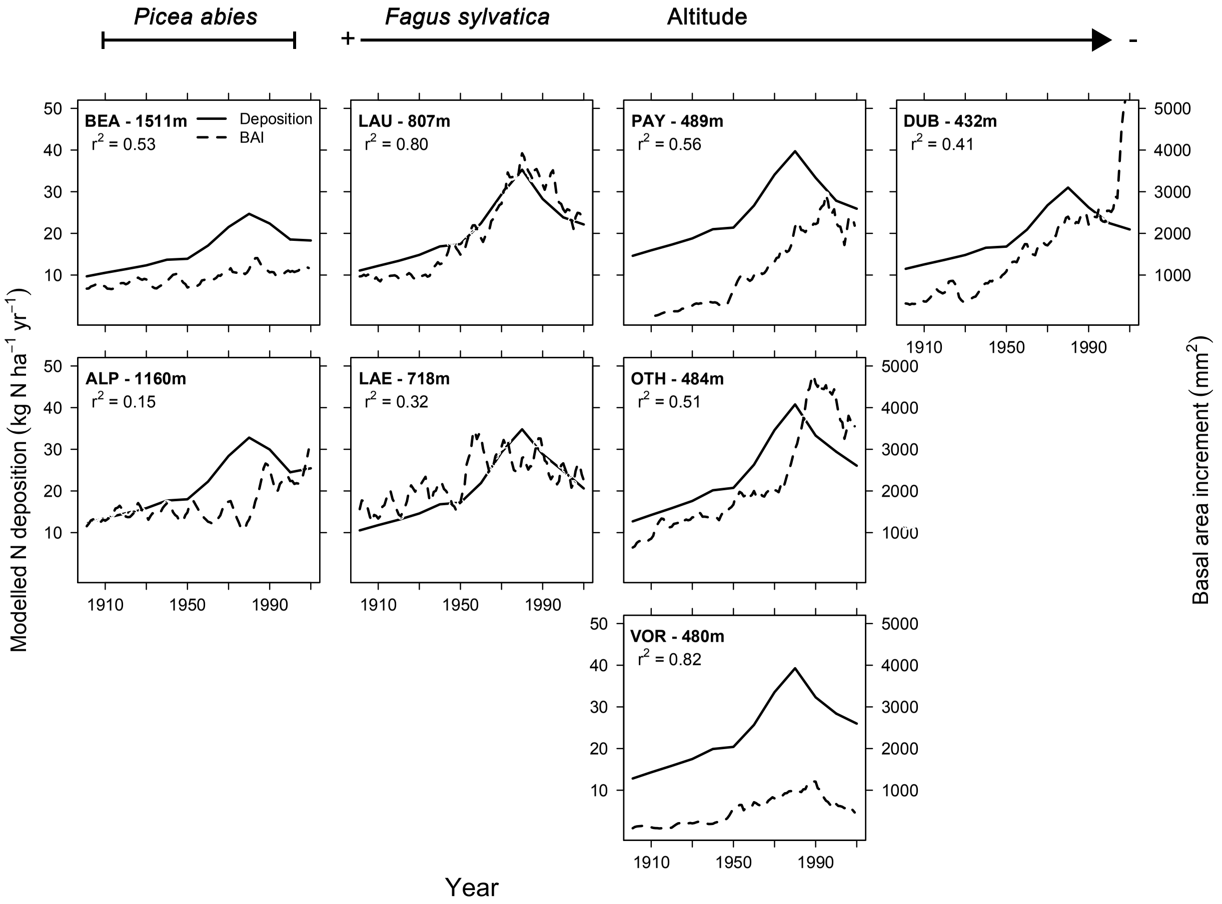
# Appendix



*Appendix, Fig. 1* Individual tree-ring $\delta^{13}$C chronologies for each of the five trees measured at Lägeren with the site average chronology between 1840-2012. All $\delta^{13}$C values have been corrected for the Suess effect.



*Appendix, Fig. 2* Correlation coefficients between standardised tree-ring width and monthly and seasonal temperature and precipitation values for each of the eight sites (all *F. sylvatica,* with the exception of ALP and BEA (*P. abies*); refer to *Table 1* for site abbreviations). Significant correlations (> 0.195) are marked as dark grey bars. Correlations were calculated between 1901 and 2011 for all sites, with the exception of PAY (1909-2011). Climate data were taken from the publicly available observational Climate Research Unit (CRU) TS 3.2 worldwide dataset, in which climate observations were constructed from meteorological stations on a 0.5° x 0.5° grid covering the global land surface (Mitchell & Jones 2005).



*Appendix, Fig. 3* Modelled N deposition and a 5-year moving average of BAI between 1900 and 2010 for each of the eight sites.

# General discussion

The aim of this thesis was not only to contribute to our understanding of how forest ecosystems have changed to past changes in environmental conditions, but also to the methods by which we can measure these changes through the use of tree stem growth measurements and stable isotopic composition analyses. Previous investigations have demonstrated that tree-ring N stable isotopic composition ($\delta^{15}N$) may be a useful tool in investigating changes in regional N cycles and subsequent changes in tree physiology, particularly when used in combination with isotopic measurements of carbon (C) and oxygen (O) (Bukata & Kyser 2007, Guerrieri *et al.* 2009). The use of tree-ring $\delta^{15}N$ in environmental investigations, however, has been limited in part by (1) a number of technical problems arising during isotope measurement due to low tree-ring N concentrations, (2) a limited understanding of the fractionation events and movement of N within the tree, and (3) a limited knowledge of the relationship between tree-ring and foliage $\delta^{15}N$ and both tree physiological processes and site-specific environmental conditions. *Chapters 1* and *2* sought to address these issues and determine the suitability of using both tree-ring and foliar $\delta^{15}N$ in environmental investigations. *Chapter 3* demonstrated the possibilities of using tree growth measurements, complemented with tree-ring isotope data ($\delta^{13}C$), to measure how trees have responded to environmental changes during the 20th century, and determine the implications of further environmental perturbations on forest ecosystems.

## *Tree nitrogen recycling*

Tree-rings have very low N concentrations (relative to tree-ring C concentrations), and the recycling of N within the tree is an important reallocation process to satisfy N sink demands within the tree (Merrill & Cowling 1966). In *Chapter 1*, we assessed the movement of N compounds between tree-rings within the tree stem. This was done using *Picea abies* trees (felled in 2010) from a long-term elevated N deposition experimental site that had previously been labelled with $^{15}N$ in 1995/96. The $^{15}N$ label application increased tree-ring $\delta^{15}N$ values for all tree-rings formed after labelling, and was found in tree-rings as far back as 1951 relative to control trees, up to 44 years prior to the label application. This indicates a significant movement of N within the tree stem both towards the heartwood and towards newer tree-rings. Label recovery was highest in the tree-ring formed the year after label application (1996), while the continued uptake of the $^{15}N$ label from the soil following label application also contributed to the higher tree-ring $\delta^{15}N$ values in tree-rings formed after label application than before. While previous investigations have demonstrated the bidirectional movement of N within the tree stem (Elhani *et al.* 2003, Hart & Classen 2003), these investigations did not measure the magnitude of the diffusion effect of N up to the heartwood-sapwood boundary, such as was demonstrated in our study. This translocation process may be partially responsible for the lack of a clear relationship between tree-ring $\delta^{15}N$ chronologies and selected environmental variables shown in *Chapter 2*, while the presence of the $^{15}N$ label throughout the tree sapwood may help to partially explain the ‘missing’ N sinks associated with most forest $^{15}N$ labelling experiments (Templer *et al.* 2012).

In *Chapter 2*, the importance of tree N recycling processes to meet various tree N demands within the tree stem and canopy is further emphasised in an investigation on intra-tree isotope variation. In this chapter, we investigated the relationship between tree-ring and foliar $\delta^{15}N$ and $\delta^{13}C$ in both *F. sylvatica* and *P. abies*. Our results demonstrated that both tree-ring $\delta^{15}N$ and $\delta^{13}C$ values were significantly less negative than foliage $\delta^{15}N$ and $\delta^{13}C$ values for both species. Perhaps more importantly, there was no significant relationship between tree-ring and foliar $\delta^{15}N$ within either species. Foliage is a much greater N sink within the tree than the tree stem, highlighted by the much higher concentration of N in foliage than tree-ring samples. Consequently, recycling processes within the tree will reallocate N to the foliage, both from the wood and, in the case of *P. abies*, from older foliage age-classes. This would explain the less negative tree-ring $\delta^{15}N$ relative to foliage, as molecules containing the heavier isotope are discriminated against during the movement of N within the tree from wood to foliage. Tree-ring $\delta^{15}N$ values also showed much higher variation relative to foliar $\delta^{15}N$ values from the same site, which may be indicative of inconsistent patterns of N transfer from tree stem to foliage over time. Similarly, Gerhart & McLauchlan (2014) suggested that the lack of a consistent correlation between tree-ring $\delta^{15}N$ and tree-ring N concentrations (as often seen in the foliage) was primarily due to non-environmental processes, such as N recycling processes within the tree.

*Methodological considerations*

In *Chapter 1*, we assessed the effectiveness of an extraction procedure that is often used to remove mobile N compounds prior to tree-ring $\delta^{15}N$ measurements. The movement of N compounds between tree-rings may obscure any environmental signals present in the tree-ring $\delta^{15}N$ chronologies. Subsequently, it was suggested that the removal of these mobile N compounds would aid in the interpretation of tree-ring $\delta^{15}N$ results in environmental investigations (Sheppard & Thompson 2000). We demonstrated that the extraction of mobile N compounds prior to isotope analysis had no effect on the tree-ring isotope composition of either $\delta^{15}N$ or $\delta^{13}C$ in either labelled or control trees. This is in agreement with other recent investigations on the effectiveness of mobile N extraction on tree-ring samples of *Picea abies*, *Fagus sylvatica* and *Pinus thunbergii* (Caceres *et al*. 2011, Doucet *et al*. 2011). Our results, however, demonstrate that this procedure will alter the tree-ring C/N ratio, the extent to which may depend on the proportion of latewood to earlywood in the individual tree-ring. Consequently, with no observable benefits of removing the mobile N compounds in the results of the $\delta^{15}N$ measurements, and with potential complications resulting from inconsistent changes in C concentration during extraction, we were able to conclude that this extraction procedure is unnecessary for the described purpose.

*Tree growth under changing environmental conditions*

We assessed both the short- (between 10 and 20 years) and long-term (over 100 years) effects of a number of climate variables, primarily associated with water availability, on tree growth of both *F. sylvatica* and *P. abies* in Switzerland. In *Chapter 2*, analyses covering a maximum of 14 years at the Swiss long-term monitoring sites (LWF) found that there was no significant effect of a growing-season drought response index (defined as potential evapotranspiration minus precipitation) on tree-ring width. The results of *Chapter 3*, however, revealed that tree-ring width was often significantly affected by precipitation and/or temperature at the same LWF sites when using longer datasets spanning the 20th century (1901-2011), demonstrating the importance of water availability on the tree growth of both species in Switzerland. A previous investigation on tree growth at the Swiss LWF sites by Etzold *et al.* (2014) found that site-specific disturbance events may have a greater impact on tree growth than climate in short-term investigations. Our results support the conclusions made by Etzold *et al.* (2014) that long-term datasets are necessary in the investigation of the response of tree growth to changing climatic conditions.

In addition, we assessed the influence of a variety of N deposition variables on tree growth (*Chapters 1, 2* and *3*). The results of the short-term investigations (*Chapters 1* and *2*) found no increase in tree-ring width of either *F. sylvatica* or *P. abies* with increasing N deposition, even under simulated elevated N deposition. Furthermore, there was no evidence of differences in tree growth of either species with increased soil N availability, assessed using soil C/N measurements. These results can be interpreted in two ways: (1) tree growth at these sites was not primarily limited by N availability, or (2) short-term investigations fail to fully capture the relationship between tree growth and increased N availability, as seen when analysing tree growth response to changes in water availability. The second hypothesis is supported by the fact that, using measurements of basal area increment (BAI), we were able to demonstrate that the increase in N deposition throughout the 20th century significantly increased tree growth of both *F. sylvatica* and *P. abies* at eight sites across Switzerland (*Chapter 3*). Significantly, we were able to show that, at many of the sites, the highest values of BAI corresponded to periods when N deposition was at its highest (1960-1990), and thus that N deposition was a more significant driver of tree growth in the second half of the 20th century than rising atmospheric $CO_2$ concentrations. Increased stem and canopy growth inevitably leads to an increase in tree water demand, and thus, as the frequency of drought events is predicted to increase throughout the 21st century, trees under elevated N deposition may be particularly susceptible to drought stress during dry spells.

*Tree stable isotopic composition under changing environmental conditions*

In order to better understand tree response to changing environmental conditions, we also assessed the relationship between selected environmental variables, including N deposition, and chronologies of both $\delta^{13}C$ (*Chapters 1, 2* and *3*) and $\delta^{15}N$ (*Chapters 1* and *2*) in tree-rings and foliage. Foliar $\delta^{13}C$ values were significantly related to both soil N availability and N deposition, attributed to differences in the effect of each on stomatal conductance. A decrease in foliar $\delta^{15}N$ over our measurement period (1997-2011) was suggested to be the result of changing atmospheric $\delta^{15}N$ values over time due to changes in the N isotopic composition of N emissions. The lack of data on past N deposition isotopic composition, however, means we were unable to confirm this hypothesis, while there was no significant effect of our considered environmental variables on foliar $\delta^{15}N$ composition.

Neither tree-ring $\delta^{15}N$ nor $\delta^{13}C$ were significantly affected by any of the selected environmental variables considered in our short-term investigations (covering between 10 and 20 years; *Chapters 1* and *2*). We did, however, demonstrate that tree intrinsic water-use efficiency ($WUE_i$; derived from tree-ring $\delta^{13}C$ measurements) from one mixed-forest site (Lägeren) increased significantly with both increasing N deposition and summer temperatures during the 20th century (*Chapter 3*). The increase in BAI at Lägeren with increasing N deposition implies that this increase in tree $WUE_i$ values can be partially attributed to increases in photosynthetic activity (i.e. a fertilization effect), while stomatal conductance was likely reduced in order to reduce increased transpiration rates associated with N-induced increases in tree leaf area. By contrast, the significant increases in $WUE_i$ with increasing summer temperatures in the more recent decades (2000-2010) were most likely the result of reduced stomatal conductance in order to reduce leaf-level water loss, while precipitation had no significant effect on tree $WUE_i$. These results imply that the primary environmental variable causing high $WUE_i$ in *F. sylvatica* at Lägeren has changed from N deposition between 1950 and 1990 to temperature at the end of the 20th century. This is due to both decreasing N deposition rates in Switzerland, as technological advancements are reducing $NO_X$ emissions, and increasing temperatures associated with climate change. The results of *Chapter 3* highlight the difficulty in disentangling the respective influence of different environmental conditions (and interactions therein) on tree growth and physiology.

### *Implications for future tree-ring $\delta^{15}N$ investigations*

The results of this thesis have highlighted the complexities arising from the use of tree-ring $\delta^{15}N$ in environmental investigations. Previous investigations have shown the potential use of foliar $\delta^{15}N$ values in environmental investigations (e.g. Vallano & Sparks 2007), but the use of foliar N isotopic composition in long-term environmental investigations is hindered by the short foliage life-span. Here we demonstrated a significant uncoupling occurring between corresponding tree-ring and foliar $\delta^{15}N$ values for both *F. sylvatica* and *P. abies*, primarily caused by fractionation events associated with N translocation and recycling processes, and thus that tree-ring $\delta^{15}N$ does not reflect foliar $\delta^{15}N$ at natural abundance (*Chapter 2*). In addition, neither tree-ring nor foliar $\delta^{15}N$ values showed any significant relationships with any of the environmental/climatic variables considered in our investigations. Previous investigations, however, have shown that tree-ring $\delta^{15}N$ values provide information on the origin of forest N inputs, particularly from anthropogenic emissions. As discussed in *Chapter 2*, the relationship between tree-ring $\delta^{15}N$ and N deposition in these investigations was often demonstrated under high levels of N deposition with a distinctly different isotopic signature from that of the soil, or with trees that had undergone experimental N fertilization. At background levels of N deposition, such as in our investigations, the quantification of atmospheric N deposition based on tree-ring $\delta^{15}N$ data is not possible in the absence of $\delta^{15}N$ data on atmospheric N-sources. Similarly, Gerhart & McLauchlan (2014) have previously suggested that the link between tree-ring $\delta^{15}N$ and N deposition is speculative without data on the isotopic composition of source N. This is complicated further by the highly regional and seasonal variations in N deposition. The lack of a significant relationship between N deposition and tree-ring $\delta^{15}N$ chronologies, together with the intra-stem movement of N within the tree stem demonstrated in *Chapter 1*, suggests that the application of tree-ring $\delta^{15}N$ in environmental investigations is inappropriate at low to moderate levels of N deposition.

## *Recommendations for future research*

The interpretation of tree-ring and foliar $\delta^{15}N$ chronologies would be improved by long-term datasets on the $\delta^{15}N$ isotopic signature of N sources, particularly of atmospheric N deposition. The isotopic signature of N deposition varies highly regionally as it depends greatly on the proximity to N emission sources, such as refineries, motorways or agricultural activities (Guerrieri *et al.* 2010, Saurer *et al.* 2004). With datasets on the temporal development of $\delta^{15}N$ of the source N, however, it may become possible to determine the strength of respective N sources to the N composition of both tree-rings and foliage (and other tree compartments). This would be complemented greatly with $^{15}N$ labelling experiments that could be used to quantify the movement of N within the forest ecosystem and within the trees themselves. Combined, these investigations would increase our understanding of the fractionation events associated with various tree physiological processes. However, the numerous fractionations during N translocation and other physiological processes within a tree make the applicability of N isotope ratios at natural abundance unsuitable for tracing the temporal variation of N availability and N deposition rates.

## *Overall conclusions*

The results of this thesis can be used to answer the primary research questions outlined in the *General introduction*:

1. *What is the extent of the mobility of N within the tree stem? Is the extraction of mobile N compounds prior to the isotopic analysis of tree-ring $\delta^{15}N$ necessary? How does this extraction procedure affect tree-ring $\delta^{13}C$? Are these effects the same in both trees labelled with $^{15}N$ and un-labelled trees?*

   Tree-ring N is highly mobile between tree-rings, and through a $^{15}N$-labelling experiment we were able to show that, not only is the $^{15}N$ label present in tree-rings formed after labelling, but also in tree-rings up to 44 years prior to labelling. Furthermore, the extraction of these mobile N compounds had no significant effect on either the tree-ring $\delta^{15}N$ or $\delta^{13}C$ isotopic composition in both labelled and un-labelled wood samples of *P. abies*.

2. *How does tree-ring isotopic ratio of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ relate to the isotopic ratio of corresponding foliage? Is this effect species-specific? What is the effect of site-specific environmental conditions on both tree-ring and foliar isotopic composition? And is it possible to use tree-ring $\delta^{15}N$ values as proxies of foliar $\delta^{15}N$ values in long-term environmental investigations?*

   Using archived foliage samples we were able to show that there was no significant relationship between tree-ring and foliar $\delta^{15}N$ in either *F. sylvatica* or *P. abies*. In addition, none of our selected environmental variables had a significant effect on tree-ring or foliar $\delta^{15}N$, as well as tree-ring $\delta^{13}C$. Foliar $\delta^{13}C$, however, became less negative with both decreasing soil N availability and increasing N deposition. With these results, we came to the conclusion that tree-ring $\delta^{15}N$ would be an unsuitable proxy for foliar $\delta^{15}N$ and thus not applicable for the quantification of the development of N deposition.

3. *How did tree growth respond to changing climatic conditions and N deposition throughout the 20th century in Switzerland? Were these effects either species or site-specific? What was the physiological response of trees to changes in N deposition and climate during this time? And what does this mean for trees during future changes in climate and N deposition?*

   Tree-ring width in the 20th century was often influenced by both precipitation and temperature at the eight Swiss sites included in this part of the thesis, highlighting the importance of water availability on tree growth, while N deposition significantly increased tree basal area increment at all sites during the second half of the 20th century. At the mixed forest site Lägeren, we were able to show that tree water-use efficiency increased as a result of increased water stress associated with N-induced increases in tree growth between 1960 and 1990. As N deposition declined between 1990 and 2010, however, summer temperature became a more significant driver of drought stress in these *F. sylvatica* trees. This implies that trees at Lägeren may become more susceptible to drought during dry spells under predicted climate change, even with decreases in N deposition.

Together, these results demonstrated that the role of tree-ring $\delta^{15}N$ analyses in environmental investigations is currently inappropriate to determine the development of N deposition and availability in forest ecosystems. This is primarily due to N recycling processes within the tree and the related non-systematic fractionation of tree-ring N, as well as the subsequent dampening of any environmental signal in the tree-ring $\delta^{15}N$ chronology. Furthermore, we were able to show how the response of tree physiology to changes in environmental conditions shifted from N deposition to temperature at the end of the 20th century. Long datasets, however, are required in order to fully understand and capture the effect of changing environmental conditions on both tree growth and physiology.

## References

Bukata, A. R. & Kyser, T. K. (2007) Carbon and nitrogen isotope variations in tree-rings as records of perturbations in regional carbon and nitrogen cycles. *Environmental Science & Technology,* **41,** 1331-1338.

Caceres, L. L. M., Mizota, C., Yamanaka, T. & Nobori, Y. (2011) Effects of pre-treatment on the nitrogen isotope composition of Japanese black pine (*Pinus thunbergii*) tree-rings as affected by high N input. *Rapid Communications in Mass Spectrometry,* **25,** 3298-3302.

Doucet, A., Savard, M. M., Begin, C. & Smirnoff, A. (2011) Is wood pre-treatment essential for tree-ring nitrogen concentration and isotope analysis? *Rapid Communications in Mass Spectrometry,* **25,** 469-475.

Elhani, S., Lema, B. F., Zeller, B., Brechet, C., Guehl, J. M. & Dupouey, J. L. (2003) Inter-annual mobility of nitrogen between beech rings: a labelling experiment. *Annals of Forest Science,* **60,** 503-508.

Etzold, S., Waldner, P., Thimonier, A., Schmitt, M. & Dobbertin, M. (2014) Tree growth in Swiss forests between 1995 and 2010 in relation to climate and stand conditions: Recent disturbances matter. *Forest Ecology and Management,* **311,** 41-55.

Gerhart, L. & McLauchlan, K. (2014) Reconstructing terrestrial nutrient cycling using stable nitrogen isotopes in wood. *Biogeochemistry,* **120,** 1-21.

Guerrieri, M. R., Siegwolf, R. T. W., Saurer, M., Jaggi, M., Cherubini, P., Ripullone, F. & Borghetti, M. (2009) Impact of different nitrogen emission sources on tree physiology as assessed by a triple stable isotope approach. *Atmospheric Environment,* **43,** 410-418.

Guerrieri, R., Siegwolf, R., Saurer, M., Ripullone, F., Mencuccini, M. & Borghetti, M. (2010) Anthropogenic $NO_X$ emissions alter the intrinsic water-use efficiency ($WUE_i$) for *Quercus cerris* stands under Mediterranean climate conditions. *Environmental Pollution,* **158,** 2841-2847.

Hart, S. C. & Classen, A. T. (2003) Potential for assessing long-term dynamics in soil nitrogen availability from variations in $\delta^{15}N$ of tree rings. *Isotopes in Environmental and Health Studies,* **39,** 15-28.

Merrill, W. & Cowling, E. B. (1966) Role of nitrogen in wood deterioration: amounts and distribution of nitrogen in tree stems. *Canadian Journal of Botany,* **44,** 1555-1580.

Saurer, M., Cherubini, P., Ammann, M., De Cinti, B. & Siegwolf, R. (2004) First detection of nitrogen from $NO_X$ in tree rings: a $^{15}N/^{14}N$ study near a motorway. *Atmospheric Environment,* **38,** 2779-2787.

Sheppard, P. R. & Thompson, T. L. (2000) Effect of extraction pretreatment on radial variation of nitrogen concentration in tree rings. *J. Environ. Qual.,* **29,** 2037-2042.

Templer, P. H., Mack, M. C., Iii, F. S. C., Christenson, L. M., Compton, J. E., Crook, H. D., Currie, W. S., Curtis, C. J., Dail, D. B., D'Antonio, C. M., Emmett, B. A., Epstein, H. E., Goodale, C. L., Gundersen, P., Hobbie, S. E., Holland, K., Hooper, D. U., Hungate, B. A., Lamontagne, S., Nadelhoffer, K. J., Osenberg, C. W., Perakis, S. S., Schleppi, P., Schimel, J., Schmidt, I. K., Sommerkorn, M., Spoelstra, J., Tietema, A., Wessel, W. W. & Zak, D. R. (2012) Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of $^{15}N$ tracer field studies. *Ecology,* **93,** 1816-1829.

Vallano, D. M. & Sparks, J. P. (2007) Foliar $\delta^{15}N$ values as indicators of foliar uptake of atmospheric nitrogen pollution. In: Dawson, T. E. & Siegwolf, R. T. W. (eds.) *Terrestrial Ecology*. Elsevier, 93-109.

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